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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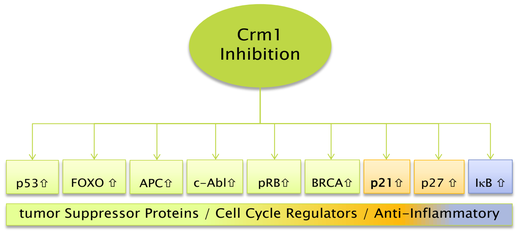
SUMMARY

CRM1 (Xpo1) is the major exporter of proteins from the nucleus to the cytoplasm, including tumor suppressor (TSP) and growth regulatory (GRP) proteins e.g. **p53**, **FOXO**, **pRB**, nucleophosmin1 (NPM1) and **IκB,** as well as **Topoisomerase IIA** (Topo IIA). Here, we describe KPT-0127, a novel small molecule, water soluble, drug-like, selective inhibitor of nuclear export (SINE) which irreversibly blocks CRM1. KPT-0127 exerts potent (EC50 ~350nM) and prolonged inhibition of CRM1-mediated HIV-1 Rev, forkhead (FOXO), and p53 nuclear export in a variety of normal and transformed cell lines:

- In cytotoxicity assays, KPT-0127 showed potent cytotoxicity in most hematologic cancer cell lines (EC50 <200nM, with myeloma and lymphoma lines often <100nM). By contrast, normal cells were largely unaffected by treatment with KPT-0127 (EC50 >5-10μM).
- In normal peripheral blood mononuclear cells (PBMCs) and in Hut78 leukemia cells, KPT-0127 potently increased the nuclear levels of IκB. However, KPT-0127 induced cell death of Hut78 cells with no effect on normal PBMCs.
- In drug combination studies, KPT-0127 showed additive or synergistic cytotoxicity activity with either bortezomib, 5-FU, carboplatin, or doxorubicin.
- Treatment of human myeloma cell lines with KPT-0127 and the topo II inhibitor doxorubicin at high cell densities resulted in >5-fold increase in caspase activation compared with either drug alone.
- SC dosing (QDX5 weekly) for 35 days up to 150mg/kg (the highest dose tested) was well tolerated and showed robust anticancer activity in Myeloma MM.1S xenograft model. Additional xenograft studies are ongoing.

Together, these data demonstrate that KPT-0127 represents a novel, tumor-selective and well-tolerated SINE 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.

Oncogenic Pathway	Tumor Suppressor Proteins Enhanced by CRM1 inhibition
AKT↑, PI3K↑	FOXO, p27
HER2, EGF-R (HER1)	FOXO
Del p53, MDM2↑	$p21^{CIP1}$, p53
$p16^{INK4A}$ ↓and/or $p14^{ARF}$ ↓	pRB, p53
mTOR↑	p53
ß-Catenin ↑	APC, HMGBP1
Del Rb	p27
CDK2-Cyclin E-E2F1	pRb, p27, $p21^{CIP1}$
NPM Mutation	p53, $p14^{ARF}$
c-Myc ↑	$p21^{CIP1}$, HMGBP1
Bcr-Abl	Abl
NF-kB ↑	IkB
Bcl2 ↑, Bcl-xL ↑	p53, $p16^{INK4A}$

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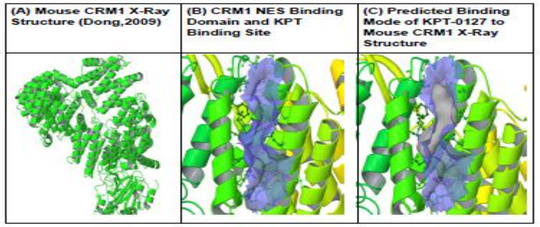
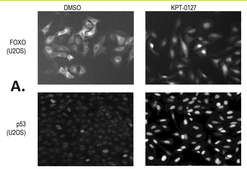
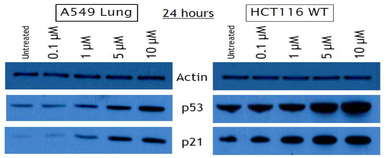
KPT Compounds Block the Interaction of CRM1 and Cargo Proteins



KPT-0127 creates an irreversible covalent bond with Cys528 of CRM1. (A) The mouse X-ray structure was recently solved to a resolution of 2.9Å (Dong, 2009). (B) Small molecule binding pocket was identified in the NES binding domain, permitting a covalent bond with Cys528. (C) In silico docking of KPT-0127 in CRM1: KPT-0127 is shown in a surface representation (carbon in gray, positive charge in blue, negative charge in red and halogen atoms in green).

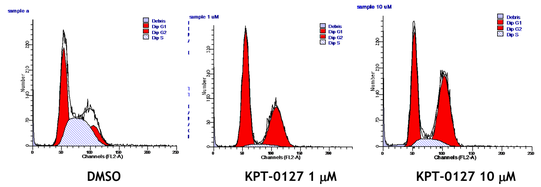
KPT-0127 Increase Nuclear Levels of p53, p21 and FOXO





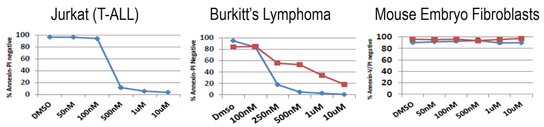
KPT-0127 forces nuclear localization of functional p53, p21 and FOXO. (A) At steady state, FOXO3A-GFP and p53 are localized to the cytoplasm in DMSO treated U2OS cells. However, in KPT-0127 treated cells FOXO3A-GFP and p53 are trapped in the nucleus due to block of CRM1 mediated nuclear export. (B) In 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 Causes Both G1/S and G2/M arrest in HCT-116 Cells



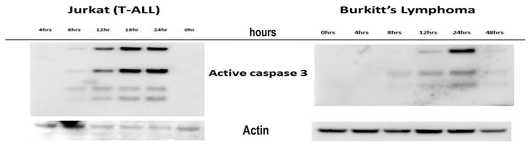
KPT-0127 causes G1/S and G2/M arrest: HCT-116 colon cancer cells were treated with vehicle (A), KPT 1 μM (B), or KPT-0127 10 μM (C) and harvested after 24 hours. Cells were fixed in ethanol and stained with propidium iodide for the analysis of light scatter/DNA content by flow cytometry.

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



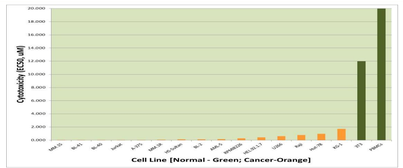
KPT-0127 kills malignant but not normal cells: Various cells were treated for 24-48 hours with KPT-0127 and apoptosis was determined by Annexin V staining. KPTI-0127 selectively kills transformed and not normal cells

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



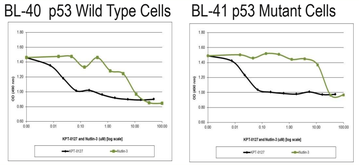
KPT-0127 Induces Caspase 3 activation: Various cell lines were treated for 0-24 hours with KPT-0127 and activated caspase was determined Western Blot.

KPT-0127 Demonstrates Potent & Selective Cancer Cell Cytotoxicity



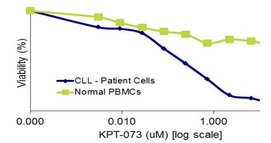
KPT-0127 has potent and selective cancer cell cytotoxicity: A panel of hematological cancer cell lines (orange) were exposed to KPT-0127 for 72 hours and half maximal cytotoxicity (EC50) was determined using MTT assay. Normal cells (3T3 and PBMC) are shown in green.

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



KPT-0127 Kills Burkitt Lymphoma (BL) Cells Independent of p53 Status: BL40 (p53WT) and BL41 (p53 mutant) were exposed to KPT-0127 for 72 hours and cytotoxicity was determined using MTT assay. Both cell lines are nutlin insensitive.

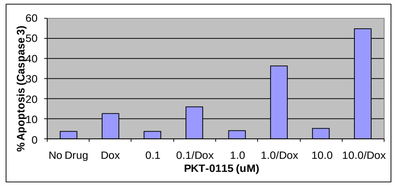
KPT-0127 Kills Fresh Human CLL Cells but not Normal PBMCs



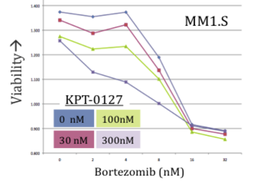
KPT-0127 Kills Human CLL Cells but not PBMC: Freshly isolated human CLL cells (relapsed after fludarabine-cyclophospham-ide-rituximab) or normal PBMCs were treated ex vivo with varying doses of KPT-0127 and viability determined by MTT assay. KPT-0127 selectively kills human CLL cells but not normal human lymphocytes.

KPT-0115 Rapidly Restores Sensitivity to Anthracyclines in Quiescent Resistant Myeloma H929 Cells

KPT-0115 traps Topo II in the nucleus. H929 myeloma cells are grown to high density and become anthracycline resistant.
KPT-0115 traps Topo II in the nucleus permitting Adriamycin to act on Topo II, rapidly inducing apoptosis, 16 hours after drug treatment.

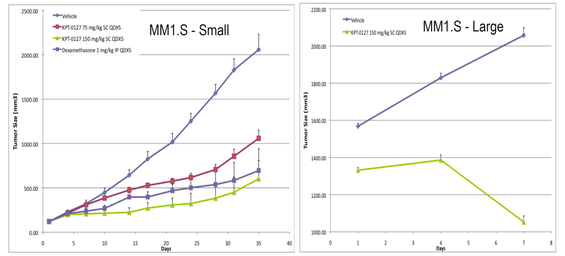


KPT-0127 Enhances the Cytotoxicity of Bortezomib



KPT-0127 enhances the cytotoxicity of bortezomib in MM1.S myeloma cells. MM1.S cells were grown in vitro and treated with 0-32nM bortezomib for 42 hours, in the presence of 0-300nM KPT-0127.
KPT-0127 enhances killing of MM1.S cells by the proteasome inhibitor bortezomib. Similar data were obtained for MM1.R cells (not shown). As a single agent, KPT-0127 induces minimal cytotoxicity at 42 hours on MM1.S; cytotoxicity of KPT-127 is potent at 72 hours after exposure (EC50 ~50nM).

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



KPT-0127 150mg/kg QDx5 Inhibits the Growth of Small (130mm^3) and large (1300mm^3) MM1.S Xenografts: MM1.S cells were grown as xenografts to 130mm^3 (small) or 1350mm^3 (large) and 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 forces retention of multiple TSP 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 ability to combine with other anticancer drugs