Selective inhibition of nuclear export in patients with neuroblastoma at high risk for treatment failure

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Key Points

- About 15% of patients have neuroblastoma at ultra-high risk for treatment failure, and therefore nearly universally fatal disease.
- XPO1, a nuclear export protein, is highly abundant in diagnostic tumor material from patients with high-risk neuroblastoma who die rapidly from disease (i.e., neuroblastoma at ultra-high risk for treatment failure)
- Selective inhibition of nuclear export with Selinexor targets XPO1 and leads to apoptosis of neuroblastoma cells *in vitro*

Background

Neuroblastoma is a highly lethal childhood cancer of the peripheral sympathetic nervous system. It is the most common extracranial solid tumor of childhood. The disease is remarkable for its broad spectrum of clinical behavior which can range from spontaneous regression to incurable aggressive metastatic disease, despite intensive multi-modal therapy.

Proteomics to Pathways

Mass spectrometry-based proteomic analyses are being conducted to identify a differential signature in ultra-high-risk neuroblastoma. This will refine our ability to identify functional proteins within neuroblastoma cells and elucidate biologically relevant targets.

Controls	vs	Cases
-diagnostic tumors from patients with high-risk NB who survive more than 3 years		-diagnostic tumors from patients with high-risk NB who die of disease within 18 months

High XPO1 expression in patients is associated with inferior outcome

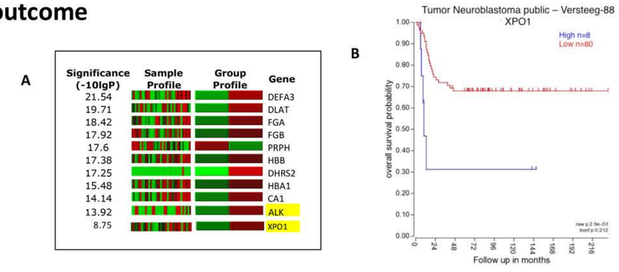

Figure 1: A) Proteomic analysis shows differentially abundant proteins, including XPO1 which is more abundant in those with poor outcome. Validating our method is the presence of ALK, previously shown to be a prognostic biomarker. B) High expression of *XPO1* is associated with inferior outcome (http://hgserver1.amc.nl/cgi-bin/r2/main.cgi).

XPO1 transports proteins from the nucleus into the cytoplasm

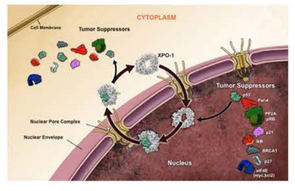

Figure 2: XPO1 translocation of cargo (Image from Karyopharm Therapeutics)

- XPO1 exports over 200 proteins, including cancer-relevant proteins such as **FOXO, p53, p73, p21 and p27,** with specific nuclear export sequences into the cytoplasm
- Selinexor (Karyopharm Therapeutics) is an orally bioavailable XPO1 selective inhibitor of nuclear export with variable cytotoxicity in neuroblastoma cell lines
- Patient-derived xenograft models demonstrate response to Selinexor treatment (tumor size and survival) (Attiyeh et al, 2015)

Methods

Neuroblastoma cell lines IMR5, NB-EBC1, NLF, and SKNSH were maintained with standard tissue culture techniques. cDNA was created from RNA extracted from untreated cell lines for RT-qPCR. Whole protein cell lysates were extracted from untreated and treated neuroblastoma cell lines and analyzed by immuno-blots. Treatment with Selinexor was for 24-72 hours (IC50 100nM-1000nM).

Results

Treatment with Selinexor decreases cellular proliferation and abundance of XPO1

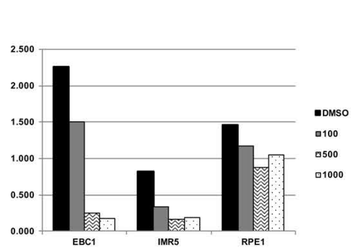

Figure 3: MTT Proliferation Assay was carried out at 48 hours of Selinexor treatment. Neuroblastoma cells and a control cell line (RPE1) were treated with increasing concentrations of Selinexor. Proliferation decreased in the neuroblastoma cell lines but was not changed in the RPE1 control cell line.

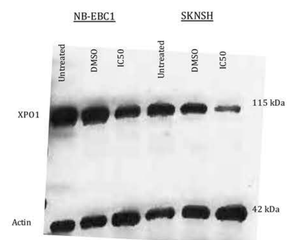

Figure 4: Western Blot Image of NB-EBC1 and SKNSH samples. Samples are untreated, DMSO treated (vehicle control), and IC50 of Selinexor treatment extracted at 24 hour time point. XPO1 and Actin are shown. XPO1 is decreased with Selinexor treatment compared to untreated and DMSO controls.

Cell Line	MYCN status	P53	ALK mutations	Selinexor IC50 concentration (nM)
NB-EBC1	Not Amplified	WT	WT Amp	325
IMR5	Amplified	WT	WT	100
SKNSH	Not Amplified	WT	F1174L	100
NLF	Amplified	Mutation	WT	875

Table 1: Neuroblastoma cell lines show molecular differences that recapitulate the diversity of prognostic variables among patients. Features of cell lines are shown in the table that are used to assess risk stratification in patients. Commonly *MYCN* amplification status, chromosomal aberrations and *ALK* mutations are used to determine prognosis. Neuroblastoma cell lines are treated with a range of Selinexor doses and measured using the MTT Proliferation Assay to calculate the IC50 dose per cell line.

Neuroblastoma cells lines show variable *XPO1* expression

- The expression profiles of neuroblastoma cell lines: NLF, SKNSH, IMR5, and NB-EBC1, show variable amounts of XPO1 mRNA.
- Evaluation of neuroblastoma cell lines with differential *XPO1* expression as well as heterogeneous molecular features show the spectrum of disease that represents the patient population

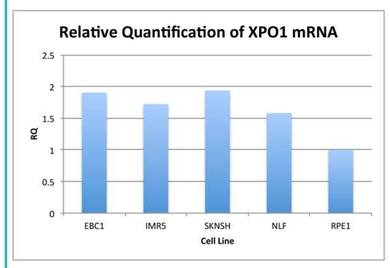

Figure 5: Neuroblastoma cells show a variable spectrum of XPO1 mRNA expression levels as shown by RT-qPCR. The Ct values of the housekeeping gene GAPDH to the gene of interest, *XPO1*, were compared in each cell line. Relative quantification was made of each neuroblastoma cell line to control non-cancer cell line RPE1.

XPO1 staining is positive in nearly all neuroblastoma diagnostic tumor samples

- Applying an anti-XPO1 antibody to a neuroblastoma tumor tissue microarray containing over 100 diagnostic samples, staining is nearly universal across neuroblastoma tumors with primarily nuclear uptake based on review by a board-certified pediatric solid tumor pathologist with expertise in neuroblastoma.

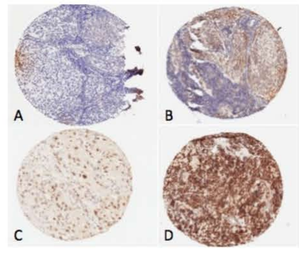

Figure 6: Representative images for IHC staining of XPO1 in neuroblastoma patient tumors, 0+ (none) to 3+ (strong) intensity, A to D, respectively. XPO1 staining was positive overall in 112 of 126 (89%) samples analyzed. (Images courtesy of B. Pawel, CHOP)

Conclusions and Future Work

- Selinexor treatment of neuroblastoma cell lines leads to decreased proliferation
- Treatment also leads to an decrease in XPO1 protein, but gene expression changes are not known
- Mechanistic understanding of XPO1 overexpression can help elucidate the biology of the most highly aggressive neuroblastoma and provide rationale for combinatorial treatment approaches
- Assessment of the correlation between XPO1 immuno-histochemical staining on primary tissue, XPO1 protein expression, and *XPO1* gene expression will provide additional insight about the potential utility of XPO1 as a prognostic and potentially therapeutic biomarker in neuroblastoma

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