KPT-0127, a Novel, Potent and Selective Inhibitor of CRM1-Mediated Nuclear Export Shows Selective Cytotoxicity for The Treatment of Hematological Cancers

*Sharon Shacham(1), **Tami Rashal (1)** Raphael Nir(2), Dirk Daelemans(3), Vincent Sandanayaka (1) Sharon Shechter(1), Ivana Vancurova (4), Ashish Juvekar (4) Winnie Lau (5), Guy Higgins(5), Giulio Draetta(6) and Michael Kauffman(1)*

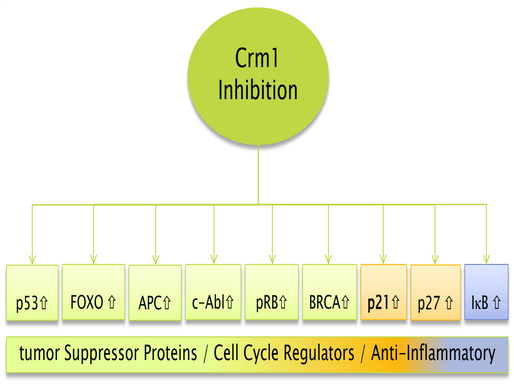
(1) Karyopharm Therapeutics,, Newton, MA (2) SBH Sciences, Natick, MA (3) Rega Institute, Leuven, Belgium (4) St. John's University New York NY (5) Cancog Technologies, Toronto, Canada (6) Dana Farber Cancer Institute, Boston, MA

Introduction

CRM1 (Xpo1) is the major export factor for proteins from the nucleus to the cytoplasm, including tumor suppressors (TSPs) and other modulators of proliferative responses such as **p53**, **FOXO**, **pRB**, **Topoisomerase IIA** (Topo IIA), nucleophosmin1 (NPM1) and **IκB**. Here, we describe our lead compound KPT-0127 a novel small molecule, water soluble, drug-like, selective, irreversible CRM1 antagonist. KPT-0127 exerts a potent (EC50 300-400nM) 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 high potency 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 velcade, 5-FU, carboplatin, or doxorubicin.
- Treatment of human myeloma cell lines with KPT-0127 and exposed to doxorubicin at high cell densities resulted in >5-fold increased sensitivity to topo II inhibitors as determined by an activated caspase assay.
- The selectivity of KTP-0127 was demonstrated across a panel of 37 proteins including several cysteine proteases. Moreover, KPT-0128, the trans isomer of KPT-0127, shows little effect on both HIV-Rev nuclear export and in cytotoxicity assays (EC50s > 10 μM), supporting the specificity of KPT-0127 for CRM1.
- SC dosing daily for 5 days up to 100mg/kg (the highest dose tested) showed no behavioral, clinical chemistry, or hematogical effects in mice;
- KPT-0127 showed robust anti cancer activity in Myeloma MM.1S xenograft model. Additional xenograft studies are on going.

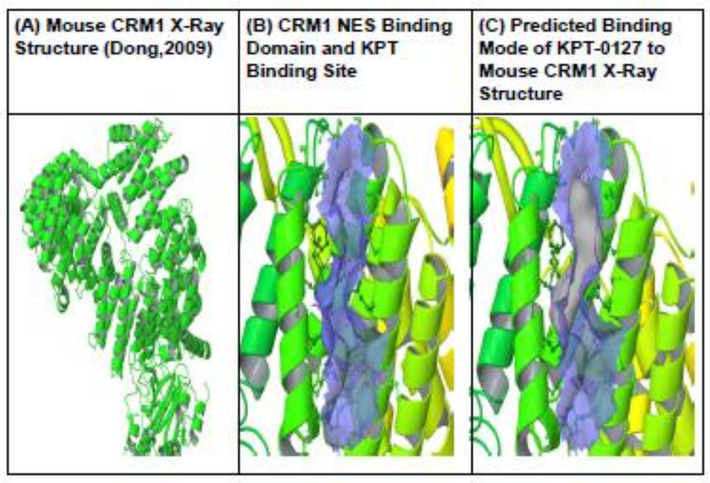
All together, these data demonstrate that KPT-0127 represents a novel, tumor selective and well-tolerated irreversible Crm1 inhibitor which may be suitable for clinical development both as a single agent and in combination with standard therapies for hematological cancers.



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, $p16^{INK4A}$

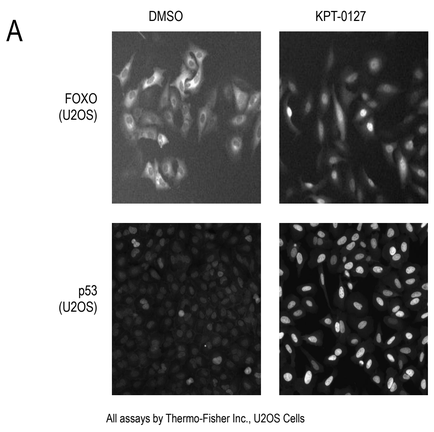
CRM1 controls the nuclear to cytoplasmic export of the majority of tumor suppressor (TSP) and growth regulatory proteins.

KPT Compounds Inhibit Binding of Cargo Proteins to CRM1

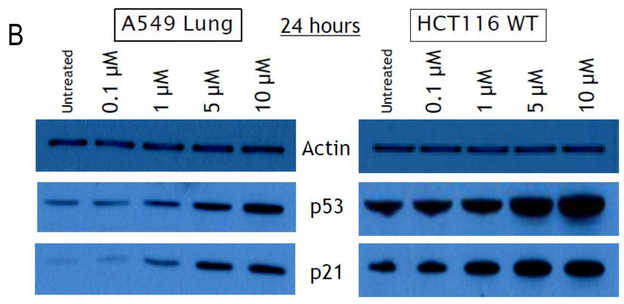


KPT-0127 is an N-azolylacrylate analog that creates an irreversible covalent bond with Cys528 of CRM1. (A) The mouse X-ray structure was recently solved to a resolution of 2.9Å (Dong, 2009). (B) Small molecule binding pocket was identified in the NES binding domain, permitting a covalent bond with Cys528. (C) 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

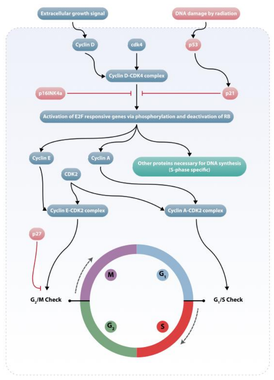


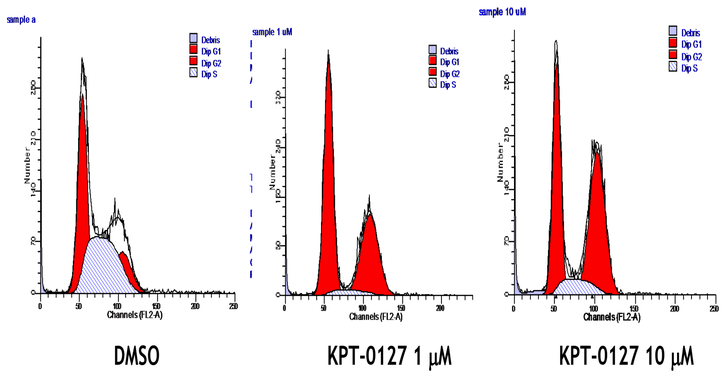
All assays by Thermo-Fisher Inc., U2OS Cells



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.

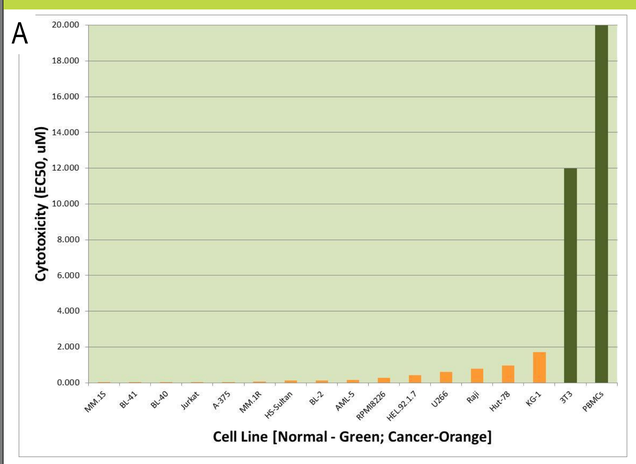
KPT-0127 Causes G1 and G2 arrest in HCT-116 Cells

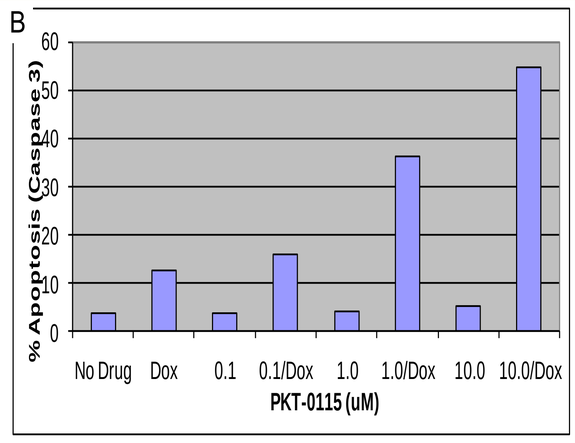




KPT-0127 causes G1 and G2/M arrest: HCT-116 colon cancer cells were treated with vehicle (A) KPT 1 μM (B) or KPT-0127 10 μM (C) and harvested after 24 hours. Cells were fixed in ethanol and stained with propidium iodide for the analysis of light scatter/DNA content by flow cytometry.

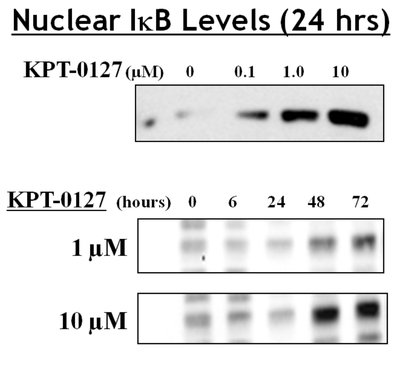
KPT-0127 Demonstrates Potent Cytotoxicity in Hemem Cancer Cell Line Alone and in Combination with Doxorubicin

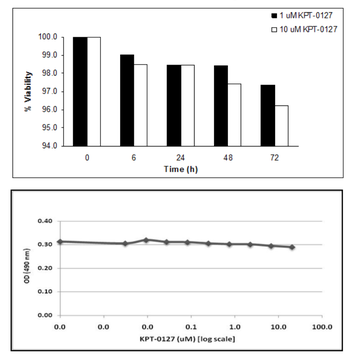




KPT-0127 has potent and selective cancer cell cytotoxicity: (A) A panel of hematological cancer cell lines were exposed to KPT-0127 for 72 hours and half maximal cytotoxicity (EC50) was determined using MTT assay. (B) KPT-0115 traps Topo II in the nucleus. H929 myeloma cells are grown to high density 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.

Pharmacodynamic Assay: KPT-0127 Enhances IkB Nuclear Expression in Human PBMC

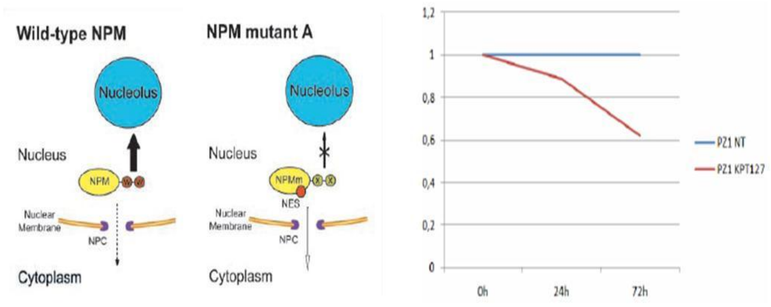




Nuclear IkB levels increased in human and mouse PBMC 24 hours after dosing. This IkB assay is being developed for determination of biologically active doses in preclinical and clinical studies. PBMCs are resistant to at least 10μM KPT-0127

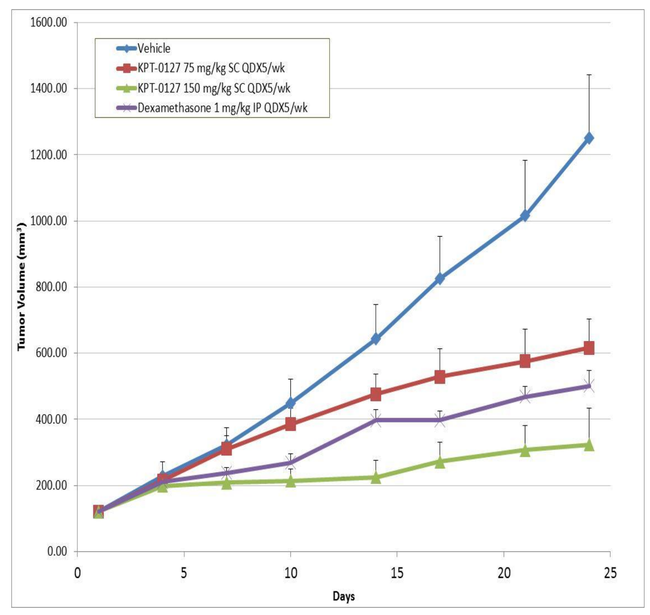
KPT-0127 Induces Apoptosis in NPM1 mutant AML Cells

- 30% of de novo AML carry NPM1 gene mutations
- These mutations cause NPM1 to accumulate in the cytoplasm due to increased CRM1 mediated nuclear export
- Inhibition of CRM1 export can restore normal NPM trafficking and induce apoptosis



KPT-0127 Inhibits Tumor Growth in Myeloma MM.1S Model

- Myeloma MM1.S cells (10^7) were injected into the flanks of SCID mice and grown to $125mm^3$
- KPT-0127 was administered SC in the opposite flank at 75 or 150mg/kg QDx5 each week
- Dexamethasone 1mg/kg IP QDx5 was the control
- KPT-0127 150mg/kg showed 79% inhibition of tumor growth vs. vehicle, compared with 63% tumor growth inhibition with dexamethasone
- KPT-0127 showed 36% increased growth inhibition compared with dexamethasone



Conclusions

- Human tumors exhibit dysfunction of multiple tumor suppressor proteins and pathways (TSP) which are required for their malignant phenotype
- Inhibition of nuclear export through blockade of CRM1 forces retention of multiple TSP and growth regulators in the nucleus
- KPTI selective CRM1 inhibitors kill a broad range of hematologic tumor cells and enhance cytotoxicity by other anti-cancer therapies in vitro with minimal effects on normal cells
- KPTI CRM1 inhibitors showed potent anti cancer activity in-vivo at tolerated doses
- KPTI small molecule CRM1 inhibitors may represent a novel approach to treating human cancers both as single agents and in combination