Poster 501

INHIBITION OF EXPORTIN 1 (XPO 1) BY SELINEXOR (KPT-330) SYNERGISTICALLY SUPPRESSES GROWTH OF NEUROBLASTOMA IN COMBINATION WITH DOXORUBICIN OR BROMODOMAIN INHIBITION

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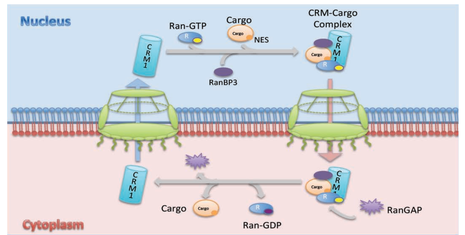
Background

- 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 XPO1 (CRM1), and **inhibition of XPO1 is therefore an attractive target in neuroblastoma**.
- **Selinexor (Karyopharm Therapeutics) is a Selective Inhibitor of Nuclear Export** (SINE) that irreversibly binds XPO1 and inhibits its function. Inhibition of XPO1 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.
- Selinexor is currently in Phase 1 clinical trials in both hematologic and solid adult malignancies.



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

Methods

- **Micro-Array:** Four cell lines were plated at three different time points in triplicate and read on the Affymetrix Human Transcriptome 2.0 Array. The 60 samples were analyzed through R packages maSigPro, Limma, BETR and Ingenuity Pathway Analysis.
- **Cell lines:** A panel of 14 well-characterized NB cell lines was tested with selinexor across a 5-log range. Gene set enrichment analysis showed potential synergistic effects with doxorubicin and bromodomain inhibitors (JQ1). Drug efficacy was measured using CellTiter-Glo (Promega) viability assays and combination indices were analyzed in Calcusyn.
- **Xenograft models:** CB17 Scid female mice were treated orally with with selinexor and one time i.p. injections of doxorubicin. Tumor size and weights were closely monitored.
- **Cell Cycle Analysis:** Four cell lines were harvested at three different time points to investigate selinexor's effect on the cell cycle. Cells were stained with FxCycle Violet and analyzed in FloJo.

Results

Neuroblastoma Cell Lines Show A Range Of Sensitivities To Selinexor, Doxorubicin And JQ1 *in-vitro*

Cell Line	MYCN Status	P53 Status	Selinexor	JQ1	Doxorubicin
IMR5	Amplified	Wild-Type	54.5	247	6.7
SKNSH	Non-Amplified	Wild-Type	107.4	190	107.1
SMSSAN	Amplified	Wild-Type	126.1	1183	33.54
NB1691	Amplified	Wild-Type	151.8	1301	
NB69	Non-Amplified	Wild-Type	291.3	667	49.6
EBC1	Non-Amplified	Wild-Type	337.9	497	111.4
BE2C	Amplified	Mutant	370.2	511	1151.55
BE2	Amplified	Mutant	384.4	431	783.2
LAN5	Amplified	Wild-Type	384.4	625	49.3
NB16	Non-Amplified	Wild-Type	395.6	220	
SKNDZ	Amplified	Mutant	415.1	434	853.9
NLF	Amplified	Mutant	460.2	435	476.8
SKNAS	Non-Amplified	Wild-Type	502.6	178	784.9
Kelly	Amplified	Mutant	676.5	405	208.85

Table 1. Neuroblastoma cell lines show a range of sensitivities to Selinexor, JQ1 and doxorubicin *in vitro*. Sensitivity did not appear to be associated with *MYCN* amplification status, however p53 mutant cell lines show resistivity. Viability was measured after 48 hours.

G1 Arrest Across 3 Cell Lines Treated With Low Dose Selinexor Treatment

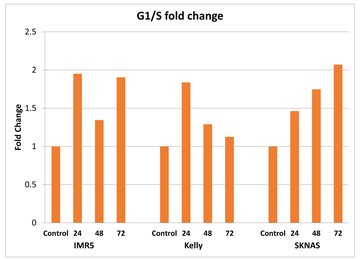


Figure 1. Cell cycle analysis on four cell lines show G1 arrest after 24 hours. Cell cycle analysis shows an increased G1/S ratio within 3 cell lines of varying sensitivity to Selinexor at different time points.

Combining Selinexor with either Doxorubicin or Bromodomain Inhibitors Results in Synergistic Neuroblastoma Inhibition *In Vitro*

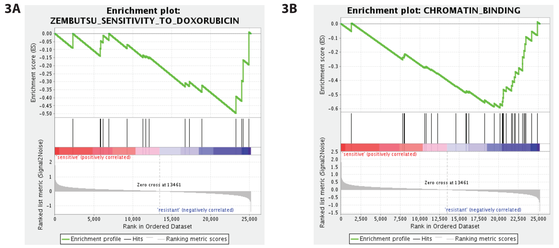


Figure 3A. GSEA plot showing potential sensitivity to doxorubicin.
Figure 3B. GSEA plot depicting potential increased drug efficacy with the inhibition of chromatin binding. Bromodomains are found in proteins that regulate chromatin structure and binding. Synergy between JQ1 and Selinexor was investigated.

Cell Line	ED50 JQ1	ED50 Doxorubicin
IMR5	0.545	2.763
SKNSH	0.510	0.010
SKNAS	0.388	0.002
Kelly	0.174	0.018
NB69	0.588	0.607
EBC1	0.314	1.229
Be2C	0.506	1.442
SKNDZ	0.390	0.429
NLF	0.712	0.245
NB16	0.716	0.825

Table 2. Combination Indicies at the EC50 for JQ1 and doxorubicin in combination with Selinexor.

Microarray Analysis Reveals Potential Gene and Pathway Targets

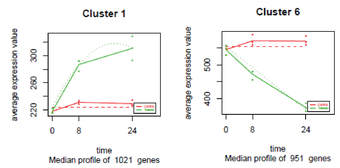
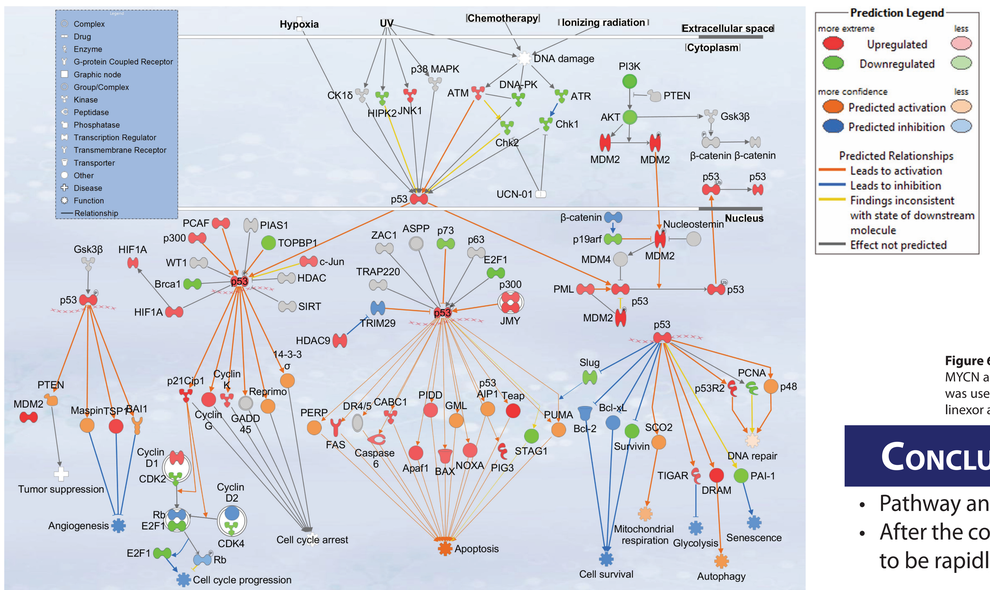


Figure 5. MaSigPro results group significant (p < .01) genes into clusters of similar profiles. GPRIN1, SMARCD2, SENP5 and MAST3 were the most significant genes between the four cell lines in the micro-array. However, at low doses of Selinexor, there was no significant correlation between the genes and sensitivity to Selinexor in an expanded panel of cell lines. Current studies investigate these genes in Ingenuity Pathway Analysis.

Figure 4. Selinexor upregulates apoptosis and inhibits cell growth via the P53 signaling pathway after 24 hours of treatment. At low dose, Selinexor inhbitis Bcl2 and Bcl-xL while upregulating p53 and BAX activating apoptosis pathways.



Combination Treatment of *MYCN* Amplified Cell Lines with Selinexor and JQ1 Shows Significant Decreases in MYCN Expression

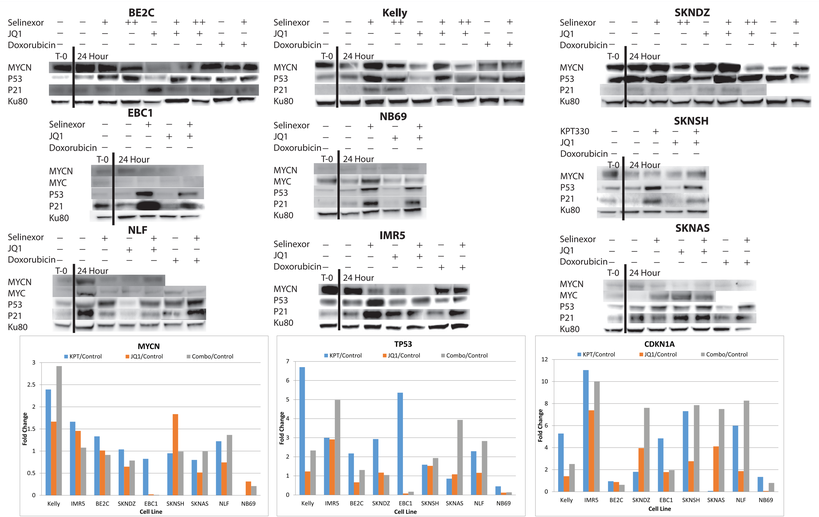


Figure 6. Drug Combination Protein and RNA results. Selinexor combinations were dosed at 100 nM selinexor and the IC50 value for JQ1 and doxorubicin. MYCN amplified cell lines show decreased protein expression for MYCN at 100 nM of selinexor, except for SKNDZ and Kelly, where the IC50 dose of selinexor was used to knockdown MYCN (indicated by ++). Selinexor and doxorubicin show increased p21 and p53 protein. The corresponding RNA results for the selinexor and JQ1 combination are shown for each gene.

Conclusions and Future Directions

- Pathway analysis after Selinexor exposure can discover potential synergistic drug combinations
- After the completion of the pediatric phase I trials, Selinexor containing regimens have the potential to be rapidly translated into a phase II clinical trial for children with neuroblastoma