Selinexor (KPT-330) and melphalan combination therapy for the treatment of multiple myeloma

Joel G. Turner[1], Jana L. Dawson[1], Chris Cubitt[2], Taiga Nishihori[3], Claudio Anasetti[3], Melissa Alsina[3], Rachid C. Baz[1,3], Michael Kauffman[4], Sharon Shacham[4], William S. Dalton[1] and Daniel M. Sullivan[1,3]

[1] Chemical Biology and Molecular Medicine Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL
[2] Translational Research Laboratory, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL
[3] Department of Blood and Marrow Transplantation, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL
[4] Karyopharm Therapeutics, Natick, MA

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
High-dose melphalan (MEL) followed by autologous stem cell transplant remains the standard of care for the treatment of multiple myeloma (MM). However, patients eventually develop drug resistance and die from progressive disease despite newer therapies using proteasome inhibitors and immunomodulatory drugs. The incurable nature of MM demonstrates the need for novel treatments. Our aim was to investigate whether MEL therapy could be improved by the addition of the XPO1 inhibitor selinexor (SEL) in drug-resistant and parental MM cells both *in vitro* and *ex vivo*.

Materials and Methods
SEL/MEL-treated human MM cells were assayed for cell viability (CT-Blue) and apoptosis by flow cytometry (activated caspase 3). Proximity ligation assays (PLD) were used to assess if XPO1-p53 binding was inhibited by SEL. Western blots of SEL-treated MM cells were performed for nuclear and total p53. MEL-resistant U266 (LR6) and 8226 (LR5) MM cell lines were developed by incremental exposure to MEL. MEL resistant and parental MM cells were treated *in vitro* with SEL +/- MEL and assayed for apoptosis and cell viability. Cells isolated from patients with newly diagnosed or relapsed MM, were treated with SEL +/- MEL and assayed for apoptosis.

Results
MM cell viability was decreased synergistically by SEL when used in combination with MEL, as shown by combination index (CI) values. Drug sequencing assays showed that concurrent treatment with MEL (10 μM) and SEL (300 nM) for 48 hours produced synergistic results in human H929 MM cells (CI value 0.079, n=3). Sequential treatment, SEL for 24 hours followed by MEL for an additional 24 hours or the reverse sequence, also demonstrated synergy with CI = 0.208 (n=3) and 0.142 (n=3), respectively. Normal PBMCs (control) were unaffected by SEL/MEL treatment as shown by viability and apoptotic assays. PLD demonstrated that SEL blocks XPO1/p53 binding. Western blot data showed that the SEL treatment of MM cells increased nuclear and total p53. Drug-resistant LR5 and LR6 MM cells were found to be resistant to MEL when compared to parental cell lines. Both resistant MM cell lines were sensitized by SEL to MEL as shown by apoptosis assay (20-fold). CD138+/light chain+ MM cells derived from newly diagnosed and relapsed MM patients were also sensitized (5 to 10-fold) by SEL to MEL as demonstrated by apoptosis assay.

Conclusions
SEL synergistically improved the response of drug-resistant and parental MM cells to MEL *in vitro* and *ex vivo*. It is possible that this synergy may be due to an increase of nuclear p53 by SEL and the reported activation of p53 by MEL. Ongoing studies include *in vitro* experiments to investigate whether this drug combination reverses MEL resistance by the Fanconi Anemia/BRCA pathway, *in vivo* treatment of MM in NSG mice with SEL/MEL and a clinical trial using high-dose MEL in combination with SEL. Combination therapies using SEL and MEL may significantly improve the treatment outcomes of MM.

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P53 and XPO1 Inhibitors

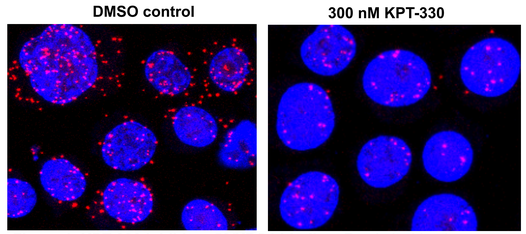

Figure 1. KPT-330 inhibits p53/XPO1 binding. Proximity Ligation Assay. H929 human MM cells ($3x10^6$) were incubated for 6 hours with DMSO control or 0.3 μM KPT-330. A red fluorescent signal was generated only when XPO1and p53 are in close proximity (<40nM). DAPI (blue) was used to detect the nuclei.

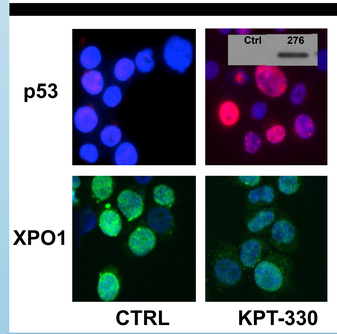

Figure 2. KPT-330 increases nuclear and total p53. Human H929 myeloma cells were treated for 6 hours with the XPO1 inhibitor KPT-330 (0.3 μM. Intracellular localization by fluorescence microscopy and protein expression (western blot, inset) of p53 were assayed. KPT-330 increased p53 protein expression(red) and the protein was localized to the nucleus.

Cell Viability Assay

KPT-330 plus Melphalan – Combination Index (CI) Results

Cell line	CombiGrp	Time1	Time2	MRatio	CI(mean)	CI(SEM)	CI(n)
NCI-H929 plateau	KPT 330 & Melphalan						
	Concurrent	48	0	0.01	0.584	0.023	2
				0.02	0.447	0.204	2
				0.04	0.079	0.025	2
	Sequential	24	24	0.01	0.561	0.168	2
				0.02	0.616	0.137	2
				0.04	0.208	0.031	2

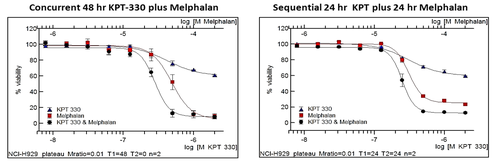

Figure 3: Cell Viability Assay: KPT 330 was found to synergize with melphalan in NCI-H929 human myeloma cell cultures. Combination index values of log phase cells co-treated 48 hours with KPT-330 and melphalan. CI, combination index. <1 = synergy. Sequential, or concurrent addition of the drug worked equally well.

In Vitro Myeloma Cell apoptosis

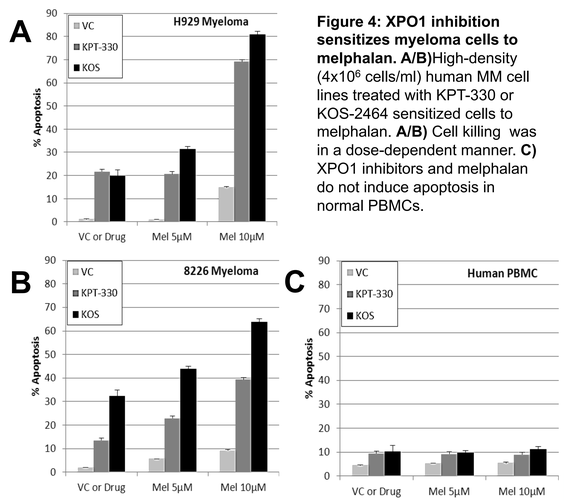

Figure 4: XPO1 inhibition sensitizes myeloma cells to melphalan. A/B) High-density ($4x10^6$ cells/ml) human MM cell lines treated with KPT-330 or KOS-2464 sensitized cells to melphalan. **A/B)** Cell killing was in a dose-dependent manner. **C)** XPO1 inhibitors and melphalan do not induce apoptosis in normal PBMCs.

Melphalan Resistant MM Cells

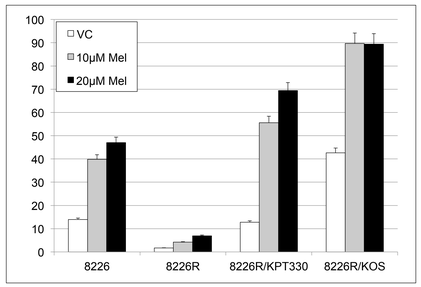

Figure 5: XPO1 inhibition sensitizes melphalan-resistant human myeloma cell lines to melphalan. Human LR5 melphalan resistant and parental myeloma cell lines were treated concurrently for 20 hours with KPT-330 (300nM) or KOS-2464 (10nM) with and without melphalan and assayed for apoptosis by flow cytometry (activated caspase 3). LR5 (8226R) melphalan resistant cells were up to 10-fold resistant to bortezomib as compared to 8226 parental cells. The addition of the XPO1 inhibitors, KPT-330 or KOS-2464 sensitized drug resistant cells to melphalan. All cells were grown at log-phase growth conditions ($5x10^5$ cells/ml).

Ex vivo Apoptosis Assay

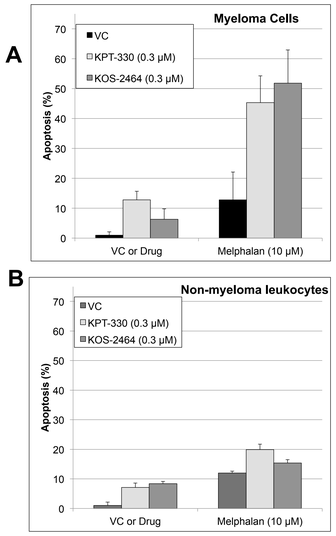

Figure 6. KOS-2464 and KPT330 sensitize newly diagnosed patient myeloma cells to melphalan. Bone marrow mononuclear cells were isolated and treated with KPT-330 or KOS-2464 (0.3 μM) +/-melphalan or DMSO (VC) for 20 hours. Treated cells were fluorescently labeled with antibodies against activated caspase 3, CD138, and light chain (kappa or lambda). **A)** KPT330 and KOS-2464 sensitized CD138/light-chain positive patient cells patient myeloma cells (n=4) when compared to the vehicle control. **B)** Non-myeloma CD138/light-chain negative patient cells were not sensitized by XPO1 inhibitors.

Conclusions

- XPO1 inhibitors synergistically improved the response of myeloma cells to melphalan both *in vitro* and *ex vivo*.
- Synergy may be due to an increase of nuclear p53 by XPO1 inhibitors and the reported activation of p53 by melphalan.
- Future studies include *in vivo* experiments using human myeloma cells in NOD-SCID-gamma mice and clinical trials using melphalan in combination with the XPO1 inhibitor selinexor.
- Combination therapies using selinexor and melphalan may significantly improve the treatment of myeloma.