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

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ABSTRACT

Introduction: Selinexor is an oral, first-in-class SINE compound that binds to the primary nuclear exporter XPO1 (CRM1). XPO1 exports major tumor suppressor proteins (TPS) leading to their nuclear retention. Inhibition of XPO1 results in nuclear retention of TPS 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 modulators (PI3K-AKT and RAS-MAPK pathways) and effectors (FOXO3A, MAD2) of the HER pathways are upregulated in BC of XPO1.

Methods: The effects of selinexor and trastuzumab alone or in combination on cell viability were tested on the BT474 and SK-BR-3 breast cancer cell lines using MTT assays. DMSO and water, vehicle cell lysates were generated and analyzed by qPCR and by Western. In vivo, BT474 cells were used to derive a xenograft mouse model. Mice were treated with or without combinations of selinexor and trastuzumab alone or in combination with the therapeutic dose of selinexor. Tumor growth (FG) was monitored for 60 days. Xenografts were normalized and analyzed by Western immunoblotting (I/W).

Results: Selinexor-trastuzumab combination was highly effective in vitro and in vivo. In MTT assays, both selinexor and trastuzumab depleted cell viability and when combined, they synergically inhibit proliferation. In vivo, the combination resulted in significantly reduced survival benefit and enhanced TGI inhibition of 88% compared to the monotherapy groups 59% (selinexor) and 36% (trastuzumab). Interestingly, in a preclinical epithelialoid BC xenograft model, FOXO3A was observed to be upregulated in the trastuzumab treated group. However, in the combination group, FOXO3A, which in 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 phospho-H3 and an increase in the cleaved caspase 3-4 staining of the derived xenograft. As a consequence, in p27Ink4a, the G1 phase arrest mediator as well as in proliferation is the phosphorylated marker MKT-177 staining was observed in the selinexor-trastuzumab-treated group.

INTRODUCTION

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 I/IV trials in patients with a wide range of malignancies in Phase I and II clinical trials with promising efficacy, tolerability and safety.

B. Trastuzumab is a monoclonal antibody that leads to the HER2 receptor and is restricted to downstream signaling pathways. Both FOXO3A and p27 are tumor suppressor proteins that regulate the cell cycle through the cell cycle inhibitors and cyclins. They are also targets of XPO1.

RESULTS

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

Figure 1. Mechanisms of action of selinexor (a) and trastuzumab (b). The effects of selinexor and trastuzumab alone or in combination on cell viability were tested on the BT474 and SK-BR-3 breast cancer cell lines using MTT assays. Selinexor was found to cause significant cell death in SK-BR-3 cells in a dose-dependent manner compared to BT474 cells.

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

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

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

Figure 3. Synergistic antitumor effect of selinexor and trastuzumab in BT474 breast cancer mouse models. Tumor growth inhibition (TGI) of the xenografts treated with the combination of selinexor and trastuzumab was 86% on day 31 of study, whereas the TGI for selinexor was 57% and the TGI for trastuzumab was 25%.

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

Figure 4. Survival curves of mice carrying BT474 xenografts treated with selinexor and trastuzumab alone and in combination. All mice treated with selinexor alone or trastuzumab showed significant delay in tumor growth compared to control. However, mice treated with selinexor and trastuzumab showed significantly improved survival compared to control, with 50% survival at day 31.

Discussion and Conclusions

Selinexor-trastuzumab combination treatment resulted in elevated FOXO3A protein levels in the nucleus. FOXO3A acts as a tumor suppressor by promoting cell cycle arrest and apoptosis. Indeed, p27Ink4a, which is phosphorylated in response to apoptosis signals and is required for DNA fragmentation during apoptosis, was elevated by selinexor-trastuzumab 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 CDK1, it is thought that upregulation of p27 in the combination treatment group entrains CDK1 inhibition.

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

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

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