COMBINED TREATMENT WITH THE CHECKPOINT ABROGATOR UCN-01 AND MEK1/2 INHIBITORS POTENTLY INDUCES APOPTOSIS IN DRUG-SENSITIVE AND -RESISTANT MYELOMA CELLS THROUGH AN IL-6-INDEPENDENT MECHANISM

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ABSTRACT

The effects of combined exposure to the checkpoint abrogator UCN-01 and pharmacologic MEK1/2 inhibitors were examined in human multiple myeloma (MM) cell lines. Treatment of RPMI8226, NCI-H929, and U266 MM cells with a minimally toxic concentration of UCN-01 (150 nM) for 24 hr resulted in MAP kinase activation, an effect that was blocked by co-administration of the MEK1/2 inhibitor PD184352. These events were accompanied by enhanced activation of p34cdc2 and a marked increase in mitochondrial damage (loss of ΔΨm; cytochrome c and Smac/DIABLO release), PARP cleavage, and apoptosis. PD184352/UCN-01 also dramatically reduced clonogenic survival in each of the MM cell lines. In contrast to As2O3, apoptosis induced by PD184352/UCN-01 was not blocked by the free radical scavenger N-acetyl-L-cysteine. Whereas exogenous IL-6 substantially prevented dexamethasone-induced lethality in MM cells, it was unable to protect them from PD184352/UCN-01-induced apoptosis despite enhancing Akt activation. IGF-1 also failed to diminish apoptosis induced by this drug regimen. MM cell lines selected for a high degree of resistance to doxorubicin, melphalan, or dexamethasone, or displaying resistance secondary to fibronectin-mediated adherence, remained fully sensitive to PD184352/UCN-01-induced cell death. Finally, primary CD138+ MM cells were also susceptible to UCN-01/MEK inhibitor-mediated apoptosis. Together, these findings suggest that simultaneous disruption of cell cycle and MEK/MAP kinase signaling pathways provides a potent stimulus for mitochondrial damage and apoptosis in MM cells, and also indicate that this strategy bypasses the block to cell death conferred by several other well-described resistance mechanisms.
Introduction

Multiple myeloma, the most common of the plasma cell dyscrasias, is a progressive and generally incurable disorder of mature B-lymphocytes. The mainstay of treatment for myeloma involves chemotherapy, utilizing agents such as steroids (e.g., dexamethasone), alkylating agents (e.g., melphalan), and topoisomerase inhibitors (e.g., doxorubicin). Although most myeloma patients respond to such approaches, at least initially, the pre-existence or emergence of drug-resistant cells represents a formidable barrier to cure.

Recently, attempts to understand the pathophysiology of multiple myeloma has focused on apoptosis, a genetically regulated program of cell suicide that is particularly involved in hematopoietic cell homeostasis. For example, there is accumulating evidence that IL-6, a growth and survival factor for myeloma cells, acts, at least in part, by blocking apoptosis. While the downstream signaling cascades responsible for the anti-apoptotic actions of IL-6 remain to be fully elucidated, JNK/SAPK-, Stat-, NFκB-, PI3K/Akt-, Mcl-1-, and Bcl-xl-related pathways have been implicated. Recently, attention focused on the p42/44 mitogen-activated protein kinase (MAP kinase) cascade as a key regulator of neoplastic cell survival. MAP kinase represents one of a family of parallel signaling modules, which includes the c-Jun N-terminal kinase (JNK) and the p38 MAP kinase. Although exceptions exist, activation of JNK and p38 MAPK is generally associated with pro-apoptotic actions, whereas MAP kinase exerts cytoprotective functions. The observation that in MM cells, IL-6- and vascular endothelial growth factor (VEGF)-related proliferative actions involves MAP kinase activation argues that the latter signaling pathway may play an important role in MM cell survival. The potential clinical implications of such findings are highlighted by the recent development of MEK1/2 inhibitors (e.g., PD184352) that display activity in vivo, and the introduction of PD184352 into Phase I trials.

UCN-01 (7-hydroxystaurosporine) is a staurosporine derivative with in vivo activity that was originally developed as a selective protein kinase C (PKC ) inhibitor. Subsequently, UCN-01 has been reported to act as an inhibitor of Chk1 and an abrogator of the G2M checkpoint. In human leukemia cells, UCN-01 interacts synergistically with antimetabolites such as ara-C and gemcitabine, and induces apoptosis in human leukemia cells, including those of lymphoid origin. Phase I/II trials of UCN-01 are currently underway, and preliminary evidence suggests that it may have activity, particularly when combined with other agents, in B-cell malignancies. The potential activity of UCN-01 against multiple myeloma cells remains largely unexplored.

In a recent communication, we reported that exposure of multiple human myeloid leukemia cell lines to sub-toxic, pharmacologically achievable UCN-01 concentrations (i.e., ~150 nM) induced activation of MAP kinase, and that interference with the latter process (e.g., by co-administration of MEK1/2 inhibitors) resulted in a dramatic potentiation of mitochondrial damage and apoptosis. In view of evidence that MAP kinase activation plays an important role in multiple myeloma cell proliferation/survival, the notion that combined exposure of myeloma cells to UCN-01 and MEK1/2 inhibitors might lead to enhanced apoptosis appeared plausible. To test this possibility, the effects of co-treatment with UCN-01 and MEK1/2 inhibitors on survival have been examined in a variety of multiple myeloma cell lines. Our results indicate that concurrent exposure of myeloma cells to UCN-01 and MEK1/2 inhibitors synergistically induces mitochondrial damage, caspase activation, and apoptosis, and that these lethal effects are undiminished by administration of exogenous IL-6 or IGF-1. Moreover, myeloma cells selected for resistance to dexamethasone, melphalan, or doxorubicin, or displaying cell adherence-related
drug resistance, retain full susceptibility to UCN-01/MEK1/2 inhibitor-induced lethality. Taken together, these findings suggest that combined treatment with a checkpoint abrogator and MEK1/2 inhibitor may warrant further investigation as a therapeutic strategy in multiple myeloma.

Materials and methods

Cells and reagents

Human MM cell lines RPMI8226, NCI-H929, and U266 were purchased from ATCC. The dexamethasone-sensitive (MM.1S) and -resistant (MM.1R) human MM cell lines26 were maintained in RPMI 1640 medium containing 10% FBS, 200 units/ml penicillin, 200 µg/ml streptomycin, minimal essential vitamins, sodium pyruvate, and glucose. Doxorubicin (Dox40) and melphalan (LR5)-resistant sublines27,28 of 8226 cells were maintained in RPMI 1640 medium as described above containing 400 nM doxorubicin and 5 µM melphalan, respectively.

The selective MEK inhibitor PD184352 was purchased from Upstate Biotechnology (Lake Placid, NY), and PD98059 and UO126 were supplied by Calbiochem (San Diego, CA) as powders. The inhibitors were dissolved in sterile DMSO, and stored frozen under light-protected conditions at -20°C. UCN-01 was kindly provided by Dr. Edward Sausville (Developmental Therapeutics Program/CTEP, NCI), dissolved in DMSO at a stock concentration of 1 mM and stored at -20°C, and subsequently diluted with serum-free RPMI medium prior to use. Arsenic trioxide (As2O3) was supplied by Sigma Chemicals (St. Louis, MO), dissolved in 1.65M NaOH at 50mM as a stock solution. N-acetyl-L-cysteine (L-NAC, Calbiochem) was prepared in sterile water immediately before use. Recombinant human IL-6 and IGF-1 were purchased from Sigma and R&D Systems (Minneapolis, MN), rehydrated in PBS and 10 mM acetic acid, respectively, both of which contained 0.1% BSA, aliquoted and stored at -80 °C. The PI3 kinase inhibitor LY294002, dexamethasone, and doxorubicin, were purchased from Sigma, dissolved in DMSO, aliquoted and stored at -20 °C. Melphalan (Sigma) was dissolved in HCl-ethanol (47:1000), aliquoted and stored at -80 °C. In all experiments, the final concentration of DMSO did not exceed 0.1%.

Experimental format

All experiments were performed utilizing logarithmically growing cells (4-6 x 10^5 cells/ml). Cell suspensions were placed in sterile 25 cm² T-flasks (Corning, Corning, NY), and incubated with PD184352 for 30 min at 37°C. At the end of this period, UCN-01 was added to the suspension, and the flasks placed in 37°C/5% CO2 incubator various intervals, generally 24 hr. In some studies, IL-6 or IGF-1 were added concurrently with PD184352. After drug treatment, cells were harvested and subjected to further analysis as described below.

Assessment of apoptosis

The extent of apoptosis was evaluated by assessing Wright-Giemsa stained cytopsin slides under light microscopy and scoring the number of cells exhibiting classic morphological features of apoptosis. For each condition, 5-10 randomly selected fields per slide were evaluated, encompassing at least 800 cells. To confirm the results of morphologic analysis, in some cases cells were also evaluated by TUNEL staining and Annexin V-FITC Staining.
For TUNEL staining, cytospin slides were fixed with 4% formaldehyde/PBS for 10 min, treated with acetic acid/ethanol (1:2) for 5 min, and incubated with terminal transferase reaction mixture containing 1x terminal transferase reaction buffer, 0.25U/l terminal transferase, 2.5 mM CoCl2, and 2 pmol fluorescein-12-dUTP (Boehringer Mannheim, Indianapolis, IN) at 37°C for 1 hr. The slides were mounted with Vectashield with propidium iodide (Vector Laboratories, Burlingame, CA) and visualized using fluorescence microscopy.

For Annexin V-FITC staining, 1 x 10^6 cells were washed twice with cold PBS and then resuspended in 1x binding buffer (10mM Hepes/NaOH, pH7.4, 140mM NaOH, 2.5mM CaCl2). The cells were incubated with Annexin V-FITC (BD PharMingen, San Diego, CA) and 5 µg/ml propidium iodide (PI), and incubated for 15 min at room temperature in the dark as per the manufacturer’s instructions. The samples were analyzed by flow cytometry within 1 hr to determine the percentage of cells displaying Annexin VI staining (early apoptosis) or both Annexin V and PI staining (late apoptosis).

### Mitochondrial membrane potential (ΔΨm) assay

After drug treatment, 2 x 10^5 cells were incubated with 40 nM 3,3-dihexyloxacarbocyanine (DiOC6, Molecular Probes Inc., Eugene, OR) in PBS at 37°C for 20 min, and then analyzed by flow cytometry. The percentage of cells exhibiting low level of DiOC6 up-take, which reflects loss of mitochondrial membrane potential, was determined using Becton-Dickinson FACScan (Becton-Dickinson, San Jose, CA).

### Western blot assay

After drug treatment, whole-cell pellets were lysed by sonication in 1x sample buffer (62.5 mM Tris base, pH6.8, 2% SDS, 50 mM DTT, 10% glycerol, 0.1% bromophenol blue, and 5 µg/ml each chymostatin, leupeptin, aprotitin, pepstatin, and soybean trypsin inhibitor) and boiled for 5 min. For analysis of protein phosphorylation, 1mM each Na vanadanate and Na pyrophosphate was added to 1x sample buffer. Protein samples were harvested as the supernatant following centrifugation of the samples at 12,800g for 5 min, and amount of protein quantified using Coomassie Protein Assay Reagent (Pierce, Rockford, IL). Equal amount of protein (30 µg) were separated by SDS-PAGE and electro-transferred onto nitrocellulose membrane. For blotting phospho-proteins, no SDS was included in the transfer buffer. The blots were blocked with 5% milk in PBS-Tween 20 (0.1%) at room temperature for 1 hr and probed with the appropriate dilution of primary antibody in 5% BSA/PBS-Tween 20 overnight at 4°C. The membranes were washed twice in PBS-Tween 20 for 30 min and then incubated with a 1:2000 dilution of HRP-conjugated secondary antibody (Kirkegaard & Perry, Gaithersburg, MD) in 5% milk / PBS-Tween 20 at room temperature for 1 hr. After washing twice in PBS-Tween 20 for 30 min, the blots were visualized by Western Blot Chemiluminescence Reagent (NEN Life Science Products, Boston, MA). For blots of phospho-proteins, TBS was used instead of PBS throughout. Where indicated, the blots were reprobed with antibodies against β-actin (BD PharMingen) to ensure equal loading and transfer of proteins. The following antibodies were used as primary antibodies: phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (1:1000, rabbit polyclonal, Cell Signaling Technology, Beverly, MA), p44/42 MAP kinase antibody (1:1000, rabbit polyclonal, Cell Signaling Technology), phospho-cdc2 (Tyr15) antibody (1:1000, rabbit polyclonal, Cell Signaling Technology), cdc2 antibody (1:1000, rabbit polyclonal, Cell Signaling Technology), phospho-cdk2 (Thr160) antibody (1:500, rabbit polyclonal, Cell Signaling Technology), cdk2 antibody (1:1000, mouse monoclonal, Transduction Laboratories, Lexington,
KY), PARP antibody (1:2500, mouse monoclonal, Biomol Research Laboratories, Plymouth Meeting, PA), phospho-Akt (Ser473) antibody (1:1000, rabbit polyclonal, Cell Signaling Technology), Akt antibody (1:1000, rabbit polyclonal, Cell Signaling Technology).

Analysis of cytosolic cytochrome c and Smac/DIABLO

After drug treatment, 4 x 10^6 cells were washed in PBS and lysed by incubating for 30 seconds in lysis buffer (75 mM NaCl, 8 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1 mM EDTA, and 350 µg/ml digitonin). The lysates were centrifuged at 12,000g for 1 min, and the supernatant was collected in an equal volume of 2x sample buffer. The proteins were quantified, separated by 15% SDS-PAGE, and subjected to Western blot as described above. Cytochrome c antibody (1:500, mouse monoclonal, Pharmingen) and Smac/DIABLO antibody (1:500, rabbit polyclonal, Upstate Biotechnology) were used as primary antibody.

CDK kinase assay

Following drug treatment, 1 x 10^7 cells were disrupted in RIPA buffer by repeated aspiration through a 21 gauge needle. Cell extracts were collected by centrifugation at 10,000g for 10 min and protein levels quantified. For each condition, 200 µg protein was immunoprecipitated with 20 µl of cdc2 p34 (mouse monoclonal, Santa Cruz Biotechnology Inc, Santa Cruz, CA) or cdk2 agarose conjugate (rabbit polyclonal, Santa Cruz Biotechnology Inc.) at 4°C overnight. The agarose was washed 4 times with RIPA buffer and incubated in Assay Dilution Buffer containing 400 µg/ml histone H1 (Upstate Biotechnology), 100 µM ATP, 15 mM MgCl_2 and 2 µCi [\gamma-^{32}P]ATP at 30°C for 20 min. An equal volume of 2x sample buffer was added in the reaction mixture and boiled for 3 minutes. [\gamma-^{32}P]-histone H1 was separated by 12% SDS-PAGE and visualized by exposure of the dried gels to X-ray film (KODAK) at -80°C for 1 hr.

Cell survival and clonogenic assays

For cell viability assays, CellTiter 96® AQueous One Solution (Promega, Madison, WI) was used as per the manufacturer’s instructions, and the absorbance at 490 nm was recorded using a 96 well plate reader (Molecular Devices, Sunnyvale, CA).

Colony forming ability following drug treatment was evaluated using a soft agar cloning assay as described previously. Briefly, cells were washed three times with serum-free RPMI medium. Subsequently, 500 cells/well were mixed with RPMI medium containing 20% FBS and 0.3% agar, and plated on 12 well plates (three wells per condition). The plates were then maintained in a 37°C/5% CO_2, fully-humidified incubator. After 10 days incubation, colonies consisting of >50 cells were scored using an Olympus Model CK inverted microscope, and colony formation for each condition calculated in relation to values obtained for untreated control cells.

Assessment of effects of fibronectin adherence on drug-induced apoptosis

96-well plates were coated with 50 µg/ml human cellular fibronectin (FN, Sigma) overnight, and the wells were washed twice with serum-free RPMI 1640 media. 4 x 10^4 cells/well were added to FN-coated plates and incubated at 37°C/5% CO_2 for 1 hr in serum-free media, and nonadherent cells were removed by washing the wells twice with serum-free media as previously described. FN-adhered cells were then treated with the drugs for 24 hr. The
percentage of apoptotic cells was determined by assessing Wright-Giemsa stained cytospin slides as described above.

**Isolation of CD138+ myeloma cells**

Bone marrow mononuclear cells were obtained with informed consent from patients with multiple myeloma undergoing routine diagnostic aspirations. Cells were collected in syringes containing preservative-free heparin and diluted 1:4 in RPMI 1640 medium. The mononuclear cell fraction was isolated by centrifugation at 400g for 38 min over Histopaque-1077 (Sigma Diagnostics). The interface layer was extracted with a Pasteur pipette and the cells washed twice in buffer (PBS containing 2mM EDTA and 0.5% BSA). The cells were incubated with MACS CD138 Microbeads (Miltenyi Biotec, Auburn, CA) at 4°C for 15 min. CD138+ and CD138- cells were separated using an MS+/LS+ column and a magnetic separator according to the manufacturer’s instructions (Miltenyi Biotec). The purity of isolated CD138+ cells was assessed by CD138-PE staining and flow cytometry and was determined to be > 90% positive. Viability of the cells was regularly > 95% by trypan blue exclusion. CD138+ cells were cultured in RPMI 1640 medium containing 10% FCS in 96-well plates under the same condition described above. In addition, parallel studies were performed utilizing the CD138- cell population. Following drug treatment, the percentage of apoptotic cells was determined by examining Wright-Giemsa stained cytospin slides under light microscopy.

**Statistical analysis**

For morphological assessment of apoptotic cells, analysis of ΔΨm, and clonogenic and cell survival assays, experiments were repeated at least three times. Values represent the means ± SD for at least three separate experiments performed in triplicate. The significance of differences between experimental variables was determined using the student’s T test.

**Results**

**UCN-01 and PD184352 interact synergistically to promote mitochondrial damage and apoptosis in MM cells**

To assess the effects of combined exposure to UCN-01 and MEK inhibitors on MM cell survival, three MM cell lines (8226, H929, and U266) were exposed to 150 nM UCN-01 ± 10 µM PD184352 for 24 hr, after which apoptosis was evaluated by morphologic criteria and loss of ΔΨm determined by monitoring DiOC6 uptake (Table 1).

**Table 1. Effects of Combined Exposure to UCN-01 and PD184352 on Apoptosis and Loss of ΔΨm in MM Cells**

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<th>Apoptotic cells (%)</th>
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8226, H929, and U266 MM cells were exposed for 24 hr to 10 µM PD184352 + 150 nM UCN-01 after which the percentage of morphologically apoptotic cells was determined by evaluating Wright Giemsa-stained cytospin preparations as described in Methods (left column). Alternatively, cells were treated as above, after which loss of mitochondrial membrane potential (ΔΨm) was monitored by analyzing DiOC6-treated cells by flow cytometry as described in Methods (right column). Values correspond to the percentage of cells displaying “low” DiOC6 uptake. In each case, values represent the means ± S.D. for three separate experiments performed in triplicate.

In each cell line, drugs administered individually were minimally toxic, whereas combined treatment resulted in a substantial increase in cell death and mitochondrial damage. Concordant results were obtained when apoptosis was monitored by Annexin V/PI staining (data not shown) or the TUNEL assay (Figure 1). In separate studies, sequential exposure to these agents also resulted in an increase in cell death, but simultaneous exposure yielded optimal lethality (data not shown). Similarly, when MM.1S myeloma cells were exposed to other MEK1/2 inhibitors (e.g., 20 µM U0126 or 50 µM PD98059) or to 150 nM UCN-01 individually for 24 hr, the percentage of annexin V/PI+ cells was < 10%. However, combined treatment of cells with UCN-01 + PD98059 or U0126 resulted in an increase in the percentage of Annexin V-stained cells to 62 and 77% respectively (data not shown). Consistent with these findings, combined (but not individual) treatment of MM cells with PD184352 and UCN-01 resulted in PARP degradation into an 85 kDa cleavage fragment and release of the pro-apoptotic proteins cytochrome c and Smac/DIABLO into the cytosolic S-100 fraction (Figure 2A). Together, these findings indicate combined treatment with UCN-01 and MEK1/2 inhibitors represents a potent stimulus for mitochondrial damage and apoptosis in MM cells.
Figure 1. UCN-01 and PD184352 interact synergistically to induce apoptosis in MM cells. Logarithmically growing 8226, H929, and U226 MM cells were exposed to 10 µM PD184352 ± 150 nM UCN-01 for 24 hr, after which cytospin preparations were obtained and apoptosis assessed by TUNEL assay as described in Methods. Slides were viewed under fluorescence microscopy under 60x magnification. An additional experiment yielded equivalent results.

Figure 2. Co-administration of PD184352 and UCN-01 in MM cells results in enhanced cytochrome c and Smac/DIABLO release, PARP degradation, and p34<sup>cdc2</sup> dephosphorylation, but diminished ERK activation. Logarithmically growing 8226, H929, and U266 MM cells were exposed to 10 µM PD184352 ± 150 nM UCN-01 for 24 hr, after which cells were lysed, the proteins separated by SDS-PAGE, and Western blot analysis performed to monitor expression of PARP (A), phospho-ERK (B), or phospho-p34<sup>cdc2</sup> (B) as described in Methods. Alternatively, S-100 cytosolic fractions were obtained as described in Methods, and expression of cytochrome c and Smac/DIABLO assessed by Western blot analysis (A). For each condition, lanes were loaded with 30 µg of protein; blots were subsequently stripped and reprobed for expression of β actin to ensure equivalent loading and transfer of protein. The results of a representative experiment are shown; an additional study yielded equivalent results. CF = PARP cleavage fragment.
MEK1/2 inhibition blocks UCN-01-mediated ERK phosphorylation while promoting p34^cdc2 activation in MM cells

Exposure of 8226 cells to 150 nM UCN-01 for 24 hr resulted in a striking increase in ERK activation, whereas in H929 and U266 cells, which displayed constitutive ERK phosphorylation, the increase was more modest (Figure 2B). However, co-exposure to PD184352 abrogated ERK activation in each cell line, comparable to results initially observed in human myeloid leukemia cells. UCN-01 also induced dephosphorylation of p34^cdc2, consistent with its role as an inhibitor of Chk1. Moreover, in each MM cell line, co-administration of PD184352 resulted in a pronounced increase in p34^cdc2 dephosphorylation. Similar findings were obtained when other MEK1/2 inhibitors (e.g., U0126 and PD98059) were employed (data not shown). These findings indicate that MEK1/2 inhibition blocks UCN-01-mediated ERK activation and markedly potentiates the capacity of UCN-01 to induce dephosphorylation of p34^cdc2 in MM cells.

To confirm that the diminished phosphorylation of p34^cdc2 was specific for this CDK and corresponded to increased activity, p34^cdc2 and CDK2 activities were assessed in drug-treated U266 and MM.1S cells (Figure 3). Consistent with the previous results in which PD184352 was employed, co-administration of U0126 and UCN-01 resulted in a marked decrease in p34^cdc2 phosphorylation, but no change in total p34^cdc2 expression. Moreover, immune kinase assays demonstrated a clear increase in p34^cdc2 activity in cells exposed to both drugs. In contrast, combined exposure to U0126 and UCN-01 did not modify CDK2 phosphorylation status or activity.

Figure 3. Co-administration of UO126 and UCN-01 in MM cells results in dephosphorylation and increased activation of p34^cdc2, but not CDK2. U266 and MM.1S cells were exposed to 20 µM UO126 ± 150 nM UCN-01 for 24 hr, after which cells were lysed, the proteins separated by SDS-PAGE, and total/phosphorylated p34^cdc2 and CDK2 were monitored by Western blot analysis. For each condition, lanes were loaded with 30 µg of protein. Alternatively, kinase assays were performed after immunoprecipitation with p34^cdc2- and CDK2-specific antibodies as described in Methods. The activity of p34^cdc2 and CDK2 was determined
by monitoring incorporation of γ-32P into histone H1. The results of a representative experiment are shown; an additional study yielded equivalent results.

**Combined exposure to UCN-01/PD184352 potently inhibits the self-renewal capacity of MM cells**

To gain insights into the combined effects of UCN-01 and MEK1/2 inhibitors on the survival and self-renewal capacity of MM cells, parallel studies were performed utilizing a pharmacologically relevant concentration of As2O3 (i.e., 1 µM), an agent that has shown promising activity against MM cells34 (Figure 4). For each of the endpoints examined (i.e., induction of apoptosis, MTS dye reduction, loss of clonogenicity), the MM cell lines displayed a differential sensitivity to a 48-hr exposure to As2O3, with H929 cells the most sensitive, 8226 cells the least sensitive, and U266 cells displaying an intermediate susceptibility (Figure 4). In particular, 8226 cells were quite resistant to As2O3, in that apoptosis was induced in only ~20% of cells after 48 hr exposure, and clonogenicity was reduced by only ~40%. This general sensitivity pattern was also observed in cells exposed to UCN-01/PD184352. However, in each of the lines, the UCN-01/PD184352 combination was highly toxic, inducing apoptosis in the large majority of cells, and essentially abrogating clonogenic survival.
Figure 4. Co-administration of PD184352 and UCN-01 potently induces loss of viability and clonogenic survival in MM cells.  8226, H929, and U266 MM cells were exposed to either 1 µM As$_2$O$_3$ or 10 µM PD184352 + 150 nM UCN-01 for 24 or 48 hr, after which the extent of morphological apoptosis (A) or loss of viability, reflected by MTS dye reduction (B), was determined as described in Methods. Alternatively, cells were washed free of drug and clonogenic assays performed as described in Methods (C). Values represent the means ± S.D. for three separate experiments performed in triplicate.

UCN-01/MEK1/2 inhibitor-mediated apoptosis in MM cells, in contrast to that induced by As$_2$O$_3$, is not antagonized by a free radical scavenger

The results of previous studies suggested that As$_2$O$_3$, induced apoptosis in MM cells through generation of reactive oxygen species (ROS). To determine whether a similar mechanism might be responsible for UCN-01/MEK1/2 inhibitor-induced apoptosis, MM cells were exposed to these agents for 48 hr in the presence or absence of the free radical scavenger L-NAC, after which apoptosis was assessed (Figure 5). Co-administration of L-NAC essentially abrogated the lethal effects of As$_2$O$_3$ in each of the MM cell lines, consistent with the results of earlier studies. In marked contrast, L-NAC exerted no effect on UCN-01/MEK1/2 inhibitor-mediated lethality. These findings suggest that the lethal actions of UCN-01/MEK1/2 inhibitors in MM cells involve factors other than or in addition to generation of reactive oxygen species.
Figure 5. The free radical scavenger L-NAC blocks As$_2$O$_3$- but not PD184352/UCN-01-induced apoptosis in MM cells. 8226 (A), H929 (B), and U266 (C) MM cells were exposed to either 1 µM As$_2$O$_3$ or 10 µM PD184352 + 150 nM UCN-01 for 24 or 48 hr after 2 hr pretreatment of 10 mM L-NAC, after which the percentage of apoptotic cells was determined by evaluating Wright-Giemsa-stained cytospin preparations as described in Methods. Values represent the means ± S.D. for three separate experiments performed in triplicate.

UCN-01/MEK1/2 inhibitor-induced apoptosis proceeds through IL-6- and IGF-1-independent pathways

In addition to its role in promoting MM cell survival, IL-6 has also been shown to protect MM cells from the lethal actions of cytotoxic agents, including dexamethasone. To determine whether and to what extent IL-6 might exert a similar function in cells exposed to UCN-01/MEK1/2 inhibitors, MM cells were exposed to these agents for 72 hr in the presence or absence of 100 ng/ml IL-6 (Figure 6). Consistent with earlier results, exogenous IL-6 essentially abrogated the lethal effects of 10 µM dexamethasone in each of the MM cell lines, reflected by loss of viability as determined by the MTS assay. Similar results were obtained when apoptosis was monitored (data not shown). In marked contrast, IL-6 failed to attenuate UCN-01/PD184352-mediated lethality in any of the MM cell lines. Similarly, IGF-1 (400 ng/ml), which as also been shown to act as survival factor in MM, was unable to block apoptosis in UCN-01/MEK1/2 inhibitor-treated cells (Figure 6D). These findings indicate that the lethal actions of the UCN-01/MEK1/2 inhibitor combination in MM cells operate downstream of IL-6 and IGF-1 cytoprotective pathways.

Figure 6. PD184352/UCN-01-induced apoptosis proceeds via IL-6- and IGF-1-independent pathways in MM cells. 8226 (A), H929 (B), and U266 (C) MM cells were exposed to 10 µM PD184352 + 150 nM UCN-01 or 10 µM dexamethasone for 24 to 72 hr in the presence or
absence of 100 ng/ml IL-6, after which the loss of viability, reflected by MTS dye reduction, was determined as described in Methods. Alternatively, cells were exposed to PD184352 + UCN-01 for 24 or 48 hr in the presence or absence of 400 ng/ml IGF-1, after which the percentage of apoptotic cells was determined by examining Wright-Giemsa-stained specimens as described in Methods (D). In all cases, values represent the means ± S.D. for three separate experiments performed in triplicate.

**IL-6 fails to block UCN-01/MEK1/2 inhibitor-induced mitochondrial damage in MM cells**

To determine whether the failure of IL-6 to attenuate UCN-01/PD184352-induced apoptosis in MM cells originated at the mitochondrial level, cytochrome c and Smac/DIABLO release were monitored in cells exposed to these agents (Figure 7A). In each of the MM cell lines, co-administration of IL-6 with UCN-01/PD failed to attenuate cytochrome c and Smac/DIABLO release, or PARP degradation. These findings suggest that UCN-01/MEK1/2 inhibitor-induced mitochondrial damage occurs independently of IL-6-related cytoprotective signaling pathways.

**UCN-01/MEK1/2 inhibitor-induced apoptosis occurs in IL-6-treated MM cells despite Akt activation**

Recent studies suggest that in MM cells, IL-6-related cytoprotective effects involve activation of the PI3K/Akt cascade. Consistent with these results, exposure of each of the MM lines to IL-6 resulted in Akt phosphorylation (Figure 7B). However, despite the failure of IL-6 to attenuate UCN-01/MEK1/2 inhibitor-induced apoptosis in MM cells (Figure 5), IL-6 administration continued to increase Akt phosphorylation over basal levels in UCN-01/PD184352-treated cells. IL-6 also increased ERK activation in each MM cell line, but this effect was abrogated in cells exposed to UCN-01/PD184352 (Figure 7B). Lastly, co-administration of the PI3K inhibitor LY294002 (10 µM) failed to modify UCN-01/PD184352-mediated lethality in any of the MM cell lines, although slight increases in UCN-01-induced apoptosis were noted (data not shown). Together, these findings argue that factors other than or in addition to perturbations in the PI3K/Akt pathway are responsible for MM cell death following exposure to UCN-01/MEK1/2 inhibitors.
Figure 7. IL-6 fails to block PD184352/UCN-01-mediated mitochondrial damage despite enhancing Akt phosphorylation. (A) 8226, H929, and U266 MM cells were exposed to 10 µM PD184352 + 150 nM UCN-01 in the presence or absence of 100ng/ml IL-6 for 24 hr after which cells were lysed and Western blot analysis performed to monitor PARP cleavage (whole cell lysates) or cytochrome c and Smac/DIABLO release (S-100 fractions) as described in Methods. (B) Alternatively, Western blot analysis was performed to assess the effects of these agents on phosphorylation of Akt and ERK as well as total ERK expression. In each case, lanes were loaded with 30 µg of protein. The results of a representative experiment are shown; a second study yielded equivalent results.

UCN-01/PD184352 effectively induces apoptosis in MM cells resistant to doxorubicin or melphalan

To determine whether MM cells resistance to standard chemotherapeutic drugs would extend to the UCN-01/MEK1/2 inhibitor combination, doxorubicin-resistant (8226/Dox40)27 and melphalan resistant (8226/LR5)28 cells were treated with 10 µM PD184352 and 150 nM UCN-01 for 24-72 hr, after which the extent of apoptosis was assessed (Figure 8). Whereas sensitive cells (8226/S) were highly susceptible to 400 nM doxorubicin and 5µM melphalan (Figure 8A), the corresponding resistant lines were essentially immune to these agents (Figures 8B and 8C). Equivalent results were obtained when loss of ΔΨₘ was monitored (data not shown). In marked contrast, both 8226/Dox40 and 8226/LR5 cells were as sensitive to the PD184352/UCN-01 combination as parental cells, with approximately 100% of cells undergoing apoptosis after 72 hr. Western analysis confirmed that induction of cytochrome c/Smac/DIABLO release and PARP degradation by the combination of UCN-01 and PD184352 in 8226/Dox40 and 8226/LR5 cells (Figure 8D) was equivalent to effects observed in parental cells (Figure 3A). These findings indicate that MM cells highly resistant to doxorubicin or melphalan remain fully sensitive to mitochondrial injury and apoptosis induced by UCN-01/PD184352.

Figure 8. MM cells resistant to doxorubicin or melphalan retain full sensitivity to UCN-01/PD184352. Logarithmically growing parental 8226 cells (8226/S; A), a doxorubicin-resistant
cell line (8226/Dox40, B), and a melphalan resistant line (8226/LR5, C) were exposed to 10 µM PD184352 + 150 nM UCN-01, 400 nM doxorubicin (Dox), or 5 µM melphalan (Mel) for 24-72 hr, after which the percentage of apoptotic cells was determined by examining Wright-Giemsa-stained cytospin preparations as described in Methods. values represent the means ± S.D. for three separate experiments performed in triplicate. (D) 8226/Dox40 and 8226/LR5 cells were exposed to PD184352 + UCN-01 as above for 48 hr and 24 hr, respectively, after which Western blot analysis was employed to monitor degradation of PARP into an 85 kDa fragment (CF) or release of cytochrome c and Smac/DIABLO into the cytosolic S-100 fraction as described above. Lanes were loaded with 30 µg of protein; blots were subsequently stripped and reprobed for expression of β actin to ensure equivalent loading and transfer of protein. The results of a representative experiment are shown; an additional study yielded equivalent results.

**MM cells highly resistant to dexamethasone retain their sensitivity to combined treatment with UCN-01 and PD184352**

Using a previously described dexamethasone-resistant myeloma cell line (MM.1R)\(^\text{26}\), attempts were made to determine whether cross-resistance to the lethal effects of UCN-01/PD184352 would occur. As shown in Figures 9A and 9B, MM.1R cells were highly resistant to dexamethasone-induced apoptosis, but retained full sensitivity to apoptosis induced by 150 nM UCN-01 + 10 µM PD184352. Furthermore, while IL-6 significantly attenuated dexamethasone-induced apoptosis, it had no effect on PD184352/UCN-01-induced cell death in either cell line. Similar results were obtained when U0126 was employed instead of PD184352 (data not shown). Concordant results were also obtained when the reduction in ΔΨm was monitored (Figure 9C).
Figure 9. IL-6 fails to protect dexamethasone-sensitive and -resistant MM cells from the lethal actions of UCN-01/PD184352. Logarithmically growing dexamethasone-sensitive (MM.1S; Figure 9A) and –resistant MM cells (MM.1R; Figure 9B) were exposed to 10 $\mu$M PD184352 + 150 nM UCN-01 or 10 $\mu$M dexamethasone in the presence or absence of 100 ng/ml IL-6 for 24-72 hr, after which the percentage of apoptotic cells was determined by examining Wright-Giemsa-stained cytospin preparations. Alternatively, MM.1S and MM.1R cells were treated as above for 24 or 48 hr, after which the percentage of cells displaying a reduction in $\Delta\Psi$m was determined as described in Methods (Figure 9C). Values represent the means ± S.D. for three separate experiments performed in triplicate.

Finally, consistent with results obtained in other MM cell lines (Figure 2B), both MM.1S and MM.1R cells displayed activation of MAP kinase following UCN-01 exposure, an effect that was abrogated by PD184352. Moreover, the combination of UCN-01 and PD184352, but not drugs administered alone, induced an equivalent increase in cytosolic release of cytochrome c and Smac/DIABLO as well as PARP degradation in dexamethasone-sensitive and resistant MM cells (data not shown). These results indicate that a high level of resistance to dexamethasone provides MM cells essentially no protection from the mitochondrial injury and lethality of the UCN-01/MEK1/2 inhibitor combination.

The UCN-01/PD18432 regimen is active against MM cells exhibiting cell adherence-associated drug resistance

Based upon recent evidence that induction of MM cell adherence (e.g., by fibronectin) confers resistance to multiple classes of cytotoxic agents$^{32}$, attempts were made to determine whether such treatment would protect MM cells from exposure to UCN-01/PD184352. As shown in Figure 10, treatment of MM.1S and MM.1R with fibronectin significantly reduced the toxicity of both doxorubicin and melphalan, consistent with results previously reported by Damiano et al., in 8226 MM cells$^{32}$. However, in marked contrast to these findings, fibronectin-adhered cells remained fully sensitive to PD184352/UCN-01-mediated apoptosis. These observations suggest that the lethal effects of checkpoint abrogation/MEK1/2 inhibition, unlike conventional cytotoxic agents, are minimally attenuated by cytoprotective signaling events associated with the adherent phenotype.

Co-administration of UCN-01 with a MEK1/2 inhibitor displays selective lethality toward primary bone marrow CD138$^+$ MM cells

To determine whether the UCN-01/MEK inhibitor strategy would be lethal toward primary MM specimens, CD138$^+$ MM cells were isolated from the bone marrow of MM patients as previously described $^{33}$. Samples #1 and #2 were obtained from patients who had progressed following therapy, whereas sample #3 was obtained from a newly-diagnosed patient. The cells were then exposed to U0126 ± UCN-01 for 24 hr, after which the extent of apoptosis was determined by morphological analysis of Wright Giemsa-stained cytospin preparations. As shown in Figure 11A-C, co-administration of U0126 + UCN-01 resulted in a clear increase in apoptosis in each of the three primary specimens evaluated. Interestingly, U0126 + UCN-01 exerted relatively minimal toxicity toward the CD138$^-$ cell population, raising the possibility that
primary MM cells may exhibit selective sensitivity to this regimen. Representative photomicrographs of pre- and post-treatment MM specimens (obtained from patient #2; Figure 11D) illustrate the striking increase in MM cell death following ex vivo UCN-01/U0126 exposure.

Figure 10. Fibronectin-adhered MM cells remain sensitive to UCN-01/PD184352-induced apoptosis. MM.1S and MM.1R cells were seeded into 96 well plates coated with fibronectin, and cells remaining in suspension removed as described in Methods. Adherent cells and cells in suspension were separately exposed to 250 nM doxorubicin, 10 µM melphalan, 10 µM dexamethasone, or 10 µM PD184352 + 150 nM UCN-01 for 24 hr, after which the extent of apoptosis was determined by evaluating Wright Giemsa-stained cytospin preparations as described previously. Values represent the means ± S.D. for three separate experiments performed in triplicate. * = significantly lower than values for cells in suspension; P < 0.01.
Figure 11. Primary CD138+ MM cells display selective sensitivity to UCN-01/UO126-induced apoptosis. CD138+ and CD138− cells were isolated from the bone marrow of MM patients as described in Methods. The cells were exposed individually or in combination to drugs as follows, UO126 (A: 25 µM; B and C: 20 µM); UCN-01 (A: 150 nM; B: 250 nM; C: 100 nM). After 24 hr treatment, the extent of apoptosis was determined by evaluating Wright Giemsa-stained cytospin preparations as described previously. Values represent the means ± S.D. for more than 20 randomly selected fields encompassing more than 300 cells. Representative microphotographs of cells obtained from patient #2 are shown in D, a: early apoptotic cells; b: late apoptotic cells; c: post-apoptotic (ghost) cells.

Discussion

The present results indicate that combined exposure to a clinically relevant concentration of UCN-01 in conjunction with pharmacologic MEK1/2 inhibitors potently induces programmed cell death in MM cells, including those selected for resistance to a variety of agents useful in the treatment of this disease, including melphalan, doxorubicin, and dexamethasone. Despite recent advances in our understanding of the molecular pathogenesis of MM, survival of patients with this disorder has not changed appreciably over the last several decades. Consequently, attention has begun to focus on the development of novel agents that target specific MM survival pathways. These have included thalidomide, an inhibitor of the VEGF signaling cascade, proteasome inhibitors such as PS341, which interfere with NFκB cytoprotective functions, and As2O3, which promotes the formation of lethal free radical species in MM cells. However, the concept of combining novel signal transduction and cell cycle inhibitors in MM or other hematologic malignancies remains relatively unexplored. The data presented here indicate that combined treatment with UCN-01, a checkpoint abrogator in clinical evaluation and pharmacologic MEK1/2 inhibitors, including PD184352, which is now in Phase I trials, represents an extremely potent stimulus for mitochondrial injury and apoptosis in MM cells. Taken in conjunction with observations involving myeloid leukemia cells, these findings raise the possibility that malignant hematopoietic cells may be particularly susceptible to a strategy in which cell cycle regulatory and cytoprotective signaling pathways are simultaneously interrupted.

While the mechanism(s) by which MEK1/2 inhibition potentiates UCN-01-induced apoptosis in MM cells remains to be defined, it is tempting to speculate that inappropriate activation of p34cdc2 may be involved. For example, consistent with its role as an inhibitor of Chk1, UCN-01 induced dephosphorylation (activation) of p34cdc2 in each of the MM cell lines (Figure 2). Moreover, this event was enhanced by co-administration of PD184352. As unscheduled activation of p34cdc2 in hematopoietic cells represents a potent stimulus for apoptosis, the cytoprotective actions of MAP kinase activation may serve to limit this process. Such a concept could help to explain the observed potentiation of p34cdc2 dephosphorylation and activation in UCN-01/PD184352-treated MM cells. In this context, a requirement for MAP kinase activation in G2M progression, an event that is regularly accompanied by p34cdc2 activation, would be fully consistent with this model.
Although activation of the MAP kinase pathway is generally known to exert anti-apoptotic actions, in MM cells it appears to play a specific role in IL-6-related cytoprotection. Moreover, the ability of UCN-01/MEK1/2 inhibitors to induce MM cell death in an IL-6-independent manner is entirely compatible with this notion. For example, the finding that the MEK1/2 inhibitor PD98059 prevents IL-6 from protecting OPM-6 MM cells from dexamethasone-mediated cell death implicates the MAP kinase pathway in IL-6 survival functions. It also suggests that activation of the latter pathway represents a downstream consequence of IL-6 actions. However, while administration of IL-6 resulted in MAP kinase activation in each of the MM cell lines examined here (Figure 7), this effect was abrogated in UCN-01/PD184352-treated cells. Thus, administration of MEK1/2 inhibitors blocked MAP kinase activation by exogenous IL-6, UCN-01, as well as the combination of these agents. It should be noted that recently histone deacetylase inhibitors (HDIs) have been shown to trigger apoptosis in MM cells in an IL-6-independent manner. The relationship between this phenomenon and effects on MAP kinase activation remains to be defined. Finally, the cytoprotective effects of IL-6 and IGF-1 in MM cells have recently been related to the PI3K/Akt pathway. The ability of UCN-01/PD184352 to trigger MM cell apoptosis in the presence of IL-6 and despite Akt activation suggests that these agents act independently of, or at a point downstream of Akt actions.

The inability of the free radical scavenger L-NAC to block UCN-01/PD184352-induced apoptosis in MM cells argues that this process proceeds through an ROS-independent mechanism. Although ROS generation has been implicated in the induction of apoptosis by various cytotoxic agents, in some cases it may represent a secondary event stemming from mitochondrial injury and disruption of the mitochondrial respiratory chain. It is noteworthy that As2O3, which also potently induces apoptosis in MM cells, including those resistant to conventional cytotoxic agents, appears to trigger cell death through a ROS-dependent process. The finding that UCN-01/PD184352 also effectively induced cell death in drug-refractory MM cell lines indicates circumvention of conventional drug resistance in MM cells can involve apoptotic pathways operating independently of ROS.

Previous studies have demonstrated that in MM cells, cell death induced by different stimuli can elicit distinct forms of mitochondrial injury. For example, in MM.1S cells, ionizing radiation primarily induced cytochrome c release, whereas dexamethasone triggered release of Smac/DIABLO. In contrast, UCN-01/PD184 induced, although to varying extents, cytosolic distribution of both cytochrome c and Smac/DIABLO in each of the MM cell lines. The relative contributions of these proteins to UCN-01/PD184352-induced lethality is unclear. In this context, recent studies in prostate cancer cells suggest that Smac/DIABLO release following PI3K/Akt inhibitor LY294002 exposure is necessary for cytochrome c-mediated activation of the apoptotic cascade. Whether similar events occur in MM cells remains to be determined.

Resistance of 8226/Dox40 cells to doxorubicin has been attributed to increased expression of P-glycoprotein (Pgp), leading to reduced intracellular drug accumulation. Although enhanced expression of Pgp has been shown to reduce, albeit weakly, uptake of small molecule inhibitors such as flavopiridol, it has been found to be somewhat more effective in diminishing the toxicity of UCN-01 in MCF-7 breast cancer cells. However, the finding that Dox40 MM cells were fully sensitive to UCN-01/PD184352 toxicity argues that Pgp-related mechanisms do not play a major role in determining the response of MM cells to either of these agents. Resistance of MM.1R cells to dexamethasone has been attributed to a mutation in the steroid receptor. In view of the presumed distal site of action for the UCN-01/PD184352...
combination (i.e., downstream of IL-6/MEK/MAP kinase signaling), it seems likely that this regimen simply bypasses the block to steroid action. The basis for alkylating agent resistance in MM or other neoplastic cells may be multifactorial, including diminished drug uptake, decreased DNA binding, increased GSH levels, or enhanced repair mechanisms, among others. The unimpaired ability of UCN-01/PD184352 to induce apoptosis in 8226/LR5 cells suggests that none of these mechanisms is involved in conferring resistance to this drug combination. Finally, the phenomenon of cell adhesion-mediated drug resistance (CAM-DR) has recently been described in MM cells, suggesting that integrins can raise the threshold for drug-induced apoptosis. Although the mechanism underlying this phenomenon remains to be elucidated, the present findings indicate that the lethal effects of the UCN-01/PD184352 combination operate independently of adhesion-related survival pathways.

In summary, the present findings indicate that combined treatment with the checkpoint abrogator UCN-01 and the MEK1/2 inhibitor PD184352, both of which are currently in clinical trials, represents a highly potent trigger for mitochondrial damage and apoptosis in MM cells. These events are associated with inhibition of UCN-01-mediated activation of MAP kinase, and enhanced activation of p34<sup>cdc2</sup>. They also indicate that UCN-01/PD184352-mediated cell death proceeds in an IL-6-independent manner, and in contrast to As<sub>2</sub>O<sub>3</sub>, is not inhibitable by free radical scavengers. In addition, this drug combination effectively induces apoptosis in MM cells that are resistant to three cytotoxic agents widely used in the MM treatment i.e., dexamethasone, melphalan, and doxorubicin, as well as in cells displaying adhesion-related drug resistance. Finally, this strategy is active against at least some primary MM cell samples <em>ex vivo</em>. A theoretical model that might provide a framework for understanding the present findings is illustrated in Figure 12. UCN-01, by inhibiting Chk1, spares cdc25C from proteasomal degradation, thereby activating p34<sup>cdc2</sup>, which is known to be a potent apoptotic stimulus. The compensatory activation of MEK1/2 and ERK1/2, which lie downstream of IL-6, IGF-1, and integrin-stimulated PKC/Ras/Raf-related pathways, may permit cells to survive the inappropriate activation of p34<sup>cdc2</sup> in UCN-01-treated cells. Conversely, abrogation of the compensatory ERK1/2 response might render cells particularly sensitive to UCN-01-induced mitochondrial injury and apoptosis. Such a model could also account for the failure of effectors such as IL-6, IGF-1, and integrins, which signal upstream of the Ras/Raf/MEK/MAP kinase cascade, to circumvent UCN-01/MEK1/2 inhibitor-associated lethality. However, the possibility that MEK inhibitors may induce perturbations in NFκB, which has been implicated in MM cell survival decision, cannot be excluded, nor can a direct effect of MEK inhibitors on cdc25C activity be ruled out. In any case, the present findings suggest that MM cells may be highly sensitive to a therapeutic strategy in which cell cycle regulatory and survival signaling pathways are simultaneously disrupted. However, the issue of therapeutic selectivity will clearly play an important role in determining the ultimate utility of this approach. In this regard, we have recently observed that UCN-01/MEK1/2 inhibitor regimens exhibit relatively modest toxicity toward normal human peripheral blood mononuclear cells, bone marrow CD34<sup>+</sup> cells, primary rodent hepatocytes, and in this study, CD138<sup>−</sup> bone marrow cells. In view of these considerations, efforts to develop this strategy in MM and possibly in other B-cell malignancies appear warranted.
Figure 12. Model of UCN-01 and MEK1/2 inhibitor interactions in MM cells. The DNA damage response genes ATM and ATR activate Chk1, which phosphorylates the Cdc25C phosphatase, leading to its proteasomal degradation. Inhibition of Chk1 phosphorylation results in activation of p34\(^{cdc2}\), which, if unscheduled, leads to apoptosis. UCN-01, by inhibiting Chk1 phosphorylation, spares Cdc25C, which in turn promotes activation (dephosphorylation) of p34\(^{cdc2}\). The putative pro-apoptotic actions of activated p34\(^{cdc2}\) may be opposed by a compensatory activation of the cytoprotective Raf/MEK/MAP kinase cascade, which is also stimulated by several MM survival factors, including IL-6, IGF-1, and integrins. Blocking the MEK/MAP kinase cascade (e.g., by pharmacologic MEK1/2 inhibitors) downstream of IL-6-, IGF-1-, and integrin-related actions (e.g., fibronectin, FN) may thus render MM cells particularly vulnerable to the lethal actions of UCN-01. The contribution of the NFκB axis, which is linked to both ERK1/2 and PI3K/Akt (dashed lines), to these events remains to be fully elucidated. Finally, the possibility that MEK1/2 inhibitors act directly on cdc2 regulatory molecules (i.e., cdc25C or Wee1) cannot be excluded.

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References


Combined treatment with the checkpoint abrogator UCN-01 and MEK1/2 inhibitors potently induces apoptosis in drug-sensitive and -resistant myeloma cells through an IL-6-independent mechanism

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