KPT-0127 Induces Selective Apoptosis of Malignant Cells by CRM1 Inhibition and Elevation of Regulatory Proteins p53, p21, FOXO and IκB

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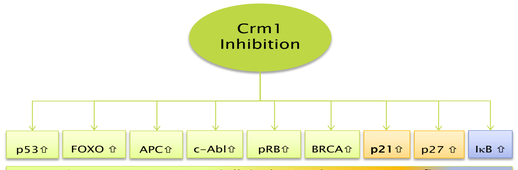
SUMMARY

CRM1 (Xpo1) is a key nuclear export protein which controls multiple tumor suppressor proteins (TSP) and cell proliferation pathways including p53, p21, FOXO, PI3K/AKT, Wnt/ß-catenin and NF-kB. It was previously reported that mislocalization can abrogate TSP functions and render chemotherapies ineffective. Induction of nuclear expression of chemotherapy targets, TSP and growth regulatory proteins by CRM1 inhibition can restore drug sensitivity and restore checkpoint control & genome surveying functions. Here, we describe the results with KPT-0127, a novel small molecule, water soluble, drug-like, selective and irreversible CRM1 antagonist.

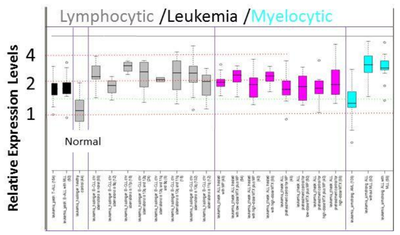
- KPT-0127 blocks CRM1 mediated nuclear export of FOXO, and p53 with an IC_{50} of ~300 nM
- KPT-0127 is selectively cytotoxic to various hematological cell lines with EC_{50}s in the 0.02-1.0 µM range, and shows limited cytotoxicity on normal PBMCs (EC_{50} >5-20 µM) or mouse embryo fibroblasts
- KPT-0127 kills Burkitt's Lymphoma cells independent of their p53 status
- KPT-0127 induces Caspase-3 activation in Jurkat and Burkitt's Lymphoma cells and causes cell cycle arrest in U937 lymphoma cells
- KPT-0127 displays additive/synergistic effects in combination with Bortezomib and Doxorubicin
- KPT-0127 administered at 150mg/kg QDx5 inhibits growth of small (130mm^3) and large (1300mm^3) MM1.S xenografts

Given all the above KPT-0127 represents a novel, tumor selective and well tolerated irreversible Crm1 Inhibitor which may be suitable for clinical development both as a single agent and in combination with standard therapies for hematological cancers.



CRM1 controls the nuclear to cytoplasmic export of the majority of tumor suppressor (TSP) and growth regulatory (GRP) proteins

CRM1 is Upregulated in Hematological Malignancies



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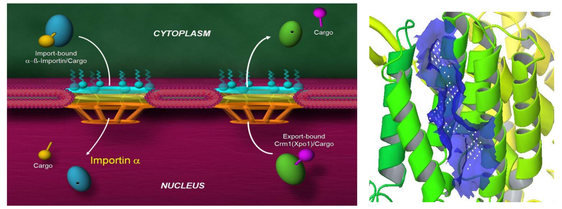
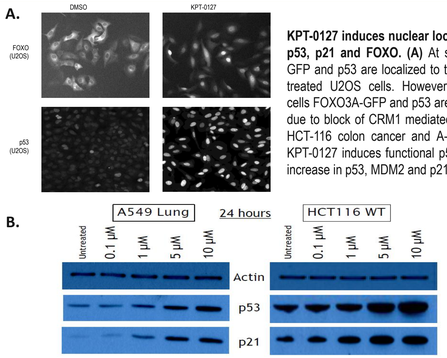
KPT Compounds Block the Interaction of CRM1 and Cargo Proteins



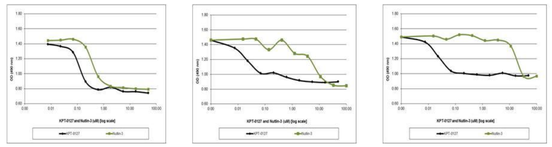
(A) CRM1 regulates the export of multiple tumor suppressor proteins (TSP) from the nucleous via the nuclear pore complex. KPT-0127 creates an irreversible covalent bond with Cys528 of CRM1 **(B)** Small molecule binding pocket was identified in the NES binding domain, permitting a covalent bond with Cys528.

KPT-0127 Increases Nuclear Levels of p53, p21 and FOXO



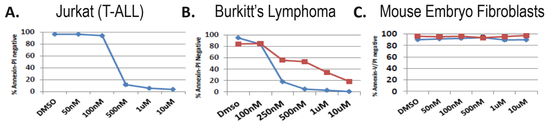
KPT-0127 induces nuclear localization of functional p53, p21 and FOXO. (A) At steady state, FOXO3A-GFP and p53 are localized to the cytoplasm in DMSO treated U2OS cells. However, in KPT-0127 treated cells FOXO3A-GFP and p53 are trapped in the nucleus due to block of CRM1 mediated nuclear export. **(B)** In HCT-116 colon cancer and A-549 lung cancer cells, KPT-0127 induces functional p53 activity as shown by increase in p53, MDM2 and p21 nuclear levels.

KPT-0127 Kills Both p53 WT and p53 Mutant BL Cells



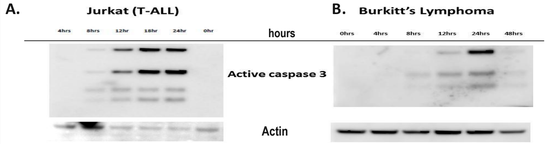
KPT-0127 kills Burkitt Lymphoma (BL) cells independent of p53 status: (A) BL-2 (nutlin sensitive, p53WT), **(B)** BL40 (nutlin resistent p53WT) and **(C)** BL41 (nutlin resistent, p53 mutant) were exposed to KPT-0127 for 72 hours and cytotoxicity was determined using MTT assay.

KPT-0127 Induces Apoptosis in Malignant But Not in Normal Cells



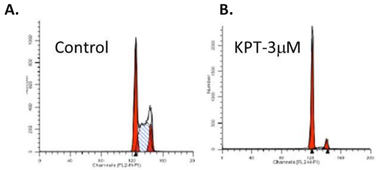
KPT-0127 kills malignant but not normal cells. (A) Jurkat and **(B)** Burkitt's Lymphoma cell lines and **(C)** normal mouse embryo fibroblasts were treated for 24-48 hours with KPT-0127 and apoptosis was determined by Annexin V staining.

KPT-0127 Induces Caspase-3 Activation in Malignant Cell Lines



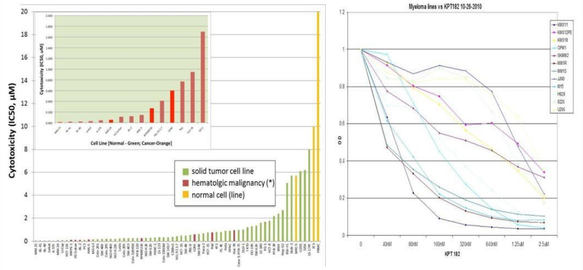
KPT-0127 Induces Caspase 3 activation. (A) Jurkat and **(B)** Burkitt's Lymphoma cell lines were treated for 0-24 hours with KPT-0127 and activated caspase was determined Western Blot.

KPT-0127 Causes Cell Cycle Arrest in U937 Lymphoma Cells



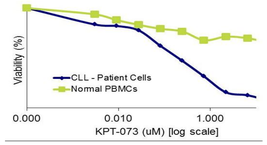
KPT-0127 causes G1/S and G2/M arrest. U937 Lymphoma cells were treated with vehicle **(A)** or KPT-0127 3 µM **(B)** Cells were fixed in ethanol and stained with propidium iodide for analysis of light scatter/DNA content by flow cytometry.

KPTI Compounds Demonstrate Potent & Selective Cancer Cell Cytotoxicity



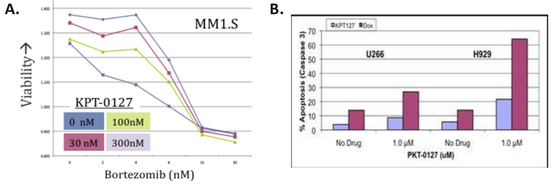
KPT-I Compounds demonstrated potent and selective cytotoxicity in hematologic and solid tumor cell lines with minimal effect on normal cells. (A) A panel of ~50 solid and hematologic cancer cell lines was exposed to KPT-0127 for 72 hours IC50 was determined. **(B)** A panel of 12 myeloma cell lines was exposed to KPT-0182 (~4X more potent than KPT-0127) for 72 hours and cytotoxicity was determined (Dr. Stewart's lab).

KPT-0127 Kills Fresh Human CLL Cells but not Normal PBMCs



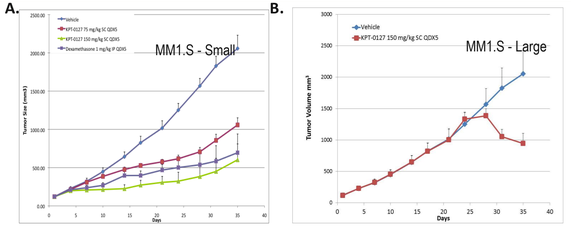
KPT-0127 kills human lymphocytic leukemic cells but not normal PBMCs. Freshly isolated human CLL cells (relapsed after fludarabine-cyclophospham-ide-rituximab) or normal PBMCs were treated ex vivo with varying doses of KPT-0127 and viability was determined using MTT assay. KPT-0127 selectively kills human CLL cells but not normal human lymphocytes.

KPT-0127 Enhances the Cytotoxicity of Bortezomib and Doxorubicin



KPT-0127 has additive and synergistic effects in combination with Bortezomib and Doxorubicin. (A) KPT-0127 enhances the cytotoxicity of bortezomib in MM1.S myeloma cells. **(B)** KPT-0127 restores sensitivity of high-density MM cells to Doxorubicin.

KPT-0127 Inhibits the Growth of Small & Large Myeloma Xenografts



KPT-0127 150mg/kg QDx5 inhibits growth of small (130mm^3) and large (1300mm^3) MM1.S xenografts. (A) MM1.S cells were grown as xenografts to 130mm^3 (small) or **(B)** 1350mm^3 (large) sizes and were treated with KPT-0127 SC or dexamethasone IP daily x 5 and growth was assessed. Treatment of large xenografts is ongoing (data shown after 5 doses of KPT-0127).

Conclusions

- Inhibition of nuclear export through blockade of CRM1 induces retention of multiple tumor suppressor proteins and growth regulators in the nucleus
- KPT-0127 is a Selective Inhibitor of Nuclear Export (SINE) with preferential toxicity for malignant versus normal cells independent of p53 status
- KPT-0127 is well tolerated *in vivo* and inhibits the growth of small (130mm^3) and large (1300mm^3) myeloma xenografts
- SINE may represent a novel approach to the treatment of hematologic malignancies with both single agent activity and in combination with other anticancer drugs

Acknowledgments

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