Preclinical activity in non-Hodgkin's lymphoma of selinexor, a Selective Inhibitor of Nuclear Export (SINE), is enhanced through combination with standard-of-care therapies

Asfar S. Azmi[1], Amro Aboukameel[1], Robert O. Carlson[2], Sivan Elloul[2], Sharon Shacham[2], Michael Kauffman[2], Ran Frenkel[3], Ramzi M. Mohammad[1]

[1]Wayne State University, Detroit, MI; [2]Karyopharm Therapeutics, Newton, MA; [3]Karyopharm Europe GmbH, Munich, Germany

ABSTRACT

Introduction: The nuclear export protein Exportin 1 (XPO1) is overexpressed in diffuse large B-cell lymphoma (DLBCL), follicular small cell cleaved lymphoma (FSCCL) and a wide variety of other cancers, which often correlates with poor prognosis. Selinexor is an oral SINE currently in Phase 1/2 clinical testing, which targets XPO1 to induce apoptosis across a broad spectrum of tumor types. This broad action is primarily due to forced nuclear retention and reactivation of tumor suppressor proteins (TSPs), resulting in selective tumor cell death. Here we report combination studies involving selinexor/dexamethasone and selinexor/mTOR inhibitor everolimus in Non Hodgkin's lymphoma relative to front-line standard-of-care therapies.

METHODS

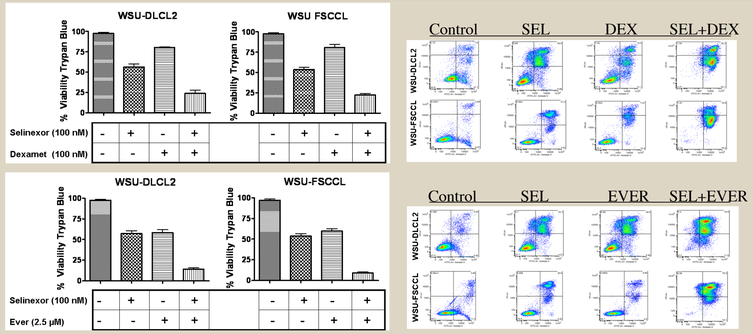
Methods: Diffuse large B cell lymphoma (WSU-DLCL2) and follicular small cleaved cell lymphoma cell lines have been previously developed at Wayne State University. All lines are GCB type with the exception of OCI-LY3 (ABC), WSU-DLCL2 (neither) and A3/KAW (unknown). Cell growth inhibition was performed using trypan blue viability assay and MTT assay (CellTiter 96® from Promega) and IC_{50} values were calculated using GraphPad Prism® software. Apoptosis was detected using Annexin V FITC assay. Changes in protein expression was evaluated using western blotting. For xenograft model of DLBCL, pieces (~50 mg) of serially passaged WSU-DLCL2 tumors were transplanted into the flanks of 4-5 wk old ICR-SCID mice and vehicle or drug treatments were started one week later. $10X10^6$ WSU-FSCCL follicular lymphoma cells were injected IV in the tail veins of ICR-SCID mice and vehicle or drug treatments were started one week later.

RESULTS

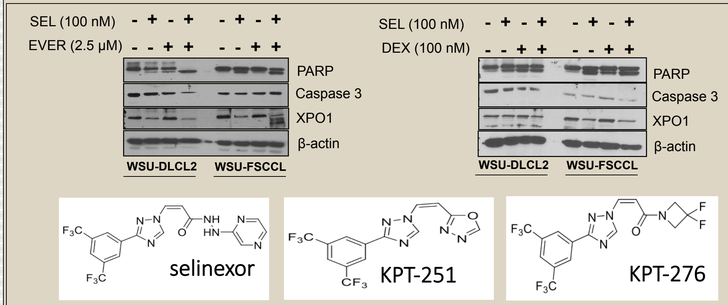
DLBCL cell line	RL	OCILY 3	A3/ KAW	OCILY 19	SUDH L5	SUDH L8	DOHH 2*	WSUD LCL2	SUDH L6	TOLE DO	PFEIF FER	DB
Selinexor IC_{50} (μM)	0.020	0.050	0.057	0.063	0.070	0.096	0.120	0.150	0.29	0.44	0.48	0.55

DLBCL cell lines were incubated with a range of selinexor concentrations over 72 hr. Resulting cell viability was determined using an MTT-based assay. (*Double hit)

RESULTS

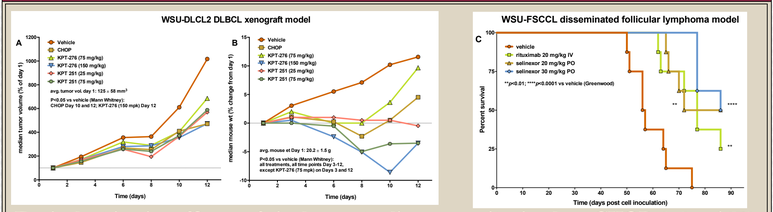


Selinexor synergizes with either DEX or EVER. WSU-FSCCL or WSU-DLCL2 cells were incubated with 100 nM selinexor (SEL) or 100 nM dexamethasone (DEX) or 1.25 μM everolimus (EVER), each drug alone, SEL+DEX or SEL+EVER. [**Left Panel**] Resulting cell viability was determined using trypan blue staining and cell counting. [**Right Panel**] Annexin V FITC apoptosis analysis (Sel 100 nM; DEX 100 nM and EV 2.5 μM).

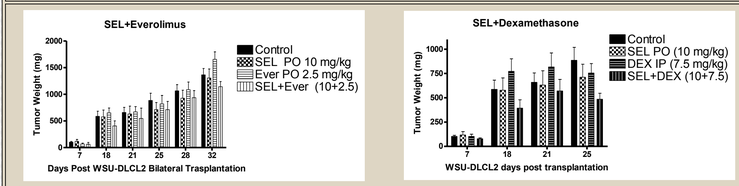


Molecular analysis of SEL-DEX and SEL-EVER synergy. WSU-DLCL2 or WSU-FSCCL were exposed to indicated concentrations of drugs for 72 hrs followed by protein isolation and western blotting. [Upper panel] Results showing enhanced PARP cleavage, full length caspase 3 reduction and decrease in XPO1 expression for combination treatments compared to single agents. β-actin was used as loading control. [**Lower panel**] Structures of SINEs.

RESULTS



Equivalent in vivo efficacy of single agent selinexor vs rituximab or CHOP. [A] and **[B]** Xenograft model of DLBCL. SINE Compounds KPT-251 and KPT-276 were administered sc and po, respectively, in cycles of once daily for ten consecutive days with a one day break prior to start of a new cycle. Cyclophosphamide, doxorubicin and vincristine (CHO) was administered once IV at MTD and prednisone (P) was administered po QDX5. **[C]** $10X10^6$ WSU-FSCCL follicular lymphoma cells were injected IV in the tail veins of ICR-SCID mice and vehicle or drug treatments were started one week later.



Selinexor at sub-optimal doses enhances the activity of DEX or EVER in subcutaneous DLBCL xenografts. WSU-DLCL2 xenograft were established as described above. Drugs were administered at indicated doses 5 days a week for three weeks. [**Left panel**] SEL +EVER and [**Right Panel**] SEL+DEX combination (study continuing beyond 25 days).

CONCLUSIONS

- **The enhanced in vitro and in vivo efficacy of selinexor in combination with DEX or the mTOR inhibitor everolimus provides rationale for further clinical investigation.**
- **The equivalent efficacy of single agent selinexor vs rituximab or CHOP is consistent with observed clinical activity of selinexor in refractory DLBCL and follicular lymphoma.**
- **A Phase 2 study of selinexor in DLBCL is currently recruiting patients. (Study of Selinexor (KPT-330) in Patients With Relapsed/Refractory Diffuse Large B-Cell Lymphoma" (SADAL – NCT02227251).**