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

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Introduction: Significant increases in response/survival have been seen over the past several years; however, multiple myeloma (MM) remains incurable. In this study we have demonstrated that the XPO1 inhibitors (XPO1i), selinexor (SEL) and KPT-8602, sensitize both wild-type and drug-resistant MM cells to melphalan (MEL) in preclinical models.

Materials and Methods: We treated the XPO1i SEL (300μM), KPT-8602 (300μM), and KOS-2464 (10μM) +/− MEL (5-20 μM) to treat human 8226, H929 and U266 MM cells, and MEL resistant 8226LR5 and U266LR6 cell lines and assayed for apoptosis, DNA damage was assayed by comet assay and g-H2AX in H929 human MM cells. XPO1i/SEL- and KPT-8602 +/− MEL, may have potential to improve the response to MEL, and patient MM cells to MEL both in vitro and in vivo. In part, to an increase in DNA damage by MEL when the XPO1i KPT-330 was added, total NFkB and IKKα/β expression was increased by MEL, and decreased by the addition of XPO1i + MEL, synergistically increased apoptosis (activated γ-H2AX, caspase 3) in MM cell lines over single agent MEL (p=0.015, 0.029 and 0.030 respectively) and increased MM cell proliferation proteins NFkB and IKKα/β,

Results: The addition of the XPO1i's KPT-330, KPT-8602 or KOS-2464 (p = 0.015, 0.029 and 0.030 respectively) synergistically increased apoptosis (activated γ-H2AX, caspase 3) in MM cell lines over single agent MEL (p=0.015, 0.029 and 0.030 respectively) and increased MM cell proliferation proteins NFkB and IKKα/β,

Conclusions: XPO1i's sensitized human MM cell lines, both parental and MEL resistant, and patient MM cells to MEL both in vitro and in vivo, and in vivo NSG mouse models. Our data show that the synergistic cell kill may be due to increased XPO1i/MEL-induced DNA damage. The mechanism of this synergy may be due to an increase in DNA damage. The mechanism of this synergy may be due to increased DNA damage.

Combination therapies using XPO1i, especially the clinical compounds SEL and KPT-8602 +/− MEL, may have potential to improve the treatment outcomes of MM. The combination of XPO1i and melphalan are being investigated in the context of high-dose chemotherapy and autologous transplant (NCT 0278009).

Abstract

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

XPO1 inhibitor/ MEL induced DNA Damage in MM

Selinexor/ MEL combination treatment decreases NFkB, IKKα, FANCF, FANCL and may prevent DNA repair.

Figure 5: Western blot of FA and FAβ protein expression with XPO1i in human MM cell lines. FA (4x10^6 cells/ml) were treated for 2 hours with 50 μM MEL in combination with XPO1i and assayed for apoptosis as described. Western blot analysis was performed immediately (Trevigen Cat#1006-01). A: H929 MM cells treated with XPO1i KPT-330, KPT-8602 or KOS-2464 (300nM) and MEL (10 μM, 30 μM and 100 μM). B: U266 MM cells treated with XPO1i KPT-330, KPT-8602 or KOS-2464 (300nM) and MEL (10 μM, 30 μM and 100 μM). C: H929 and U266 MM cells treated with XPO1i with and without MEL (10 μM, 30 μM and 100 μM). D: Western blot of FA and FAβ protein expression with XPO1i in MM cell lines. FA (4x10^6 cells/ml) were treated for 2 hours with 50 μM MEL in combination with XPO1i and assayed for apoptosis as described. Western blot analysis was performed immediately (Trevigen Cat#1006-01). A: H929 MM cells treated with XPO1i KPT-330, KPT-8602 or KOS-2464 (300nM) and MEL (10 μM, 30 μM and 100 μM). B: U266 MM cells treated with XPO1i with and without MEL (10 μM, 30 μM and 100 μM). C: H929 and U266 MM cells treated with XPO1i with and without MEL (10 μM, 30 μM and 100 μM).

Figure 6: Western blot of FA and FAβ protein expression with XPO1i in human MM cell lines. FA (4x10^6 cells/ml) were treated for 2 hours with 50 μM MEL in combination with XPO1i and assayed for apoptosis as described. Western blot analysis was performed immediately (Trevigen Cat#1006-01). A: H929 MM cells treated with XPO1i KPT-330, KPT-8602 or KOS-2464 (300nM) and MEL (10 μM, 30 μM and 100 μM). B: U266 MM cells treated with XPO1i with and without MEL (10 μM, 30 μM and 100 μM). C: H929 and U266 MM cells treated with XPO1i with and without MEL (10 μM, 30 μM and 100 μM).

Figure 7: XPO1i's sensitized newly diagnosed and relapsed/refractory MM patients to MEL. Inhibition of XPO1 sensitizes MEL-resistant human MM cell lines to MEL.

Figure 8: Ex Vivo Patient Data

Figure 9: Absolute Tumor Response to XPO1i's and MEL.

Figure 10: In vivo treatment

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