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Selinexor, a Selective Inhibitor of Nuclear Export (SINE) Compound Shows Enhanced Anti-tumor Activity When Combined with Either Venetoclax or Bendamustine in Diffuse Large B Cell Lymphoma (DLBCL) Mouse Models

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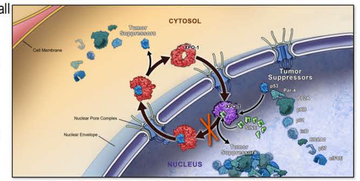
ABSTRACT

Introduction: Selinexor (KPT-330) is a small molecule inhibitor of CRM1/XPO1, the primary nuclear exporter of over 200 proteins. As such, it affects multiple cellular pathways and has been shown to be broadly synergistic with various drugs in multiple indications. We have previously shown that selinexor has marked activity in double-hit DLBCL in pre-clinical models and in a small cohort of patients with heavily pre-treated relapsed / refractory double or triple-hit DLBCL. The goal of this study was to test whether combination of selinexor with either venetoclax (ABT-199), a selective BCL2 inhibitor or bendamustine, a DNA damaging agent, can further enhance the anti-tumor effect of selinexor in DLBCL.



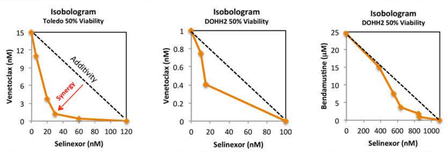
Methods: The effects of selinexor, bendamustine and venetoclax as single agents and the effects of selinexor in combination with either bendamustine or venetoclax on cell viability were tested on DLBCL cell lines including Toledo, DoHH2 and SUDHL6 using MTT assays. Whole protein cell lysates were extracted and analyzed by immunoblots. Mice were implanted with either Toledo or DoHH2 cells. Toledo inoculated mice were treated with either selinexor or venetoclax alone or in combination and DoHH2 inoculated mice were treated with selinexor, venetoclax or bendamustine alone or with selinexor-bendamustine or selinexor- venetoclax combinations. %Tumor growth inhibition (%TGI) and overall survival were determined. Xenografts were harvested and analyzed by Immunohistochemestry (IHC).

Results: Selinexor-venetoclax and selinexor-bendamustine were highly effective both *in-vitro* and *in-vivo*. Using MTT assay, we showed that each of the drugs have low IC50 values and they function synergistically/additively when combined. *In-vivo*, in the selinexor-bendamustine model, treatment with each drug showed %TGI of 37% (selinexor) and 86% (bendamustine) but in combination %TGI was 107%. Western and IHC analyses showed that selinexor reduces the expression of key DNA Damage Response (DDR) proteins presumably disabling the cells from over coming the damage induced by bendamustine. In the selinexor-venetoclax model, in both Toledo and DoHH2 mouse models, individual drugs had little effect on %TGI (Toledo: selinexor 4%, venetoclax 28%; DoHH2: selinexor 37%, venetoclax 24%) but when combined, treatment showed a synergistic effect (Toledo: 109%; DoHH2: 93%). Moreover, combination treatment of Toledo-derived large xenografts resulted in 30% tumor volume shrinkage that was sustained until the end of the study. Interestingly, BCL2 protein levels were reduced by each drug and to a further extent in the combination-treated group suggesting that the synergistic effect is induced via BCL2.

RESULTS

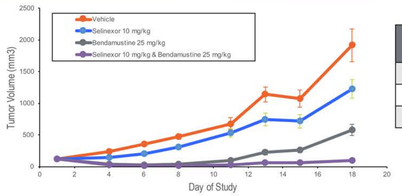
Selinexor Synergizes with Venetoclax and Bendamustine to Reduce DLBCL Cell Viability

Toledo	Viability (%) Venetoclax (nM)						
Selinexor (uM)	100	33	11	3.7	1.2	0.4	0
10	3	2	4	5	5	10	5
3.3	3	2	4	5	6	6	5
1.1	4	3	6	8	9	8	10
0.37	6	4	5	7	8	10	20
0.12	4	5	15	17	20	29	52
0.04	7	18	35	42	59	84	68
0.01	8	15	40	67	68	80	81
0.003	9	20	51	61	66	74	93
0	8	24	58	62	91	88	100



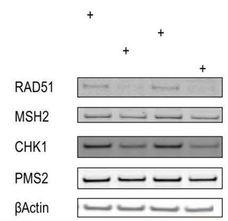
The effects of venetoclax and bendamustine, alone or in combination with selinexor, on cell viability in DLBCL cell lines using the MTT assay.

Additive Growth Inhibitory Effect and Down Regulation of DDR Proteins in Selinexor-Bendamustine Combination Treated DoHH2-Derived Xenografts



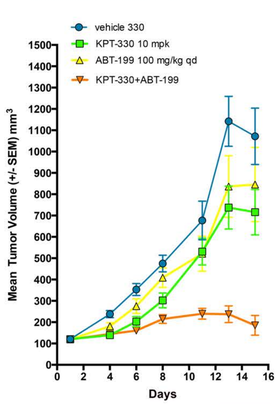
Treatment	% TGI (day 18)
Selinexor	37
Bendamustine	86
Combination	107

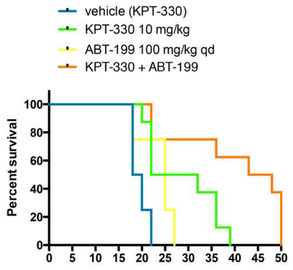
DMSO Vehicle Control
10nM Bendamustine
700nM Selinexor
700nM Selinexor +10nM Bendamustine



The effects of selinexor and bendamustine alone or in combination on DDR proteins were determined. Total protein from DoHH2 cell lysates were extracted and analyzed by Western blot analysis

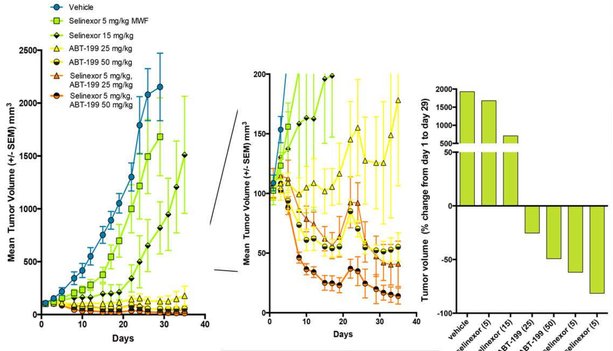
Selinexor-Venetoclax Combination is Synergistic and Prolongs Survival in DoHH2 Model of DLBCL-Derived Xenografts





Treatment	% TGI (day 16)
Selinexor	37
ABT-199	24
Combination	93

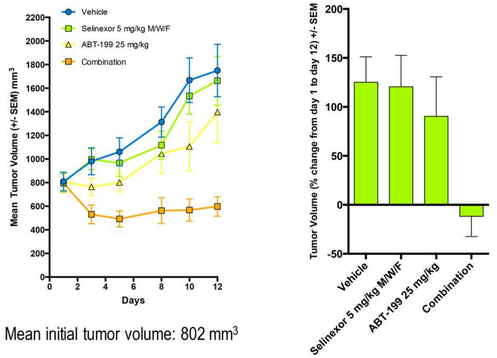
Selinexor-Venetoclax Combination is Synergistic in Toledo Model of DLBCL-Derived Xenografts



Mean initial tumor volume: 108 mm^3

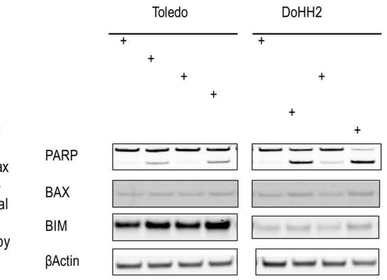
Treatment	% TGI (day 36)
Selinexor	4
ABT-199	28
Combination	109

30% Tumor Volume Shrinkage of Advanced Large Tumors Treated With Selinexor and Venetoclax

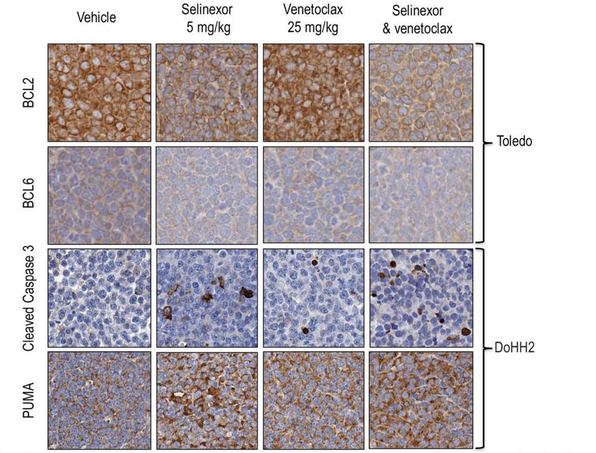


Mean initial tumor volume: 802 mm^3

Down Regulation of BCL2, BCL6 and Induction of Apoptosis in Selinexor-venetoclax Treated DLBCL Xenograft models



The effects of selinexor and venetoclax alone or in combination on apoptosis-related proteins were determined. Total protein from Toledo and DoHH2 cell lysates were extracted and analyzed by Western blot analysis



The effects of selinexor and venetoclax alone or in combination on BCL2, BCL6 and apoptosis-related proteins in Toledo-derived and DoHH2-drevied DLBCL Xenograft models were determined by IHC

CONCLUSIONS

Selinexor is an excellent candidate partner for combination therapies in DLBCL. It shows enhanced antitumor effect with both bendamustine and venetoclax modulating DDR and BCL2 pathway activity, respectively. These data provide rational support for study of selinexor-venetoclax and selinexor-bendamustine combination in clinical trials.

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