Dual inhibition of CDK4/6 and IL-6 pathways as a novel therapeutic approach for triple-negative breast cancer cells

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Abstract

Triple-negative breast cancer (TNBC) is highly aggressive and associated with poor clinical outcomes. TNBC stands as a major cause of death among breast cancer patients and offers only a few therapeutic options. Abemaciclib, a cyclin dependent kinase 4/6 (CDK4/6) inhibitor, has received FDA approval for use in hormone receptor-positive, HER2-negative, and metastatic/advanced breast cancers. However, acquired resistance to CDK4/6 inhibitors in treating TNBC is becoming an increasing concern. Our recent results indicated that CDK4/6 inhibitors can induce IL-6 levels in TNBC cells, and increased IL-6 signaling could potentially compromise the efficacy of CDK4/6 inhibitors. In this study, abemaciclib was combined with an IL-6/GP130 inhibitor (bazedoxifene). We tested the effect of the combination on cell viability, migration, and invasion of human and mouse TNBC cells. Our data demonstrated that the bazedoxifene and abemaciclib combination synergistically inhibited TNBC cell viability, migration, and invasion in vitro. These results support dual inhibition of CDK4/6 and IL-6 as a novel therapeutic approach for TNBC.

Introduction and Background

Breast cancer is the most diagnosed cancer in women worldwide and is the second leading cause of cancer death in women in the United States.

Triple-negative breast cancer (TNBC):

- Accounts for up to 20% of all breast cancer cases
- Very aggressive clinical behavior
- Worst prognosis compared to other breast cancer types
- Very limited treatment options
- Germline BRCA mutation rate: 15-37%

Abemaciclib is an orally-administered CDK4/6 inhibitor that has been FDA-approved for the treatment of hormone receptor positive, HER2- breast cancer.

However, acquired resistance to CDK4/6 inhibitors, potentially in part due to the activation of IL-6/STAT3 signaling, is a growing problem.

Our preliminary results showed that CDK4/6 inhibitors can induce IL-6/P-STAT3 levels in TNBC cells.

Bazedoxifene is an orally-administered FDA-approved drug that has demonstrated efficacy as an IL-6/GP130 inhibitor.

We investigated the ability of this bazedoxifene and abemaciclib combination treatment to suppress TNBC cell viability and inhibit cell migration and invasion *in vitro*.

Results

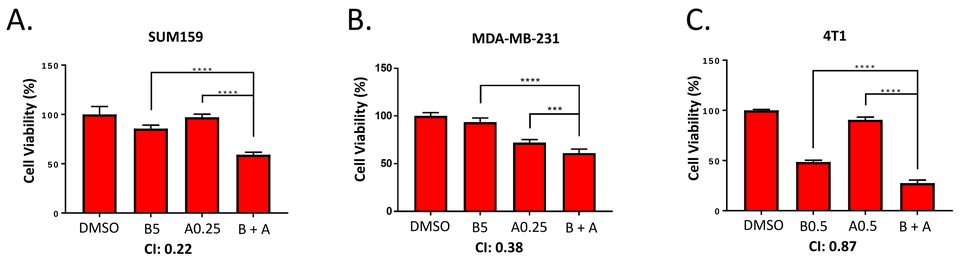

Figure 1. Effects of bazedoxifene (B), abemaciclib (A), and their combination on cell viability. MTT assays were performed to assess the cell viability of (A) SUM159 TNBC cells, (B) MDA-MB-231 TNBC cells, and (C) 4T1 TNBC cells. Dosage in micromolar (ex. B5 = 5μM). ***P < 0.001; ****P < 0.0001; CI value < 1 indicates a synergistic effect, > 1 indicates an antagonistic effect, and =1 indicates an additive effect

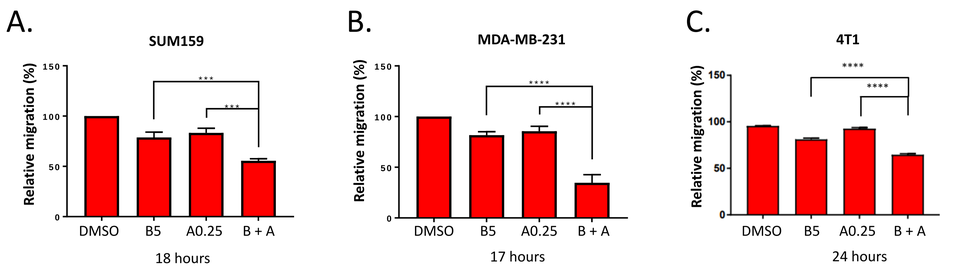

Figure 2. Effects of bazedoxifene (B), abemaciclib (A), and their combination on cell migration. Wound healing assays were performed to assess the cell migration of (A) SUM159, (B) MDA-MB-231, and (C) 4T1 TNBC cells. Dosage in micromolar (B5 : bazedoxifene 5μM; A5:). ***P < 0.001; ****P < 0.0001

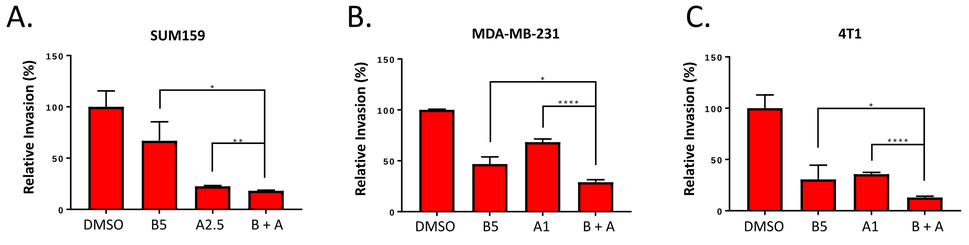

Figure 3. Effects of bazedoxifene (B), abemaciclib (A), and their combination on cell invasion. Cell invasion assays were performed to assess the relative invasion of (A) SUM159, (B) MDA-MB-231, and (C) 4T1 TNBC cells. Dosage in micromolar (ex. B5 = 5μM). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001

Materials and Methods

MTT Cell Viability Assay: 12 hours after seeding 3000 cells (SUM159 and MDA-MB-231) or 6000 cells (4T1) in a 96-well plate, the cells were treated for 3 days with respective drug(s) or DMSO. After treatment, 20μL of MTT solution was added and incubated for 4 hours followed by 100μL of DMF solubilization solution with shaking overnight in the dark. Absorbance was measured at 595 nm. Combination index (CI) was calculated using CompuSyn software.

Wound Healing (Migration) Assay: After cells reached 100% confluency in a 6-well plate, the monolayer was scratched with a 20μL pipette tip. After a PBS wash, new medium was added and cells were treated with the respective drug(s) or DMSO, then incubated again at 37°C. Pictures were taken at time zero, and when the control DMSO scratch had completely healed. Quantification was done using ImageJ.

Cell invasion assay: The inserts were coated with 1 mg/ml Matrigel (Corning™, NY, USA) at 37°C overnight. $6x10^4$ cells (SUM159, 4T1) or $6.5x10^4$ cells (MDA-MB-231) were seeded in 200 μl serum free medium in the inserts and the respective drug(s) or DMSO. 500 μl DMEM with 10% FBS was placed in the lower chamber. Cells were cultured for 41.5 hours (SUM159), 43 hours (MDA-MB-231), or 44.5 hours (4T1) and invasive cells on the lower surface of the inserts were stained with 0.1% crystal violet in 25% methanol at RT for 10 min. Invasive cells were counted and photographed using a light microscope under a ×100 magnification objective.

Conclusions and Future Directions

Conclusion:

- Abemaciclib or bazedoxifene monotherapy were able to inhibit some extent of cell viability, migration, and invasion, but the combination treatment showed synergistic inhibition to a greater extent than monotherapy.

Future Directions:

- Test the combination therapies *in vivo using* an orthotopic TNBC tumor mouse model.
- Determine the target proteins with combined treatment in CDK4/6 and IL-6/STAT3 pathway.

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