Poster Number: 4646

Synergistic Antitumor Effect of Selinexor, a Selective Inhibitor of Nuclear Export (SINE) Compound and Trastuzumab in a Mouse Model of Breast Cancer

Sivan Elloul, Hua Chang, Boris Klebanov, Trinayan Kashyap, Maxwell Werman, Margaret Lee, Yosef Landesman, Sharon Shacham, Michael Kauffman and Sharon Friedlander

Karyopharm Therapeutics, Newton MA 02459

ABSTRACT

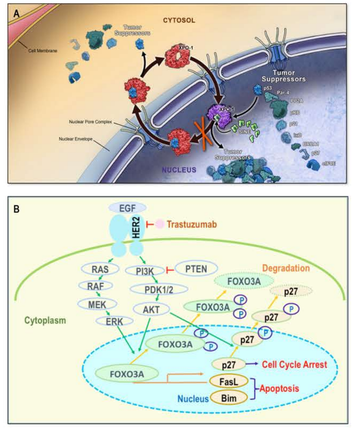
Introduction: Selinexor is an oral, first-in-class SINE compound that binds to the primary nuclear exporter XPO1/CRM1. XPO1 exports include major tumor suppressor proteins (TSPs) leading to their inactivation. Inhibition of XPO1 results in nuclear retention of TSPs and restores their normal functions. Here we tested the hypothesis that inhibition of HER2 signaling with trastuzumab synergizes with selinexor in a xenograft mouse model of breast cancer (BC) as both mediators (PI3K-AKT and Ras-Raf-MEK pathways) and effectors (FOXO3A, MDM2) of the HER2 pathway are among direct cargos of XPO1.

Methods: The effects of selinexor and trastuzumab alone or in combination on cell viability were tested on the BT474 HER2+ BC cell line using MTT assays. Total RNA and whole protein cell lysates were extracted and analyzed by qPCR and by immunoblots. *In-vivo*, BT474 cells were used to derive a xenograft mouse model. Mice were treated with sub-therapeutic doses of selinexor and trastuzumab alone or in combination and with the therapeutic dose of selinexor. Tumor growth (TG) was monitored for 60 days. Xenografts were harvested and analyzed by immunohistochemestry (IHC).

Results: Selinexor-trastuzumab combination was highly effective *in-vitro* and *in-vivo*. In MTT assays, both selinexor and trastuzumab demonstrated low IC_{50} values and when combined, they synergistically inhibit proliferation. *In-vivo*, the combination resulted in significant survival benefit and enhanced TG inhibition of 88% compared to the monotherapy groups 57% (selinexor) and 25% (trastuzumab). Interestingly, an increase in pro-survival cytoplasmic FOXO3A protein was observed in the trastuzumab-treated group however, in the combination group, FOXO3A, which is an XPO1 cargo, was restricted to the nucleus where it can trigger apoptosis. Indeed, apoptosis was evident in the combination group by an increase in total PH2AX protein in the treated cells and an increase in cleaved caspase 3 IHC staining of the derived xenografts. In addition, a synergistic increase in p27 protein, the G1 phase arrest regulator as well as a reduction in the proliferation marker KI67 IHC staining was observed in the selinexor-trastuzumab-treated group.

INTRODUCTION

Figure 1. Mechanisms of action of selinexor (A) and trastuzumab (B)



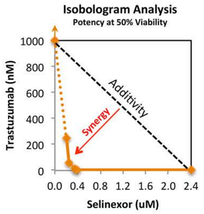
A. Selinexor is a SINE compound that increases the nuclear retention of major tumor suppressor proteins by inhibiting the nuclear export protein XPO1. Selinexor has been tested in >1400 patients to date in Phase 1 and 2/3 clinical trials with promising signs of efficacy, tolerability and safety.

B. Trastuzumab is a monoclonal antibody that binds to the HER2 receptor and inhibits its downstream signaling pathways. Both FOXO3A and p27 are tumor suppressor proteins involved in HER2 signaling pathways. They are also cargos of XPO1.

RESULTS

Selinexor-trastuzumab Combination Treatment Shows Synergistic Reduction in Viability in BT474 Cells

BT474 Viability (%)	Trastuzumab (nM) 1000	250	60	16	4	0
Selinexor (uM) 10	42	42	41	42	44	51
3.3	39	40	38	39	40	47
1.1	40	41	41	42	42	52
0.37	46	45	46	48	49	60
0.12	54	53	55	57	60	75
0.04	64	63	65	65	69	93
0.01	69	66	71	70	75	98
0.005	71	74	75	75	80	104
0	73	**76**	76	80	83	100



The effects of selinexor and trastuzumab alone or in combination on cell viability were tested on BT474 breast cancer cells using MTT assay.

Induction of Cell Cycle Regulatory and Apoptosis Proteins in Selinexor-trastuzumab Combination Treated BT474 cells

BT474 cells were treated with selinexor and trastuzumab alone or in combination for 24hrs. Total, nuclear and cytoplasmic protein cell lysates were extracted and analyzed by Western-blots.

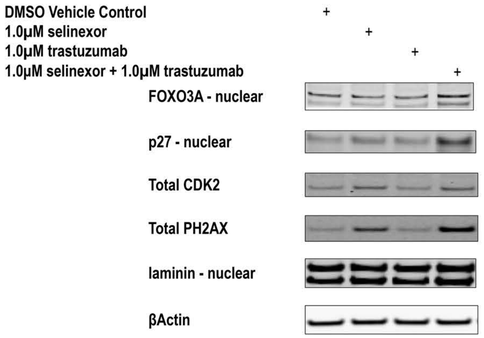
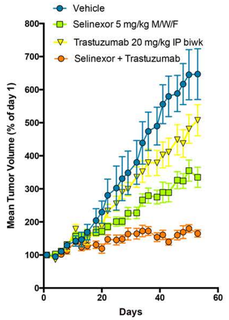


Figure 2. Enhanced effects of selinexor and trastuzumab in induction of cell cycle arrest and apoptosis proteins in BT474 cells in vitro. Note increase in nuclear FOXO3A and p27 in cells treated with selinexor and trastuzumab. In addition, total CDK2 increased in cells treated with selinexor alone or selinexor in combination with trastuzumab and an increase in the DNA damage marker pH2AX in cells treated with selinexor alone or selinexor in combination with trastuzumab.

Selinexor-trastuzumab Combination Treatment is Synergistic in a BT474 Mouse Xenograft Model



Treatment	% TGI (day 53)
selinexor	57
trastuzumab	25
Combination	88

Figure 3. Synergistic antitumor effect of selinexor and trastuzumab in BT474 breast cancer mouse models. Tumor growth inhibition (TGI) of the selinexor and trastuzumab combination treatment group was 88% on day 53 of study, whereas the TGI for selinexor alone was 57% and the TGI for trastuzumab alone was 25%.

Selinexor-trastuzumab Combination Treatment Improved Survival of Mice Carrying BT474 Xenografts

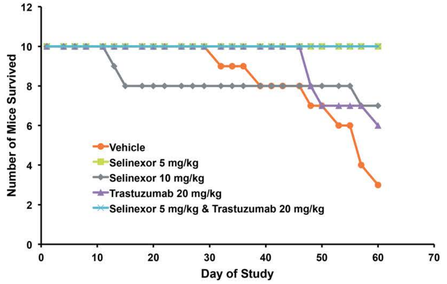


Figure 4. Survival curve of mice carrying BT474 xenografts treated with selinexor and trastuzumab alone and in combination. All mice treated with selinexor low dose and selinexor low dose in combination with trastuzumab survived the whole study period, better than the groups treated with vehicle, high dose selinexor or tratsuzumab alone.

Increased Apoptosis and Inhibition of Proliferation in BT474 Xenografts Treated With Selinexor and Trastuzumab

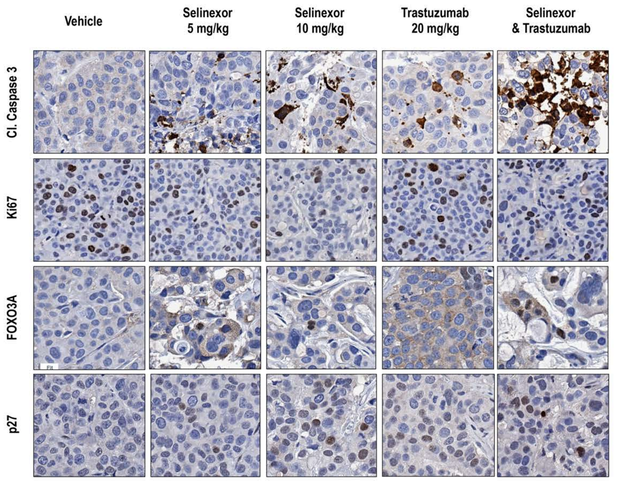


Figure 5. Immunohistochemistry analysis of BT474-derived mouse xenografts treated with selinexor and trastuzumab alone and in combination. Note induction of apoptosis (cleaved caspase 3 and nuclear FOXO3A) and decreased cell proliferation (decreased Ki67 and increased nuclear p27) in combination-treated xenografts.

Discussion and Conclusions

Selinexor-trastuzumab combination treatment resulted in elevated FOXO3A protein levels in the nucleus. FOXO3A act as a tumor suppressor by promoting cell cycle arrest and apoptosis. Indeed, pH2AX, which is phosphorylated in response to apoptotic signals and is required for DNA fragmentation during apoptosis, was elevated by selinexor treatment alone and to a higher extent in the combination treated cells. Increased expression of FOXO3A resulted in activation of the cell cycle inhibitor p27. Although treatment with selinexor contributed to elevated levels of the G1 to S transition regulator CDK2, it is thought that up regulation of p27 in the combination treatment group enforces CDK2 inhibition.

Conclusion: Selinexor-trastuzumab combination is a novel candidate therapy to HER2+ BC. It synergizes to induce survival benefit and TG inhibition. These data provide rational support for study of selinexor-trastuzumab combination in clinical trials.

Website: www.karyopharm.com contact: Sharon Friedlander, email: sfriedlander@karyopharm.com