Melphalan and XPO1 Inhibition are Synergistic in Pre-Clinical Models of Multiple Myeloma

Joel G. Turner, Jana L. Dawson and Daniel M. Sullivan

H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL

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H. LEE MOFFITT CANCER CENTER & RESEARCH INSTITUTE,
AN NCI COMPREHENSIVE CANCER CENTER – Tampa, FL
1-888-MOFFITT (1-888-663-3488) www.MOFFITT.org

Abstract

Introduction:

Multiple myeloma (MM) accounts for about 10% of all hematologic malignancies, with estimated numbers of new cases and deaths for 2015 in the US at 26,850 and 11,240, respectively. Significant increases in response/survival have been seen over the past several years; however, MM remains incurable and patients ultimately die from progressive disease refractory to anti-myeloma therapy. There is clearly a need for additional effective therapies.

Materials and Methods:

Human MM parental and melphalan (MEL) resistant cell lines were treated with XPO1 inhibitors (XPO1i) KPT330 or KPT8602 +/- MEL and assayed for apoptosis and viability by flow cytometry. Treated cells were assayed for DNA damage by comet assay and phospho-H2AX protein expression. XPO1/MEL treated cells were assayed for p53, NFkB, IKKα, FANCF, and FANCL by Western blot. Cells from patients with newly diagnosed or relapsed/refractory MM treated with XPO1i/MEL were assayed for apoptosis. NOD/SCID-gamma mice with MM tumors were treated with XPO1i/MEL and assayed for tumor growth, survival, and toxicity.

Results:

MM cell viability was decreased synergistically and apoptosis increased by XPO1i/MEL treatment in all MM cell lines tested. XPO1/MEL drug combination significantly increased DNA damage when compared to either MEL or XPO1 alone, as shown by comet assay and increased phospho-H2AX expression. Western blot showed that XPO1i treatment of MM cells increased p53 and decreased NFkB, IKKα, FANCF, and FANCL. MEL-resistant MM cell lines were found to be sensitized by XPO1i to MEL as shown by apoptosis assay (20-fold). CD138+/light chain+ MM cells from newly diagnosed and relapsed/refractory MM patients were also sensitized (5-10 fold) by XPO1i to MEL (apoptosis assay). NOD/SCID-gamma mice challenged with MM tumors demonstrated a strong synergistic antitumor effect in XPO1i/MEL-treated mice, with increased survival and no significant toxicity.

Conclusions:

XPO1i's improved the response of human MM cell lines and patient MM cells to MEL *in vitro* and *ex vivo*. It is possible that this synergy may be due to increased nuclear p53 in combination with decreased NFkB and IKKα and decreased DNA repair proteins FANCL and FANCF reversing MEL resistance by the Fanconi Anemia/BRCA pathway. These preliminary data suggest that XPO1i's augment MEL-induced DNA damage and may also block the repair of the DNA damage—both of which could result in synergistic cell kill. Combination therapies using XPO1i, especially the clinical compounds KPT330 (selinexor) and KPT8602 +/- MEL, may significantly improve the treatment outcomes of MM.

Small-molecule inhibitors of XPO1 sensitize human MM cell lines to MEL and 4-hydroperoxy-cyclophosphamide (4HC).

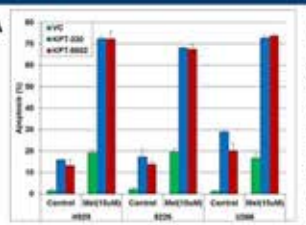

Figure 1. Small-molecule inhibitors of XPO1 sensitize human MM cell lines to MEL and 4-hydroperoxy-cyclophosphamide (4HC). High-density H929 MM cells ($3x10^6$ cells/ml), 8226 ($2x10^6$ cells/ml) and U266 ($4x10^6$ cells/ml) human MM cells were treated concurrently with KPT-330 (300nM) or KPT-8602 (300 nM) +/- (A) MEL (15 μM) or (B) 4HC (5 μM) for 20 hours. (A) The combination of KPT-330/MEL ($P = 2.2x10^{-4}$ to 0.0032) or KPT-8602/MEL ($P = 1.2x10^{-3}$ to 0.0031) synergistically increased apoptosis (activated caspase 3) in MM cell lines over single agent MEL (n=3). (B) In addition the combination of KPT-330/4HC ($P = 0.004$ to 0.019) or KPT-8602/4HC ($P = 1.2x10^{-3}$ to 0.0031) synergistically increased apoptosis (activated caspase 3) in MM cell lines over single agent 4HC (n=4).

XPO1 Inhibition Sensitizes MEL-Resistant Myeloma

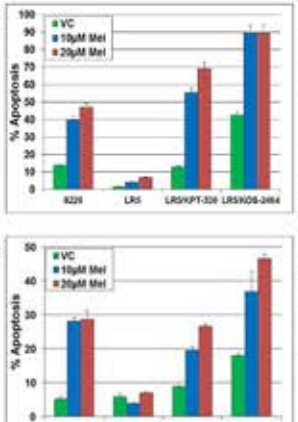

Figure 2 XPO1 inhibition sensitizes MEL-resistant human MM cell lines to MEL. Human 8226(A) and U266(B) drug-resistant (LR5 and LR6) and parental MM cell lines were treated concurrently for 20 hr with KPT-330 (300 nM) or KOS-2464 (10 nM) +/- MEL (10 or 20 μM) and assayed for apoptosis by flow cytometry (activated caspase 3). Resistant MM cell lines were 4.1 to 9.5-fold resistant to single agent MEL when compared to parental cells. The addition of the XPO1i's KPT-330 or KOS-2464 ($p = 0.015$ to 0.030) sensitized drug resistant LR5 cells to MEL when compared to single agent MEL. XPO1i's KPT-330 or KOS-2464 ($p = 0.003$ to 0.0006) sensitized drug resistant LR6 cells to MEL when compared to single agent MEL.

XPO1 inhibitor/MEL Induced DNA Damage in MM

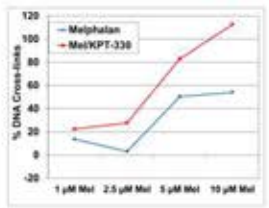

Figure 3: Alkaline Comet Assay showing increased MEL-induced DNA ICL's from the addition of KPT-330. High-density H929 MM cells (3 x 10⁶/ml) were exposed to the XPO1i KPT-330 (0.1 μM) for 20 hr followed by MEL (1, 2.5, 5, & 10 μM) for 2 hr. Drugs were removed and cells incubated another 3 hr to allow cross-links to form. Cells were exposed to 900 rads (X-Rad 160 X-ray biological irradiator) and alkaline comet assays were performed immediately (Trevigen Inc).

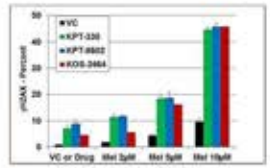

Figure 4: XPO1/melphalan induced Phospho-H2AX-γ expression. Human H929 MM cells were treated with XPO1i's +/- MEL and assayed for H2AX. Data are expressed as the percentage of treated cells expressing high-levels of H2AX. XPO1 inhibition by KPT-330, KPT-8602 and KOS-2464 increased the effectiveness of 10 μM MEL to induce double strand DNA breaks versus MEL control as shown by H2AX expression ($P = 0.005$, $P = 0.001$ *and* $P = 0.0005$ respectively) and in a dose-dependent manner.

Selinexor/MEL combination treatment decreases NFkB, IKKα, FANCF, FANCL and increases nuclear P53.

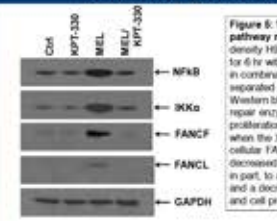

Figure 5: Western blot of FA and NFkB pathway members with XPO1i + MEL. High-density H929 MM cells (3 x10⁶/ml) were treated for 6 hr with MEL (10 μM), KPT-330 (300 nM) or in combination. Whole cell lysates were separated by SDS-PAGE and assayed by Western blot. MEL was found to increase DNA repair enzymes FANCF and FANCL, and cell proliferation proteins NFkB and IKKα. However, when the XPO1i KPT-330 was added, total cellular FANCF, FANCL, IKKα, and NFkB were decreased. MEL/KPT-330 synergy may be due, in part, to an increase in DNA damage by MEL, and a decrease in both DNA repair enzymes and cell proliferation proteins by KPT-330.

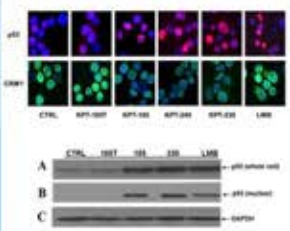

Figure 6: WT p53 and XPO1i's. XPO1 inhibitors increase total cellular and nuclear p53 as shown by immunofluorescence microscopy (red) and Western blot (Turner et al, *J Cancer*. 2013 Sep 10;4(8):614-25). MEL has been shown to kill cells through production of reactive oxygen species and activation of the p53 pathway (Surget et al, Leuk. Lymphoma. 2014 Sep;55(9):2165-73). MEL/ KPT-330 synergy may be due, in part, to an increase of nuclear p53 by KPT-330, and the reported activation of p53 by MEL.

Ex Vivo Patient Data

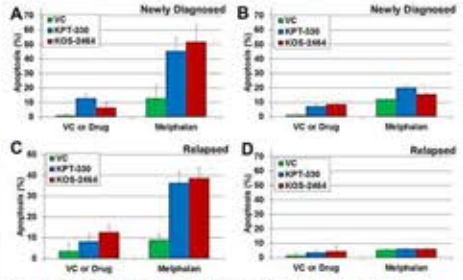

Figure 7: KOS-2464 and Selinexor (KPT-330) sensitize newly diagnosed and relapsed patient MM cells to MEL. Bone marrow mononuclear cells were isolated and treated with XPO1i's (300 nM) +/- MEL (10 μM) for 20 hours. Cells were fluorescently labeled with antibodies against activated caspase 3, CD138, and light chain (kappa or lambda). **(A)** Newly diagnosed (n=4) and **(C)** relapsed (n=2) MM patient samples were sensitized by selinexor and KOS-2464 to MEL vs single agent MEL ($P < 0.014$ to 0.008) as shown by increased apoptosis. **(B, D)** Non-myeloma CD138/light-chain double-negative patient cells were not sensitized to apoptosis by XPO1i's.

NOD/SCID In vivo Treatment

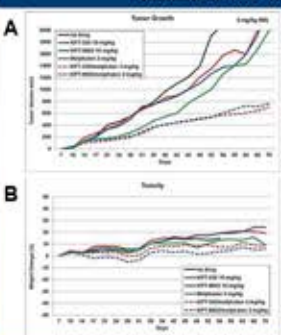

Figure 8: NSG mouse *in vivo* studies. A) Tumor growth with XPO1i's and MEL. MEL/KPT-330 or MEL/KPT-8602 treatment reduced human MM U266 tumor growth when compared to single-agent MEL ($P = 0.0024$ and 0.0030, respectively). Tumor volumes were plotted versus time. Mice were euthanized when tumor volumes reached 2000 mm³. **B) Toxicity**, as assessed by weight loss, was minimal in all treatment groups. MEL (intraperitoneal injection) and the XPO1i's (oral gavage) were both administered twice weekly (Tues, Fri), and when given in combination were on the same day and concurrently.

Conclusions

- XPO1 inhibitors improved the response of human MM cell lines and patient MM cells to melphalan *in vitro* and *ex vivo*.
- XPO1 inhibitors increased nuclear p53 in combination with decreased NFkB and IKKα and DNA repair proteins FANCL and FANCF reversing MEL drug resistance.
- XPO1 inhibitors augment MEL-induced DNA damage and may also block the repair of the DNA damage, resulting in synergistic cell kill.
- Combination therapies using XPO1 inhibitors, especially the clinical compound KPT330 (selinexor) +/- MEL, may significantly improve the treatment outcomes of MM.