XPO1 is a rational target for double and triple-hit B-cell lymphomas

Abstract # LB-062

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Background

Mutation and constitutive expression of **MYC, BCL2 and/or BCL6 (double and triple-hit lymphomas)** defines a subsets of diffuse large B-cell lymphoma (DLBCL) patients with **particularly poor outcome** due to **chemo-refractory disease, a prognosis that cannot be overcome with intense chemotherapy.**

Exportin 1 (**XPO1**/CRM1) is a well characterized mammalian export protein that facilitates the transport of large macromolecules including RNAs and **proteins across the nuclear membrane to the cytoplasm.**

XPO1 binds to a diverse array of protein cargos through their canonical leucine-rich nuclear export signals (NES) domain. XPO1 exports many tumor-suppressor proteins and thus acts as a proto-oncogene by removing oncosuppressor protein from the nucleus, where they are active, to the cytoplasm.

XPO1 overexpression is common in solid tumors and hematologic malignancies and correlates with poor prognosis and resistance to therapy.

Hypothesis

Since double-triple hit lymphomas are characterized by the concomitant deregulation of multiple oncogenic pathways, **we hypothesize that XPO1 may be an effective target for these tumors as it simultaneously impacts multiple oncogenic mechanisms**

We also hypothesize that inhibition of **XPO1 by the selective small molecule KPT-330 may also revert the chemo-refractory status** of aggressive lymphomas.

Materials and Methods

In vitro:

DLBCL cell lines
Toledo
DoHH2
SUDHL-4
OCI-Ly1
OCI-Ly10
SUDHL-6
HBL-1
SC-1
K422

In vivo:

Patient-Derived Xenograft
Double hit DLBCL
XPO1 amplification
Stage IVb
IPI:4
Chemo-refractory (relapse within 3 months)

XPO1 inhibitor: KPT-330 (a.k.a. Selinexor, Karyopharm) – Selective Inhibitor or Nuclear Export (SINE)

***In vitro* experiments:**

Viability: fluorescent assay based on the reduction of resazurin into resorufin (Cell Titer Blue)

Cell Cycle Profile: Propidium Iodide Staining and subsequent Flow Cytometry analysis

Protein expression: SDS-PAGE and Western Blot Analysis

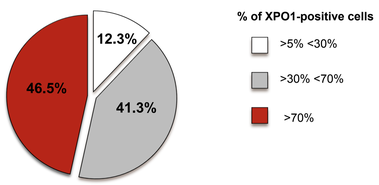
mRNA expression in nuclear vs. cytoplasm: cellular fractionation by differential centrifugation followed by RNA isolation and RT-qPCR

DNA damage repair kinetic: Alkaline Comet Assay

Results

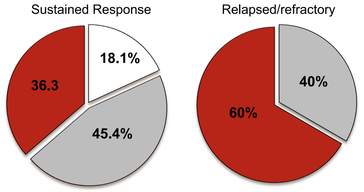
XPO1 is highly expressed in DLBCL preferentially in chemorefractory cases

XPO1 expression in DLBCL tissues by IHC



n=58

XPO1 expression in chemosensitive and chemorefractory DLBCL patients



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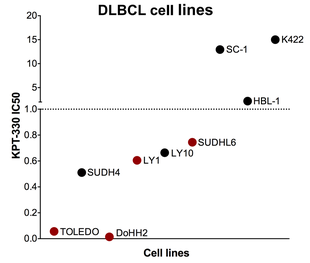
Inhibition of XPO1 by KPT-330 impairs proliferation and survival in double/triple hit DLBCLs

KPT-330 is active at nanomolar dose in DLBCL cell lines



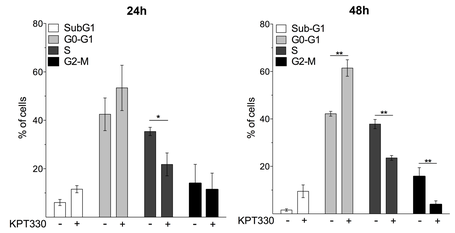
Cells were exposed to a dose range of KPT330 for 48h and viability measured by Cell Titer Blue

Double/triple hit cell lines in our study

Cell line	Myc	BCL2	BCL6
Toledo	Dup/t3,8	Rea	Del/t3,8
DoHH2	Rea	Amp	Amp
LY1	Amp/Del	t14,8	3q27
SUDHL6	Amp	T14,18	WT

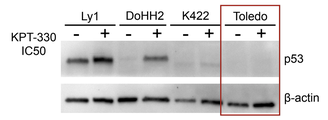
Double-Triple hit cells were identified by performing FISH analysis for MYC, BCL6 and BCL2 genes

KPT-330 induces cell cycle arrest in Toledo cells



Cells were exposed to KPT330 at IC50 dose (0.2 μM) and cell cycle profile was determined at 24h and 48h by Propidium Iodide staining and subsequent flow cytometry analysis

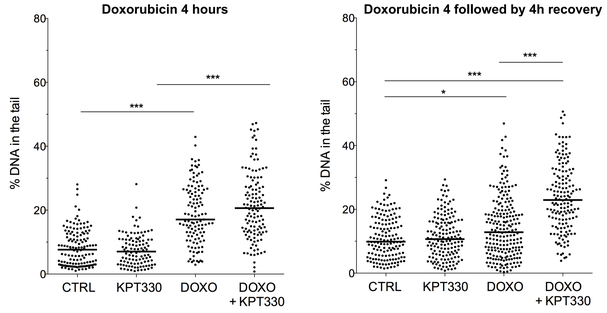
KPT-330-induced cell cycle arrest is independent of p53



Cells were exposed to KPT330 at IC50 dose and p53 accumulation was evaluated at 24h by Western Blot

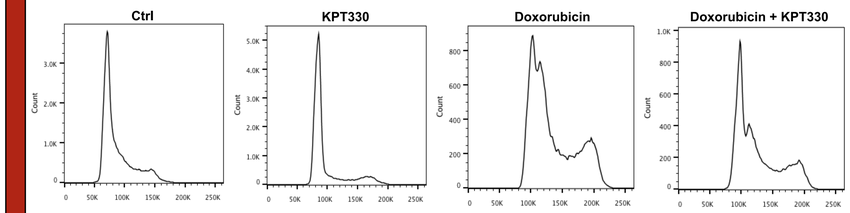
XPO1 inhibition impairs DNA damage response and repair in DLBCL

XPO1 inhibition by KPT330 impairs the repair of doxorubicin-induced DNA damage in Toledo cells



Cells were pretreated with KPT330 (IC50, 0.2 uM) for 24h and then exposed to Doxorubicin (IC50, 1.2 uM) for 4h. DNA damage was assessed by alkaline comet assay at the end of the 4h Doxorubicin treatment or after allowing cells to recover for additional 4 hours.

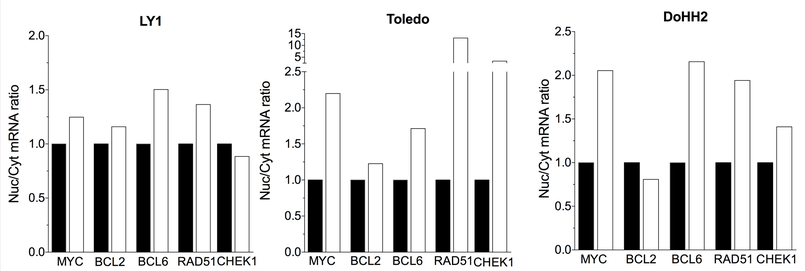
XPO1 inhibition by KPT-330 impairs doxorubicin-induced cell cycle arrest in Toledo cells



Cells were treated with KPT330 (IC50, 0.2 μM) and Doxorubicin (IC50, 1.2 μM) alone or in combination for 24h. After treatment cell cycle profile was determined by Propidium Iodide staining and subsequent flow cytometry analysis

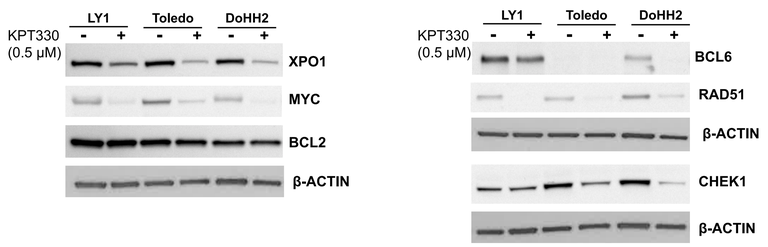
Inhibition of XPO1 by KPT-330 reduces the expression of multiple oncogenic proteins by affecting the nuclear export of their mRNA

Exposure to KPT330 results in nuclear entrapment of mRNA encoding key oncogenic proteins in DLBCLs



Cells were exposed to KPT330 (0.5 μM) for 24 h and subsequent cellular fractionation was performed by differential centrifugation. mRNA transcript for each gene were determined by RT-qPCR. Nuclear/Cytoplasm mRNA ratio in treated vs. untreated cells was computed by using total cellular RNA for each condition as calibrator

Exposure to KPT-330 for 24h reduces the expression of multiple proteins in DLBCL cell lines



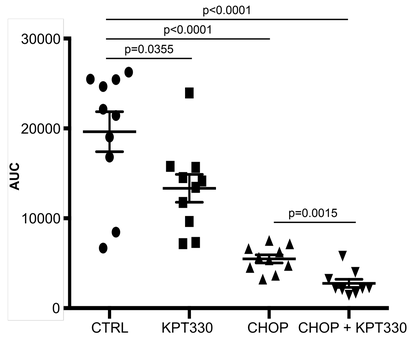
KPT-330 is active as single agent and improves the response to CHOP in a patient-derived xenograft model of triple-hit DLBCL

Experiment plan:

- 5 NSG mice engrafted in both flanks per group
- Treatment started at tumor size of 50-70 mm^3

	Mon	Wed	Fri
Group A	vehicle	vehicle	vehicle
Group B	KPT330 7.5 mg/Kg	KPT330 7.5 mg/Kg	vehicle
Group C	vehicle	vehicle	CHOP
Group D	KPT330 7.5 mg/Kg	KPT330 7.5 mg/Kg	CHOP

- Mice received 5 somministrations of KPT330 and one somministration of CHOP
- Tumor volume and weight were evaluated every two days



Conclusions

- XPO1 is required for proliferation and survival of double/triple hit lymphomas
- XPO1 regulates the nuclear export of transcripts encoding key lymphomagenesis drivers, such as MYC and BCL6; thus, exposure to KPT330 results in nuclear entrapment of MYC and BCL6 transcripts and subsequent reduction in protein expression
- XPO1 regulates the nuclear export of transcripts encoding members of DNA damage response and repair pathways, such as CHEK1 and RAD51; thus, exposure to KPT330 results in nuclear entrapment of CHEK1 and RAD51 transcripts and subsequent reduction in protein expression
- KPT-330 pretreatment increases the effectiveness of first line chemotherapy (CHOP) *in vivo* in a triple-hit patient-derived xenograft model