Abstract # 2756

TARGETED INHIBITION OF CHROMOSOMAL MAINTENANCE REGION PROTEIN (CRM1) POTENTLY SUPPRESSES GROWTH OF HUMAN NEUROBLASTOMA CELL LINE MODELS

Edward F. Attiyeh, Aaron McKeon-Fish, Yosef Landesman, William Senapedis, Dilara McCauley, Trinayan Kashyap, Sharon Shacham, Michael Kauffman, and John M. Maris

Children's Hospital of Philadelphia; Perelman School of Medicine at the University of Pennsylvania; Karyopharm Therapeutics

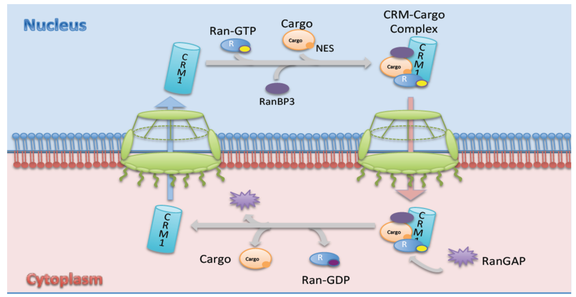
Background

- Neuroblastoma is the most common extracranial solid tumor of childhood and accounts for a disproportionately high (12%) fraction of deaths from pediatric cancer. Most patients with high-risk neuroblastoma are not cured, and **new therapies that rationally target unique vulnerabilities in neuroblastoma cells are urgently needed**.
- Although the *TP53* gene is rarely mutated in primary neuroblastoma, its protein product is thought to be sequestered in the cytoplasm. Translocation out of the nucleus of p53, FOXO, and IκB are mediated by CRM1 (XPO1), and **inhibition of CRM1 is therefore an attractive target in neuroblastoma**.
- **KPT-330 (Karyopharm Therapeutics) is a Selective Inhibitor of Nuclear Export** (SINE) that irreversibly binds CRM1 and inhibits its function. Inhibition of CRM1 results in forced nuclear retention and activation of multiple tumor suppressor proteins; this induces apoptosis in multiple tumor cell types but is tolerated with minimal effect in normal cells.
- KPT-330 is currently in Phase 1 clinical trials in both hematologic and solid adult malignancies.

Methods

- **Cell lines:** A panel of 14 well-characterized neuroblastoma cell lines was tested with KPT-330 across a 5-log range. Growth inhibition was measured using CellTiter-Glo (Promega) viability assays and a real-time growth monitoring system (xCELLigence, Roche).
- **Xenograft models:** NOD/SCID mice with neuroblastoma xenografts were treated orally with KPT-330 and tumor size was monitored.
- **Disseminated models:** Neuroblastoma cell lines stably expressing a luciferase construct were tail-vein injected into NSG mice. Treatment started after tumor luminesence exceeded 10^5 photons/s.
- **mRNA expression:** A well characterized cohort of primary neuroblastoma cases (80 high-risk and 20 low-risk) as well as a panel of cell lines were profiled using Illumina Human-6 expression beadchips.

Results



CRM1, also called exportin 1 (XPO1), is the major nuclear export protein. Protein cargoes with a canonical leucine-rich nuclear export sequence (NES) require transport through the nuclear pore. CRM1 forms a complex with the protein cargo, RanGTP and RanBP3 for export. Once traversing the nuclear pore, hydrolysis of GTP, catalyzed by RanGAP, leads to dissociation of the complex, and CRM1 is recycled.

Karyopharm Therapeutics

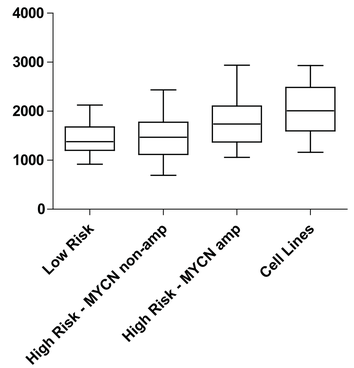


Figure 2. CRM1/XPO1 mRNA expression is higher in cell lines and MYCN amplified primary tumors. Within high-risk samples, CRM1 expression was statistically significantly higher in MYCN amplified cases (p=0.025).

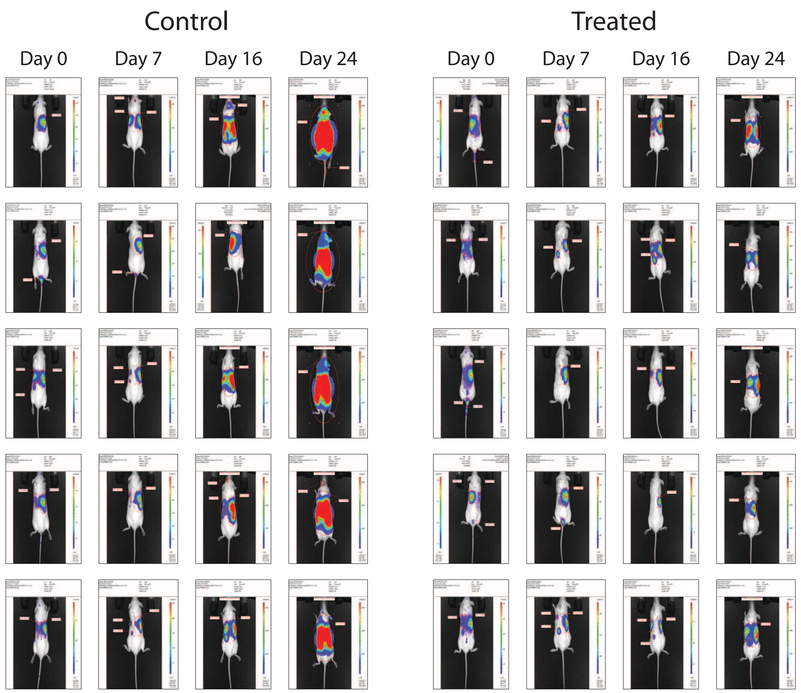


Figure 3. KPT-330 shows activity against SY5Y in a model of disseminated neuroblastoma. Mice were treated with KPT-330 10 mg/kg M-W-F or vehicle only.

Cell Line	IC50
LAN5	50.64
CHP-212	75.89
SY5Y	77.19
IMR5	95.69
SK-N-SH	110.2
NB1643	131.7
SK-N-DZ	144.4
Kelly	208.1
1691	236.4
Ebc1	253.6
NLF	325
SK-N-AS	329.7
Be2c	382.2
NGP	429.5
SK-N-FI	540.9
Be2	544.9
NB16	567.5

Table 1. Neuroblastoma cell lines show a range of sensitivities to KPT-330 in vitro. Cell lines were tested with KPT-330 across a 5-log range. Viability was measured after 48 hours (CellTiter-Glo) or continuously over 96 hours (xCELLigence).

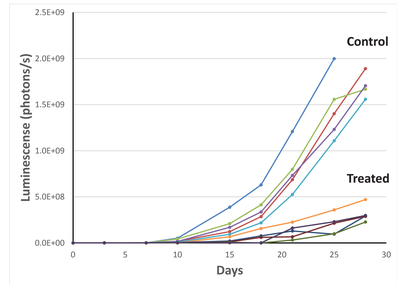


Figure 4. Tumors in mice treated with KPT-330 show significant growth arrest.

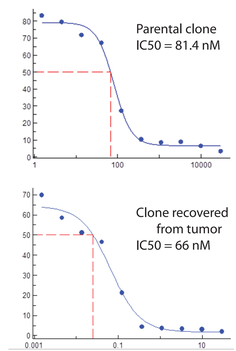


Figure 5. Cells isolated from tumors growing in treated mice retain in viro sensitivity to KPT-330.

Conclusions and Future Directions

- Neuroblastomas show sensitivity to CRM1 inhibition both in vitro and in vivo.
- Ongoing work is focused on discovering the cellular and genomic factors responsible for increased sensitivity to nuclear export inhibition.
- The mechanism of apparant drug resistance *in vivo* is unclear as the cells retain *in vitro* sensitivity.
- In other studies, higher doses (up to 20 mg/kg) of KPT-330 have been well tolerated and may provide further benefit in these models.
- With the expected completion of the first in human phase I trials by mid 2013, treatment with KPT-330 has the potential to be rapidly translated into a clinical trial for children with