Synergistic effect of bazedoxifene and abemaciclib co-treatment in triple-negative breast cancer cells *in vitro*

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Abstract. Triple-negative breast cancer (TNBC) is an aggressive disease with the capability of metastasizing quickly. However, treatment options for patients with TNBC still remain limited. CDK4/6 inhibitors have been approved by the U.S. Food and Drug Administration and are administered for the treatment of hormone receptor-positive breast cancer subtypes, but not yet for TNBC. Although pre-clinical research is being conducted on their efficacy in treating TNBC, acquired resistance to CDK4/6 inhibitors is now a growing clinical problem. One of the identified resistance mechanisms is through the IL-6/STAT3 signaling pathway. In the present study, the CDK4/6 inhibitor, abemaciclib, was tested in combination with the IL-6 inhibitor, bazedoxifene, on human (SUM159 and MDA-MB-231) and murine (4T1) TNBC cell lines. Both abemaciclib and bazedoxifene monotherapies inhibited cell cycle progression and cell viability, migration and invasion, and induced apoptosis; however, the combination treatment exerted a greater effect than either monotherapy. These findings support the concept of CDK4/6 and IL-6 dual inhibition as a novel targeted therapy against TNBC.

Introduction

Breast cancer is the most commonly diagnosed cancer worldwide, with millions of cases diagnosed annually (1). Breast cancer can be classified into five major molecular subtypes: Luminal A [estrogen receptor $(ER)^+$, progesterone receptor $(PR)^+$, human epidermal growth factor receptor $(HER2)^-$], luminal B (ER^+, PR^+, $HER2^{+/-}$), HER2-enriched, normal breast-like and triple-negative breast cancer (TNBC) (2). TNBC is characterized by its lack of ER, PR and HER2 expression. In total, 15-20% of all breast cancer cases can be attributed to TNBC, and patients with TNBC frequently harbor BRCA1 mutations (3,4). TNBC is a very aggressive disease; it metastasizes quickly and patients with TNBC have the worst 5-year survival rate of all patients with breast cancer (5). Due to its deficiency in ER, PR and HER2, traditional hormone therapies and HER2-targeted therapies cannot be used to treat TNBC, leaving patients with very limited treatment options (3). Therefore, there is currently an urgent unmet need to develop novel treatment strategies for patients with TNBC that are effective and have minimal adverse side effects.

Cyclins and cyclin dependent kinases (CDKs) have a crucial role in the regulation of cell cycle progression (6,7). When in complex with cyclin D1, CDK4 and CDK6 are activated, which in turn phosphorylate the retinoblastoma (RB) tumor suppressor protein, and CDK4/6 are essential for the transition from G_1 to S phase (8). Thus, CDK4/6 inhibitors are able to arrest cells in G_1 phase, effectively halting tumor growth and progression (7,9). Abemaciclib is an orally-administered CDK4/6 inhibitor that has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of hormone receptor-positive, $HER2^-$ breast cancer. If CDK4/6 inhibitors could also inhibit RB-proficient TNBC cells, there would be a wider utility for these drugs. Previous preclinical studies have demonstrated the potential of abemaciclib in the treatment of TNBC (6,10-13). However, acquired resistance to CDK4/6 inhibitors has also been reported. A number of different CDK4/6 inhibitor resistance mechanisms have been identified, including activation of the interleukin-6 (IL-6)/signal transducer and activator of transcription 3 (STAT3) pathway, among others (14-22). IL-6 signaling may be able to confer resistance to CDK4/6 inhibitors through an increase in cyclin D1 and cyclin E2 levels by its downstream effectors STAT3, PI3-K/AKT and MEK/ERK, which may bypass CDK4/6 inhibition via cyclin E2/CDK2 pathway-mediated or non-canonical cyclin D1/CDK2-mediated S-phase entry (14,23-25).

IL-6 signaling plays an important role in tumorigenesis and cancer cell survival and progression as IL-6 is a pro-tumorigenic factor in a number of cancer types and is associated with poor prognosis and metastasis (26-28). In total, ~50% of breast cancer cases express IL-6, and TNBC cell lines secrete the highest levels of IL-6 compared with the luminal A, luminal B and HER2 enriched subtypes (26,29-31). Bazedoxifene is an orally-administered FDA-approved drug that has been repurposed as an IL-6/glycoprotein 130 (GP130) inhibitor (28,32).

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In phase III clinical trials, bazedoxifene has been shown to have an overall favorable safety and tolerability profile, as well as a favorable endometrial, ovarian and breast-specific safety profile in postmenopausal women (33-35). This favorable profile makes bazedoxifene an excellent candidate for use in combination therapy with abemaciclib for TNBC. Hence, in the present study, the ability of this bazedoxifene and abemaciclib combination treatment to suppress TNBC cell cycle progression and cell viability, migration and invasion, and to induce apoptosis *in vitro* was investigated. The findings of the present study support the use of this combination as a novel and more effective TNBC therapeutic option compared with CDK4/6 inhibitor monotherapy.

Materials and methods

Reagents. Bazedoxifene acetate (cat. no. 102233) and abemaciclib mesylate (cat. no. 206973) were purchased from MedKoo Biosciences, Inc. Both were dissolved in sterile dimethyl sulfoxide (DMSO) to prepare a 20 mM stock solution and stored at -20˚C. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from MilliporeSigma (Merck KGaA) and N,N-dimethylformamide (DMF) was obtained from Thermo Fisher Scientific, Inc. Different doses of the drugs are used across different experimental assays as the sensitivity of the cancer cells to the drugs may be affected by several factors such as seeding cell densities, cell growth rate, and experimental culture materials (96-well plates, 10-cm plates, and cell invasion transwell chambers).

Cell culture. The human TNBC cell line, MDA-MB-231, and the murine TNBC cell line, 4T1, were purchased from American Type Culture Collection. The human TNBC cell line, SUM159, was purchased from Asterand Bioscience, Inc. (BioIVT, LLC). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Corning, Inc.; cat. no. 10013CM) with L-glutamine supplemented with 10% fetal bovine serum (FBS) (Gibco, Inc.; cat. no. 16000-044) and 1% penicillin/streptomycin (Gibco, Inc.; cat. no. 15240-062). All cells were cultured in a humidified 37˚C incubator with 5% CO_2.

MTT cell viability assay. Cells were seeded into 96-well plates in triplicate at a density of 3,000 cells (SUM159 or MDA-MB-231) or 6,000 cells (4T1) per well. After overnight incubation, the cells were treated with different concentrations of bazedoxifene and/or abemaciclib or DMSO alone for 72 h. Next, 20 μl MTT solution (MilliporeSigma; Merck KGaA; cat. no. 475989) was added for 4 h of additional culture, followed by the addition of 150 μl DMF solubilization solution (Thermo Fisher Scientific, Inc.; cat. no. 047390) with shaking overnight in the dark. The absorbance was then measured at 595 nm. The combination index (CI) was calculated using CompuSyn software (36). CI<1 represents a synergistic effect, CI>1 represents an antagonistic effect and CI=1 indicates an additive effect.

Western blotting. Cells were collected following 24 h of treatment, and total protein was extracted using cell lysis buffer (Cell Signaling Technology, Inc.). The protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Equal amounts of protein were separated by 8% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% non-fat milk at room temperature and probed overnight at 4˚C with specific antibodies against phosphorylated (P)-STAT3 (Y705) (cat. no. 9145S), STAT3 (cat. no. 12640S), cyclin D1 (cat. no. 2978S), or GAPDH (cat. no. 2118S) (all 1:1,000; Cell Signaling Technology, Inc.). Next, the membranes were incubated with an HRP-conjugated anti-rabbit secondary antibody (1:5,000; Cell Signaling Technology, Inc.; cat. no. 7074S). The protein bands were visualized using SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Inc.; cat. no. 34094) and an Amersham Imager 680 (Cytiva).

Cell cycle analysis. SUM159 and MDA-MB-231 cells were treated with different concentrations of bazedoxifene and/or abemaciclib or DMSO alone for 24 h, then fixed using ice-cold 70% ethanol at 4˚C for 1 h. The cells were washed twice in wash buffer, then stained with a 50 μg/ml propidium iodide (PI; cat no. P4170; MilliporeSigma; Merck KGaA) and 10 μg/ml RNase A (cat no. EN0531; Thermo Fisher Scientific, Inc.) solution prepared in PBS. The cell cycle distribution was determined using the ImageStream^X Mark II System (Amnis, Inc./Luminex Corp; Cytek Biosciences), which allowed the image capture of cells passing through the cytometer. Data were analyzed using the IDEAS® 6.2 software (Amnis, Inc./EMDmillipore; MilliporeSigma; Merck KGaA).

Wound healing assay. All cell lines were seeded into a 6-well plate and cultured until 100% confluency was reached. A straight wound down the monolayer was created using a 200 μl pipette tip and images of the wounds were collected using an Echo Rebel microscope (ECHO; BICO). The cells were then treated with different concentrations of bazedoxifene and/or abemaciclib or DMSO alone in triplicate. Once the wounds of the DMSO-treated cells were completely healed, images were collected again and the relative migration (%) of each cell line was calculated using ImageJ software (Version 1.54d; National Institutes of Health).

Cell invasion assay. The cell invasion assay was performed using Matrigel-coated chambers. For this, the inserts (24-well insert; CELLTREAT Scientific Products) were coated with 1 mg/ml Matrigel (Corning, Inc.) at 37˚C overnight. Then, $3x10^4$ (MDA-MB-231 or 4T1) or $6x10^4$ (SUM159) cells in 200 μl serum-free DMEM were seeded into the inserts and treated with different concentrations of bazedoxifene and/or abemaciclib or DMSO alone in triplicate, and 500 μl DMEM with 10% FBS was placed in the lower chamber. The cells were cultured for 21 (MDA-MB-231 and 4T1) or 22 (SUM159) h, then the invasive cells were counted and images were collected using an Echo Rebel microscope under a x100 magnification objective. The cell number was quantified using ImageJ software (Version 1.54d; National Institutes of Health).

Apoptosis assay. The FITC Annexin V Apoptosis Detection Kit (BioLegend, Inc.) was used for Annexin V staining. MDA-MB-231 cells were treated with different concentrations of bazedoxifene and/or abemaciclib or DMSO alone for

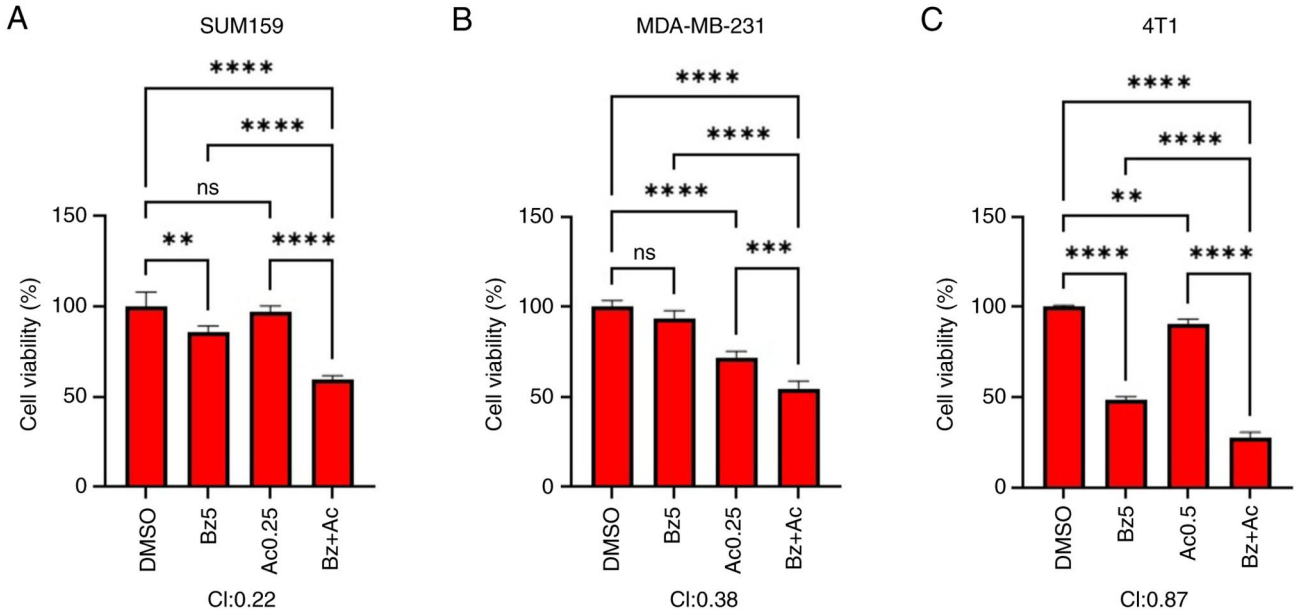


Figure 1. Effects of bazedoxifene, abemaciclib and their combination on cell viability. Co-treatment with bazeboxifene and abemaciclib in (A) SUM159, (B) MDA-MB-231 and (C) 4T1 cells exhibited a synergistic effect, and inhibited cell viability more significantly than either drug monotherapy or DMSO. Data are presented as the mean ± standard deviation. **P<0.01, ***P<0.001, ****P<0.0001. CI<1 indicates a synergistic effect, CI>1 indicates an antagonistic effect and CI=1 indicates an additive effect. Ac, abemaciclib; Ac0.25, 0.25 μM Ac; Ac0.5, 0.5 μM Ac; Bz, bazedoxifene; Bz0.5, 0.5 μM Bz; Bz5, 5 μM Bz; CI, combination index; ns, not significant.

24 h. Then, the cells were harvested and stained with Annexin V-FITC and PI according to the manufacturer's instructions. Cell apoptosis was determined using an ImageStream^X Mark II System. Data were analyzed using the IDEAS® 6.2 software.

Statistical analysis. All data are presented as the mean ± standard deviation. The difference between groups was analyzed by one-way ANOVA followed by Tukey's post hoc test. All statistical calculations were performed using GraphPad Prism 5 software (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Bazedoxifene combined with abemaciclib synergistically inhibits the viability and growth of TNBC cells. In the present study, the monotherapy effects of bazedoxifene and abemaciclib were confirmed and compared with the combination treatment across a variety of experimental assays. Different doses of the drugs are used across these different experimental assays as the sensitivity of cancer cells to the drugs may be affected by several factors such as seeding cell densities, cell growth rate, and experimental culture materials (96-well plates, 10-cm plates, and cell invasion transwell chambers). Since CDK4/6 inhibitors are less effective in RB-deficient and androgen receptor (AR)$^-$ TNBC cells, the RB-proficient AR$^+$ TNBC cell lines, MDA-MB-231 and SUM159, were used in the present study (18,37,38).

As shown by MTT cell viability assay, the viabilities of the SUM159, MDA-MB-231 and 4T1 cell lines were inhibited by bazedoxifene alone, abemaciclib alone and/or the combination treatment (Fig. 1). Furthermore, the combination treatment of bazedoxifene plus abemaciclib inferred a greater inhibition of cell viability than either monotherapy treatment.

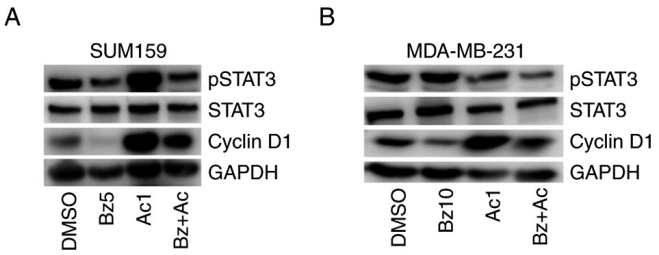
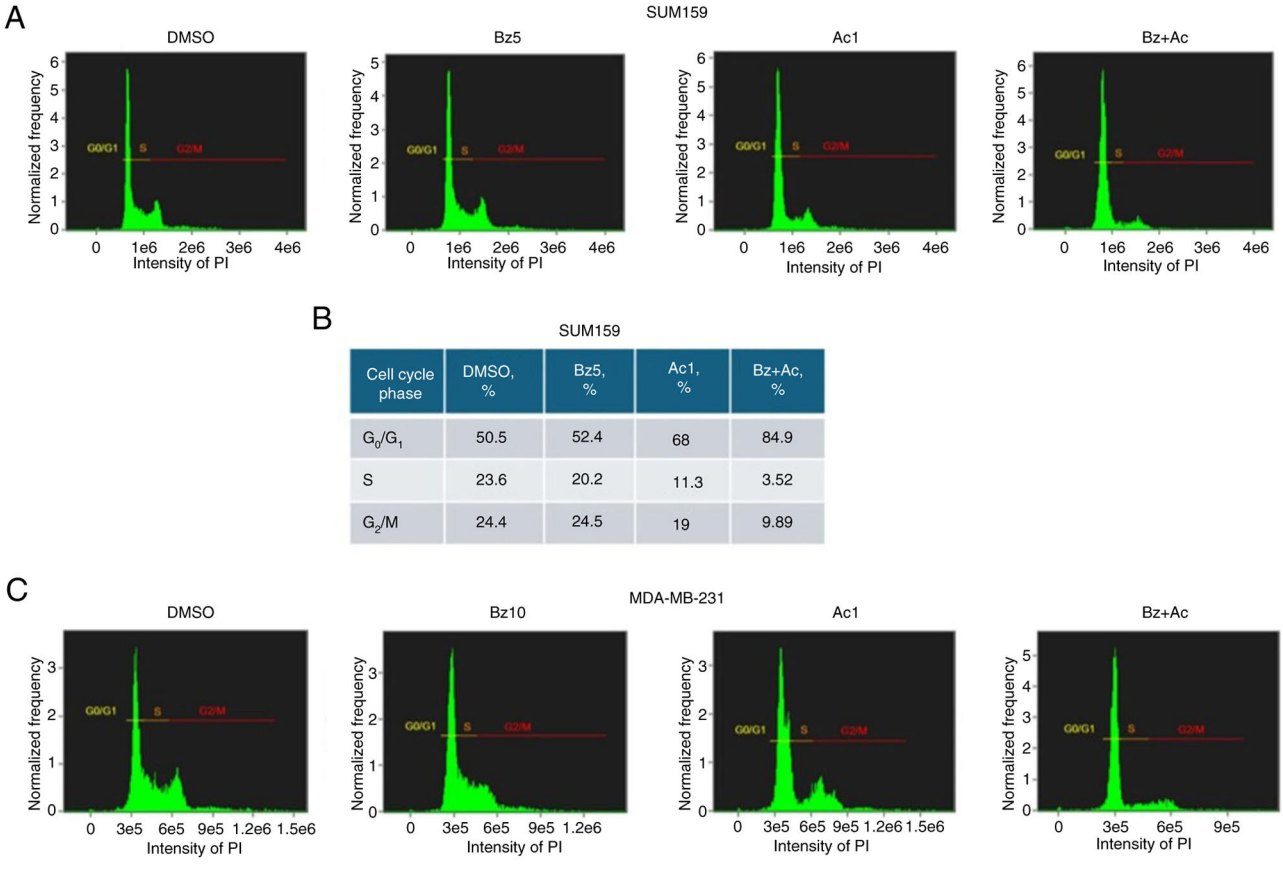


Figure 2. Effects of bazedoxifene, abemaciclib and their combination on IL-6/STAT3 signaling and downstream targets. Western blot analysis of pSTAT3, STAT3, cyclin D1 and GAPDH after treatment with DMSO, bazedoxifene, abemaciclib or bazedoxifene plus abemaciclib in (A) SUM159 and (B) MDA-MB-231 cells. Abemaciclib monotherapy induced pSTAT3 and cyclin D1, while the bazedoxifene plus abemaciclib combination combated this induction. Ac, abemaciclib; Ac1, 1 μM Ac; Bz, bazedoxifene; Bz5, 5 μM Bz; Bz10, 10 μM Bz; p, phosphorylated.

Additionally, the CI of bazedoxifene plus abemaciclib was found to be <1.0 for all cell lines tested, which was indicative of the two drugs having a synergistic effect in inhibiting cell viability (39).

The mechanism of this bazedoxifene plus abemaciclib combination was assessed by investigating the IL-6/STAT3 pathway via western blotting (Fig. 2). Abemaciclib treatment alone resulted in an increase in cyclin D1 expression, while the combination of bazedoxifene plus abemaciclib resulted in decreased pSTAT3 and cyclin D1 expression compared with abemaciclib monotherapy. This supported the hypothesis that IL-6/STAT3 signaling may be able to confer resistance to abemaciclib through the induction of cyclin D1, and that the dual inhibition of IL-6 and CDK4/6 with bazedoxifene and abemaciclib combination therapy may have a positive effect in inhibiting tumor cell viability and growth.



Cell cycle phase	DMSO, %	Bz5, %	Ac1, %	Bz+Ac, %
G_0/G_1	50.5	52.4	68	84.9
S	23.6	20.2	11.3	3.52
G_2/M	24.4	24.5	19	9.89

Cell cycle phase	DMSO, %	Bz10, %	Ac1, %	Bz+Ac, %
G_0/G_1	48.2	55.9	67	78.1
S	26.1	22.9	8.31	7.72
G_2/M	24.3	21.3	23.8	12.4

Figure 3. Effects of bazedoxifene, abemaciclib and their combination on cell cycle progression. Cell cycle analysis of (A) SUM159 cells, including (B) quantification, and (C) MDA-MB-231 cells, including (D) quantification, after treatment with DMSO, bazedoxifene, abemaciclib or the bazedoxifene plus abemaciclib combination. The strongest G_0/G_1 blockage was induced by the bazedoxifene plus abemaciclib combination. Ac, abemaciclib; Ac1, 1 μM Ac; Bz, bazedoxifene; Bz5, 5 μM Bz; Bz10, 10 μM Bz.

To further investigate the impact of this bazedoxifene plus abemaciclib combination on cell growth, cell cycle analysis was performed using the human SUM159 and MDA-MB-231 TNBC cell lines (Figs. 3 and S1) and representative images of MDA-MB-231 TNBC cells in the G_0/G_1, S and G_2/M phases of the cell cycle were collected (Fig. S1). Similar results for SUM159 TNBC cells in the G0/G1, S and G2/M phases of the cell cycle were observed (data not shown). Human TNBC cell lines were used in these experiments since they are more relevant to future human clinical trials. Abemaciclib treatment alone blocked more cells in the G_0/G_1 phase compared with DMSO or bazedoxifene monotherapy; however, the combination of abemaciclib plus bazedoxifene resulted in the highest G_0/G_1 blockage in the human SUM159 and MDA-MB-231 TNBC cell lines (Fig. 3).

Bazedoxifene plus abemaciclib combination treatment inhibits TNBC cell migration. Cell migration is one of the hallmarks of cancer cell metastasis. Therefore, a wound healing assay was performed using TNBC cell lines to investigate the effect of bazedoxifene and/or abemaciclib treatment on cell migration (Fig. 4). The monotherapies inhibited cell migration across all three TNBC cell lines tested. However, the combination therapy exerted a higher statistically significant inhibitory effect than either monotherapy in all three TNBC cell lines.

Bazedoxifene plus abemaciclib combination treatment inhibits TNBC cell invasion. Tumor cell invasion is another hallmark of metastatic disease. Therefore, a cell invasion assay was performed to assess the effect of bazedoxifene and/or abemaciclib treatment on the invasion of SUM159, MDA-MB-231 and 4T1 TNBC cells. As shown in Fig. 5, both bazedoxifene and abemaciclib monotherapies inhibited the invasion of all cell lines tested. However, the combination treatment of bazedoxifene plus abemaciclib inhibited cell invasion more significantly than either drug alone.

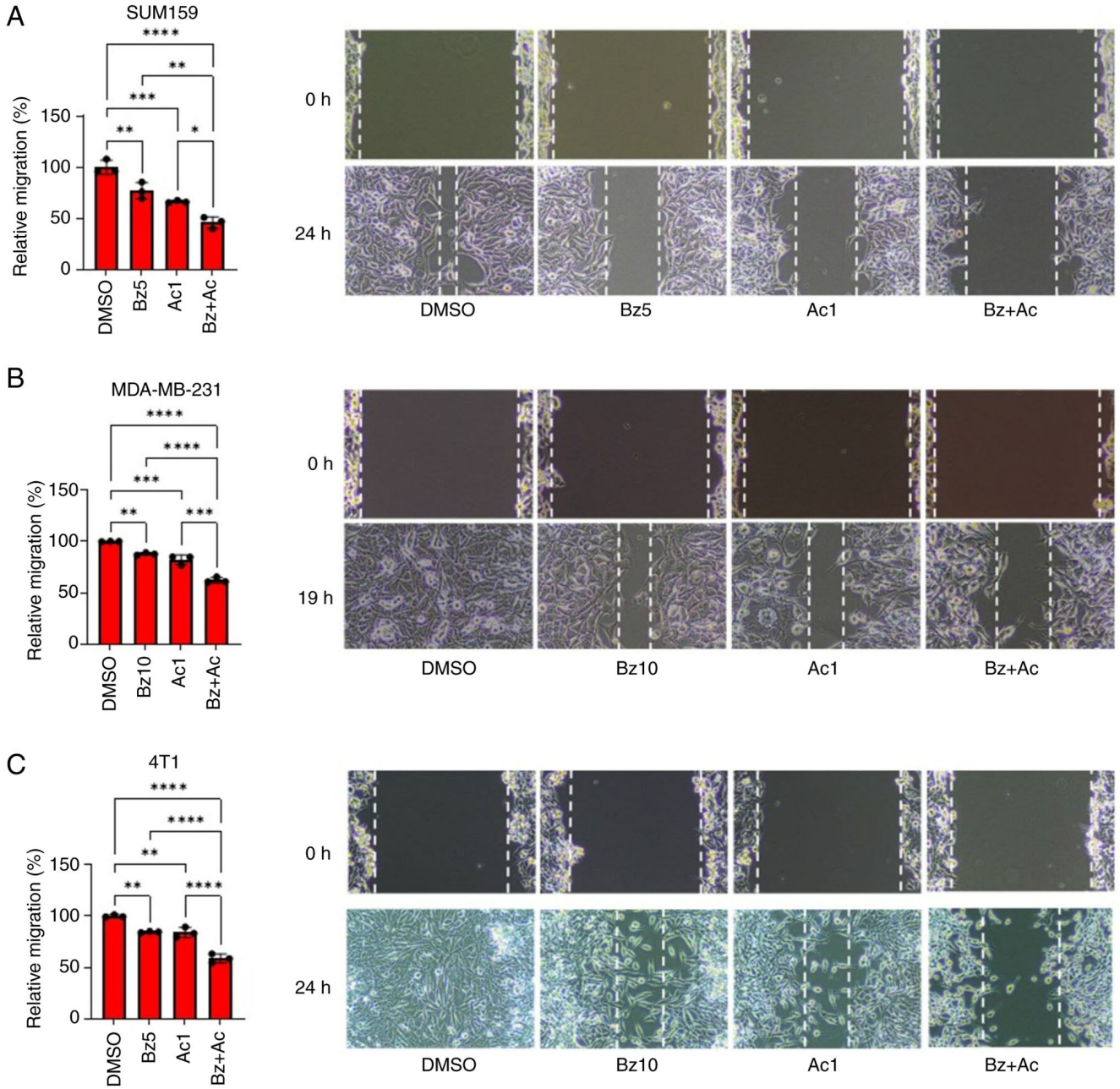


Figure 4. Effects of bazedoxifene, abemaciclib and their combination on cell migration. The migration of (A) SUM159, (B) MDA-MB-231 and (C) 4T1 cells was inhibited to a greater extent when cells were treated with bazedoxifene plus abemaciclib compared with either monotherapy or DMSO. Images were captured at a magnification of x10. Data are presented as the mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Ac, abemaciclib; Ac1, 1 μM Ac; Ac2, 2 μM Ac; Bz, bazedoxifene; Bz5, 5 μM Bz; Bz10, 10 μM Bz.

Bazedoxifene plus abemaciclib combination treatment induces the apoptosis of TNBC cells. To further evaluate the effect of combining bazedoxifene with abemaciclib, apoptosis of the MDA-MB-231 TNBC cell line was measured through Annexin V staining (Fig. 6). Compared with both monotherapies and DMSO treatment, the combination therapy induced the greatest level of apoptosis, as illustrated by the percentage of live and dead cells post-treatment. Thus, as the combination treatment significantly inhibited cell viability, migration and invasion and induced apoptosis, bazedoxifene and abemaciclib co-treatment may have a higher potential to prevent TNBC recurrence and metastasis than the single drugs alone.

Discussion

TNBC accounts for 15-20% of all breast cancer cases worldwide (3). Patients with TNBC have a more aggressive disease and a poorer prognosis compared with patients with other breast cancer subtypes, as demonstrated by TNBC typically having an earlier age of onset than other subtypes, as well as the lowest 5-year survival rate of all breast cancer subtypes (1,5). Furthermore, patients with TNBC have high rates of recurrence and metastasis to the bone, brain, lung and liver. These patients have an elevated risk of developing brain metastasis, and their median survival time after brain metastasis development is shorter compared with patients developing brain metastasis

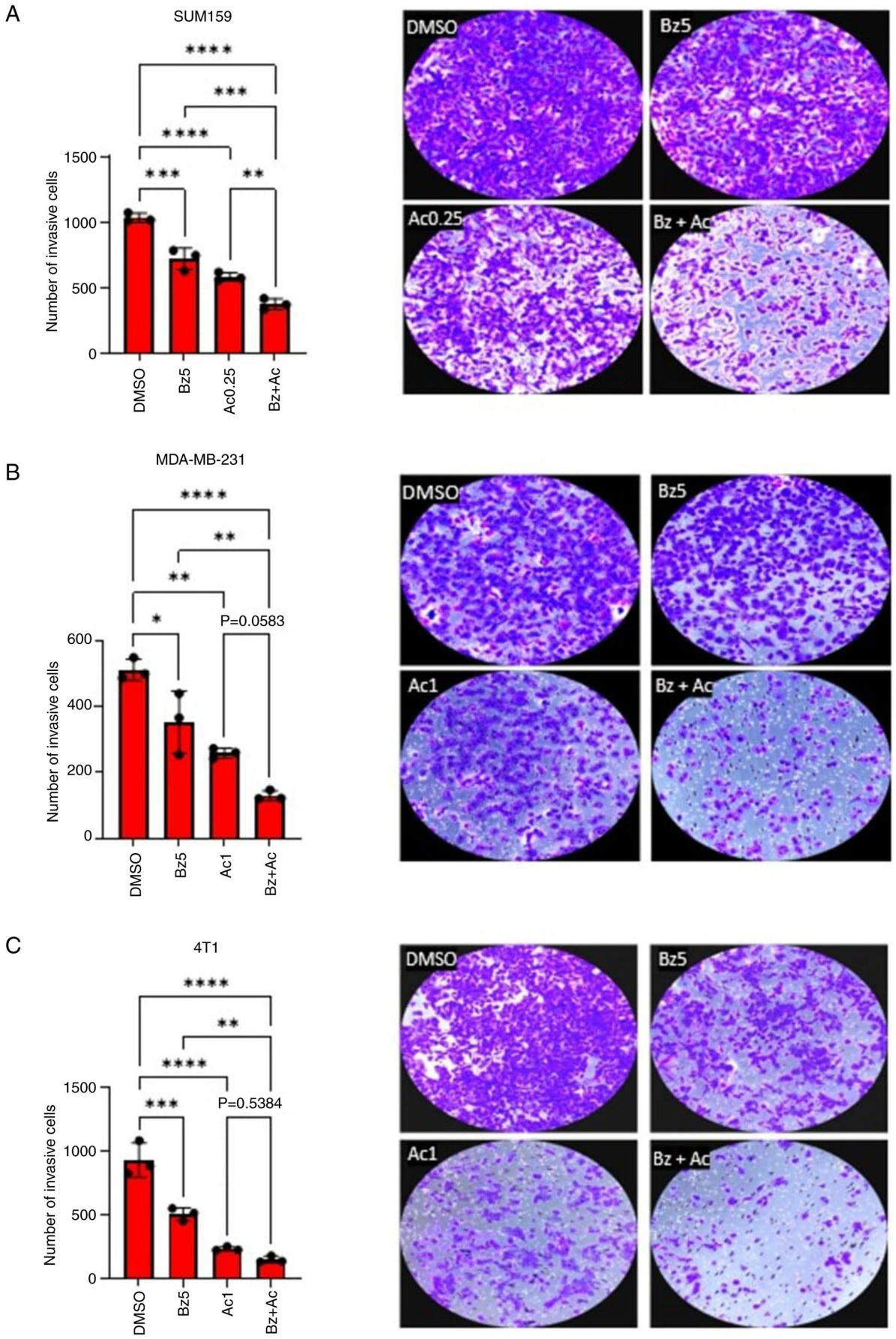
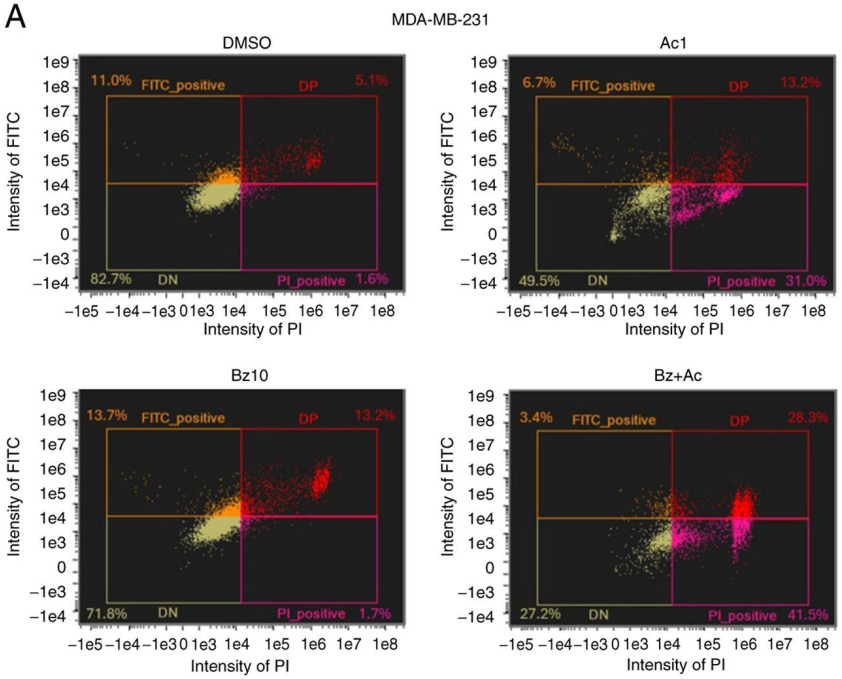


Figure 5. Effects of bazedoxifene, abemaciclib and their combination on cell invasion. The invasion of (A) SUM159, (B) MDA-MB-231 and (C) 4T1 cells was inhibited to a greater extent when cells were treated with bazedoxifene plus abemaciclib, compared with either monotherapy or DMSO. Images were captured at a magnification of x10. Data are presented as the mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Ac, abemaciclib; Ac0.25, 0.25 μM Ac; Ac1, 1 μM Ac; Bz, bazedoxifene; Bz5, 5 μM Bz.

A



B

Cell Status	DMSO, %	Bz10, %	Ac1, %	Bz+Ac, %
Live cells	82.7	71.8	49.5	27.2
Early apoptosis	11.0	13.7	6.7	3.4
Late apoptosis	5.1	13.2	13.2	28.3
Dead cells	1.6	1.7	31.0	41.5

Figure 6. Effects of bazedoxifene, abemaciclib and their combination on apoptosis. Apoptosis analysis of (A) MDA-MB-231 cells after treatment with DMSO, bazedoxifene, abemaciclib, or the bazedoxifene plus abemaciclib combination, and (B) its quantification. The greatest induction of apoptosis was achieved by the bazedoxifene plus abemaciclib combination compared with either monotherapy or DMSO treatment. Ac, abemaciclib; Ac1, 1 μM Ac; Bz, bazedoxifene; Bz10, 10 μM Bz; DN, double negative (negative for FITC and PI, indicative of live cells); FITC_positive, only positive for FITC (indicative of cells in early apoptosis); DP, double positive (positive for FITC and PI, indicative of cells in late apoptosis); PI_positive, only positive for PI (indicative of dead cells).

from other breast cancer subtypes. In addition, patients with TNBC with bone-only metastases have a poorer outcome and outlook compared with patients with luminal A or luminal B tumors and bone-only metastases (5). Traditional hormone therapies and HER2-targeted therapies cannot be used to treat TNBC; therefore, there is an urgent unmet need to develop novel therapeutic options for patients with TNBC, particularly to help combat metastatic TNBC.

Within the past decade, a new generation of CDK4/6 inhibitors have emerged for treating hormone receptor-positive breast cancer subtypes, including ribociclib, palbociclib and abemaciclib. All of these drugs function to arrest dividing tumor cells in the G_1 phase of the cell cycle to effectively stop tumor progression and growth (9,40,41). These drugs have demonstrated efficacy for some patients with breast cancer, and there is evidence to suggest potential CDK4/6 inhibitor efficacy for TNBC treatment, which could assist in the response to the current unmet need for novel TNBC therapeutic options (13). However, acquired resistance to CDK4/6 inhibitor therapy, potentially in part due to IL-6 signaling, is a growing problem that must also be addressed (14-22).

Bazedoxifene is an FDA-approved drug for the prevention of postmenopausal osteoporosis, with a favorable safety and tolerability profile. It has also been demonstrated that bazedoxifene can act as an IL-6/GP130 inhibitor, downregulating the IL-6/STAT3 pathway and the expression of its downstream genes (28,32). Limited research from previous studies has demonstrated the antitumor activity of bazedoxifene, either as a monotherapy or in combination with talazoparib, paclitaxel, cisplatin, or radiation therapy, in a variety of cancer types including ovarian cancer, gastrointestinal cancer, rhabdomyosarcoma, hepatocellular carcinoma, colon cancer, cervical cancer, pancreatic cancer, and head and neck cancer (28,42-49). There is also limited research demonstrating the efficacy of bazedoxifene as a TNBC therapeutic; however, to the best of our knowledge, there has been no previous research conducted on combining bazedoxifene with a CDK4/6 inhibitor, and most research surrounding CDK4/6 inhibitors and breast cancer, both in terms of monotherapy and combination therapy, has been focused on hormone receptor-positive breast cancer, not TNBC (7,9-12,14,21,22,41,50-52).

In the present study, the combination of bazedoxifene and abemaciclib was tested in TNBC cell lines *in vitro*. The results demonstrated that while bazedoxifene and abemaciclib monotherapies were able to exhibit a limited ability to inhibit TNBC cell cycle progression and cell viability, migration and invasion, and to induce apoptosis, the combination therapy acted synergistically to inhibit cell cycle progression and cell viability, migration and invasion, and to induce apoptosis to an even greater extent than either monotherapy in both human and mouse TNBC cell lines. Migration and invasion are hallmarks of cancer metastasis; thus, the results of the present study indicate that bazedoxifene and abemaciclib combination therapy may have the potential to prevent TNBC recurrence and metastasis. In addition, the bazedoxifene and abemaciclib combination may also have potential as a novel targeted therapy against RB^+ TNBC. Both drugs can be administered orally, which improves the quality of life of the patients. However, additional research is warranted to further investigate this combination *in vitro* and *in vivo* as a candidate for targeted anticancer therapies.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KC and CS performed the experiments. KC, CS and JL analyzed and interpreted the data. KC wrote the original draft of the manuscript. KC and JL reviewed and edited the manuscript. JL was responsible for project administration, resources and supervision. KC and CS confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S and Soerjomataram I: Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast 66: 15-23, 2022.
2. Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 490: 61-70, 2012.
3. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y and Pietenpol JA: Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 121: 2750-2767, 2011.
4. Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, Valero V, Lehmann BD, Pietenpol JA, Hortobagyi GN, *et al*: Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 19: 5533-5540, 2013.
5. Brouckaert O, Wildiers H, Floris G and Neven P: Update on triple-negative breast cancer: Prognosis and management strategies. Int J Womens Health 4: 511-520, 2012.
6. Zhu X, Chen L, Huang B, Li X, Yang L, Hu X, Jiang Y, Shao Z and Wang Z: Efficacy and mechanism of the combination of PARP and CDK4/6 inhibitors in the treatment of triple-negative breast cancer. J Exp Clin Cancer Res 40: 122, 2021.
7. de Groot AF, Kuijpers CJ and Kroep JR: CDK4/6 inhibition in early and metastatic breast cancer: A review. Cancer Treat Rev 60: 130-138, 2017.
8. Sherr CJ, Beach D and Shapiro GI: Targeting CDK4 and CDK6: From discovery to therapy. Cancer Discov 6: 353-367, 2016.
9. O'Leary B, Finn RS and Turner NC: Treating cancer with selective CDK4/6 inhibitors. Nat Rev Clin Oncol 13: 417-430, 2016.
10. Kim ES: Abemaciclib: First global approval. Drugs 77: 2063-2070, 2017.
11. Johnston SRD, Toi M, O'Shaughnessy J, Rastogi P, Campone M, Neven P, Huang CS, Huober J, Jaliffe GG, Cicin I, *et al*: Abemaciclib plus endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer (monarchE): Results from a preplanned interim analysis of a randomised, open-label, phase 3 trial. Lancet Oncol 24: 77-90, 2023.
12. Messina C, Cattrini C, Buzzatti G, Cerbone L, Zanardi E, Messina M and Boccardo F: CDK4/6 inhibitors in advanced hormone receptor-positive/HER2-negative breast cancer: A systematic review and meta-analysis of randomized trials. Breast Cancer Res Treat 172: 9-21, 2018.
13. Asghar US, Barr AR, Cutts R, Beaney M, Babina I, Sampath D, Giltnane J, Lacap JA, Crocker L, Young A, *et al*: Single-cell dynamics determines response to CDK4/6 inhibition in triple-negative breast cancer. Clin Cancer Res 23: 5561-5572, 2017.
14. Kettner NM, Vijayaraghavan S, Durak MG, Bui T, Kohansal M, Ha MJ, Liu B, Rao X, Wang J, Yi M, *et al*: Combined inhibition of STAT3 and DNA repair in palbociclib-resistant ER-positive breast cancer. Clin Cancer Res 25: 3996-4013, 2019.
15. Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, Pearson A, Guzman M, Rodriguez O, Grueso J, *et al*: Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. Cancer Res 76: 2301-2313, 2016.
16. Yang C, Li Z, Bhatt T, Dickler M, Giri D, Scaltriti M, Baselga J, Rosen N and Chandarlapaty S: Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene 36: 2255-2264, 2017.
17. Min A, Kim JE, Kim YJ, Lim JM, Kim S, Kim JW, Lee KH, Kim TY, Oh DY, Bang YJ and Im SA: Cyclin E overexpression confers resistance to the CDK4/6 specific inhibitor palbociclib in gastric cancer cells. Cancer Lett 430: 123-132, 2018.
18. Pandey K, An HJ, Kim SK, Lee SA, Kim S, Lim SM, Kim GM, Sohn J and Moon YW: Molecular mechanisms of resistance to CDK4/6 inhibitors in breast cancer: A review. Int J Cancer 145: 1179-1188, 2019.
19. Huang J, Zheng L, Sun Z and Li J: CDK4/6 inhibitor resistance mechanisms and treatment strategies (review). Int J Mol Med 50: 128, 2022.
20. Álvarez-Fernández M and Malumbres M: Mechanisms of sensitivity and resistance to CDK4/6 inhibition. Cancer Cell 37: 514-529, 2020.
21. Portman N, Alexandrou S, Carson E, Wang S, Lim E and Caldon CE: Overcoming CDK4/6 inhibitor resistance in ER-positive breast cancer. Endocr Relat Cancer 26: R15-R30, 2019.
22. Cetin B, Wabl CA and Gumusay O: CDK4/6 inhibitors: Mechanisms of resistance and potential biomarkers of responsiveness in breast cancer. Future Oncol 18: 1143-1157, 2022.
23. Leslie K, Lang C, Devgan G, Azare J, Berishaj M, Gerald W, Kim YB, Paz K, Darnell JE, Albanese C, *et al*: Cyclin D1 is transcriptionally regulated by and required for transformation by activated signal transducer and activator of transcription 3. Cancer Res 66: 2544-2552, 2006.
24. Mullany LK, Nelsen CJ, Hanse EA, Goggin MM, Anttila CK, Peterson M, Bitterman PB, Raghavan A, Crary GS and Albrecht JH: Akt-mediated liver growth promotes induction of cyclin E through a novel translational mechanism and a p21-mediated cell cycle arrest. J Biol Chem 282: 21244-21252, 2007.
25. Mirza AM, Gysin S, Malek N, Nakayama K, Roberts JM and McMahon M: Cooperative regulation of the cell division cycle by the protein kinases RAF and AKT. Mol Cell Biol 24: 10868-10881, 2004.
26. Hartman ZC, Poage GM, den Hollander P, Tsimelzon A, Hill J, Panupinthu N, Zhang Y, Mazumdar A, Hilsenbeck SG, Mills GB and Brown PH: Growth of triple-negative breast cancer cells relies upon coordinate autocrine expression of the proinflammatory cytokines IL-6 and IL-8. Cancer Res 73: 3470-3480, 2013.
27. Bachelot T, Ray-Coquard I, Menetrier-Caux C, Rastkha M, Duc A and Blay JY: Prognostic value of serum levels of interleukin 6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients. Br J Cancer 88: 1721-1726, 2003.

28. Zhang R, Wang T and Lin J: Synergistic effect of bazedoxifene and PARP inhibitor in the treatment of ovarian cancer regardless of BRCA mutation. Anticancer Res 41: 2277-2286, 2021.
29. Garcia-Tuñón I, Ricote M, Ruiz A, Fraile B, Paniagua R and Royuela M: IL-6, its receptors and its relationship with bcl-2 and bax proteins in infiltrating and in situ human breast carcinoma. Histopathology 47: 82-89, 2005.
30. Kim G, Ouzounova M, Quraishi AA, Davis A, Tawakkol N, Clouthier SG, Malik F, Paulson AK, D'Angelo RC, Korkaya S, *et al*: SOCS3-mediated regulation of inflammatory cytokines in PTEN and p53 inactivated triple negative breast cancer model. Oncogene 34: 671-680, 2015.
31. Wang K, Zhu X, Zhang K, Yin Y, Chen Y and Zhang T: Interleukin-6 contributes to chemoresistance in MDA-MB-231 cells via targeting HIF-1α. J Biochem Mol Toxicol 32: e22039, 2018.
32. Li H, Xiao H, Lin L, Jou D, Kumari V, Lin J and Li C: Drug design targeting protein-protein interactions (PPIs) using multiple ligand simultaneous docking (MLSD) and drug repositioning: Discovery of raloxifene and bazedoxifene as novel inhibitors of IL-6/GP130 interface. J Med Chem 57: 632-641, 2014.
33. de Villiers TJ, Chines AA, Palacios S, Lips P, Sawicki AZ, Levine AB, Codreanu C, Kelepouris N and Brown JP: Safety and tolerability of bazedoxifene in postmenopausal women with osteoporosis: Results of a 5-year, randomized, placebo-controlled phase 3 trial. Osteoporos Int 22: 567-576, 2011.
34. Archer DF, Pinkerton JV, Utian WH, Menegoci JC, de Villiers TJ, Yuen CK, Levine AB, Chines AA and Constantine GD: Bazedoxifene, a selective estrogen receptor modulator: Effects on the endometrium, ovaries, and breast from a randomized controlled trial in osteoporotic postmenopausal women. Menopause 16: 1109-1115, 2009.
35. Palacios S, de Villiers TJ, Nardone Fde C, Levine AB, Williams R, Hines T, Mirkin S and Chines AA; BZA Study Group: Assessment of the safety of long-term bazedoxifene treatment on the reproductive tract in postmenopausal women with osteoporosis: Results of a 7-year, randomized, placebo-controlled, phase 3 study. Maturitas 76: 81-87, 2013.
36. Chou TC: Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev 58: 621-681, 2006.
37. Konecny GE, Winterhoff B, Kolarova T, Qi J, Manivong K, Dering J, Yang G, Chalukya M, Wang HJ, Anderson L, *et al*: Expression of p16 and retinoblastoma determines response to CDK4/6 inhibition in ovarian cancer. Clin Cancer Res 17: 1591-1602, 2011.
38. Brough R, Gulati A, Haider S, Kumar R, Campbell J, Knudsen E, Pettitt SJ, Ryan CJ and Lord CJ: Identification of highly penetrant Rb-related synthetic lethal interactions in triple negative breast cancer. Oncogene 37: 5701-5718, 2018.
39. Chou TC: Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res 70: 440-446, 2010.
40. Hu Y, Gao J, Wang M and Li M: Potential prospect of CDK4/6 inhibitors in triple-negative breast cancer. Cancer Manag Res 13: 5223-5237, 2021.
41. Cejuela M, Gil-Torralvo A, Castilla MÁ, Domínguez-Cejudo MÁ, Falcón A, Benavent M, Molina-Pinelo S, Ruiz-Borrego M and Salvador Bofill J: Abemaciclib, palbociclib, and ribociclib in real-world data: A direct comparison of first-line treatment for endocrine-receptor-positive metastatic breast cancer. Int J Mol Sci 24: 8488, 2023.
42. Thilakasiri P, Huynh J, Poh AR, Tan CW, Nero TL, Tran K, Parslow AC, Afshar-Sterle S, Baloyan D, Hannan NJ, *et al*: Repurposing the selective estrogen receptor modulator bazedoxifene to suppress gastrointestinal cancer growth. EMBO Mol Med 11: e9539, 2019.
43. Yadav A, Kumar B, Teknos TN and Kumar P: Bazedoxifene enhances the anti-tumor effects of cisplatin and radiation treatment by blocking IL-6 signaling in head and neck cancer. Oncotarget 8: 66912-66924, 2016.
44. Xiao H, Bid HK, Chen X, Wu X, Wei J, Bian Y, Zhao C, Li H, Li C and Lin J: Repositioning bazedoxifene as a novel IL-6/GP130 signaling antagonist for human rhabdomyosarcoma therapy. PLoS One 12: e0180297, 2017.
45. Ma H, Yan D, Wang Y, Shi W, Liu T, Zhao C, Huo S, Duan J, Tao J, Zhai M, *et al*: Bazedoxifene exhibits growth suppressive activity by targeting interleukin-6/glycoprotein 130/signal transducer and activator of transcription 3 signaling in hepatocellular carcinoma. Cancer Sci 110: 950-961, 2019.
46. Wei J, Ma L, Lai YH, Zhang R, Li H, Li C and Lin J: Bazedoxifene as a novel GP130 inhibitor for Colon Cancer therapy. J Exp Clin Cancer Res 38: 63, 2019.
47. Kim L, Park SA, Park H, Kim H and Heo TH: Bazedoxifene, a GP130 inhibitor, modulates EMT signaling and exhibits antitumor effects in HPV-positive cervical cancer. Int J Mol Sci 22: 8693, 2021.
48. Wu X, Cao Y, Xiao H, Li C and Lin J: Bazedoxifene as a novel GP130 inhibitor for pancreatic cancer therapy. Mol Cancer Ther 15: 2609-2619, 2016.
49. Park SA, Kim LK, Park HM, Kim HJ and Heo TH: Inhibition of GP130/STAT3 and EMT by combined bazedoxifene and paclitaxel treatment in ovarian cancer. Oncol Rep 47: 52, 2022.
50. Tian J, Chen X, Fu S, Zhang R, Pan L, Cao Y, Wu X, Xiao H, Lin HJ, Lo HW, *et al*: Bazedoxifene is a novel IL-6/GP130 inhibitor for treating triple-negative breast cancer. Breast Cancer Res Treat 175: 553-566, 2019.
51. Fu S, Chen X, Lo HW and Lin J: Combined bazedoxifene and paclitaxel treatments inhibit cell viability, cell migration, colony formation, and tumor growth and induce apoptosis in breast cancer. Cancer Lett 448: 11-19, 2019.
52. Damodaran S, O'Sullivan CC, Elkhanany A, Anderson IC, Barve M, Blau S, Cherian MA, Peguero JA, Goetz MP, Plourde PV, *et al*: Open-label, phase II, multicenter study of lasofoxifene plus abemaciclib for treating women with metastatic ER+/HER2-breast cancer and an ESR1 mutation after disease progression on prior therapies: ELAINE 2. Ann Oncol 34: 1131-1140, 2023.