The Combination of Dasatinib and PD-L1 inhibitor prevents the progression of epithelial-mesenchymal transition and dramatically blocks cell invasion of HER2-positive breast cancer cells

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Introduction

- HER2-positive breast cancer overexpresses the human epidermal growth factor receptor type 2 (HER2) and comprises of 15-25% of total breast cancer cases.
- Dasatinib (DA), an oral tyrosine kinase inhibitor, successfully blocks SRC and SRC-family kinases (SFKs) which are expressed in several subtypes of breast cancers. DA in combination with trastuzumab and paclitaxel as a first-line treatment showed good efficacy in HER2-positive breast cancer.
- On the other hand, the immune checkpoint, programmed cell-death ligand-1 (PD-L1) is significantly expressed in HER2-positive breast cancer subtypes, and thus, the use of PD-1/PD-L1 inhibitors plays a vital role in the management of several types of solid tumors, including breast.
- However, there are no studies on DA and PD-1/PD-L1 inhibitors combined effect in HER2-positive breast cancer.

Materials & Methods

- We herein explored for the first time the individual and synergistic effects of DA and PD-1/PD-L1-inhibitor (BMS-202) on cell proliferation, cell invasion, and colony formation in two HER2-positive human breast cancer cell lines, SKBR3 and ZR75. Human normal mammary epithelial cells (HNME) were used as a control in this study

Results

Cell viability

- Our data revealed that both inhibitors reduced the viability of SKBR3 and ZR75 cells significantly in a dose-dependent fashion (**Figure 1**).
- The IC50 of DA was found to be 8.58±0.08µM and 13.89±0.14µM in SKBR3 and ZR75, respectively (**Figure 1A**); While IC50 of BMS-202 had a higher value of 12.84±1.09µM and 15.14±0.46µM, in SKBR3 and ZR75 cell lines, correspondingly (**Figure 1B**).
- A significant decrease in cell proliferation is observed in the presence of the combination treatment, 5 µM of DA and 5 µM of BMS-202 for both SKBR3 and ZR75 cell lines (**Figure 1C**).
- Moreover, HNME-E6/E7 cells treated with DA and BMS-202 did not show a significant reduction in cell viability at used concentrations (**Figure 1D**).

Cell invasion assay

- Subsequently, we analyzed the anti-invasion ability of DA and BMS-202, alone and in combination in both cell lines, SKBR3 and ZR75, using Matrigel® Invasion Chambers.
- The synergistic effects of DA and BMS-202 showed a dramatic decrease in the number of invasive cells upon treatment compared to each treatment individually (**Figure 2**).
- This suggests that both DA and BMS-202 can considerably downgrade cell invasion and consequently cancer progression of HER2-positive breast cancer.

Western blot

- Based on the above data, we explored the expression patterns of key marker genes of EMT and cancer invasion, E-cadherin, β-catenin, and vimentin.
- Our data pointed out that the combination of DA and BMS-202 enhances E-cadherin expression and β-catenin in SKBR3 and ZR75 cell lines. In contrast, vimentin expression was decreased compared to individual treatment and untreated control cells (**Figure 3**).

Immunofluorescence

- Our immunofluorescence analysis reveals that treatment with a combination of DA and BMS-202 results in restoration of E-cadherin and β-catenin complex (**Figure 4**).
- The dual treatment promotes the translocation of E-cadherin and β-catenin from the cytoplasm to the cytoplasmic membrane and its undercoat, respectively. However, in untreated cells, E-cadherin and β-catenin are equally distributed in the cytoplasm.
- These data indicate that DA and BMS-202 synergistically prevent the EMT progression of both cell lines, SKBR3 and ZR75, via the restoration of the E-cadherin/β-catenin complex.

Colony formation assay

- Our data show a significant decrease in colonies' number and size upon treatment compared to the control (**Figure 5**).
- Interestingly, cells treated with the two drugs combined did not form any colonies in both cell lines ($p<0.001$) (Figure 7). Quantification analysis reveals a significant decrease in colony number and size in cells treated with DA and BMS-202 individually ($p<0.001$) in comparison to treatment with their controls (Figure 7). These data indicate that treatment with DA and BMS-202 together significantly suppress colony formation of HER2-positive breast cancer and probably tumor growth in vivo.

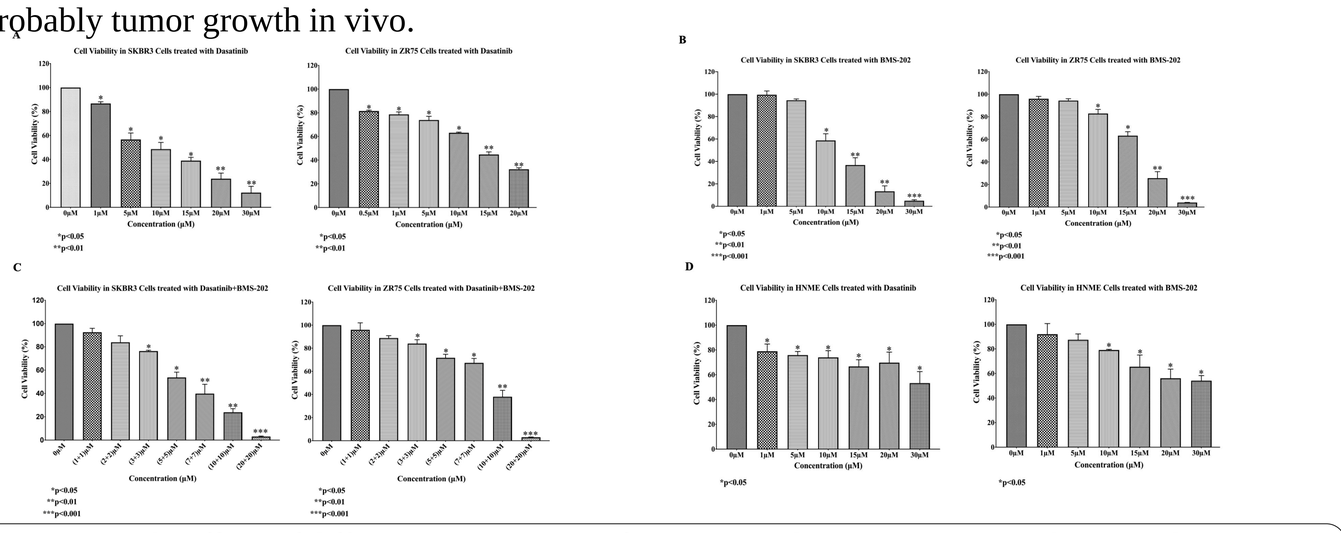

Figure 1 (A-D). The effects of different concentrations of (A). Dasatinib, (B). BMS-202, and (C). combination (DA and BMS-202) on cell proliferation of SKBR3 and ZR75. (D). The effects of different concentrations of a combination of DA and BMS-202 on human immortalized mammary epithelial (HNME) cells.

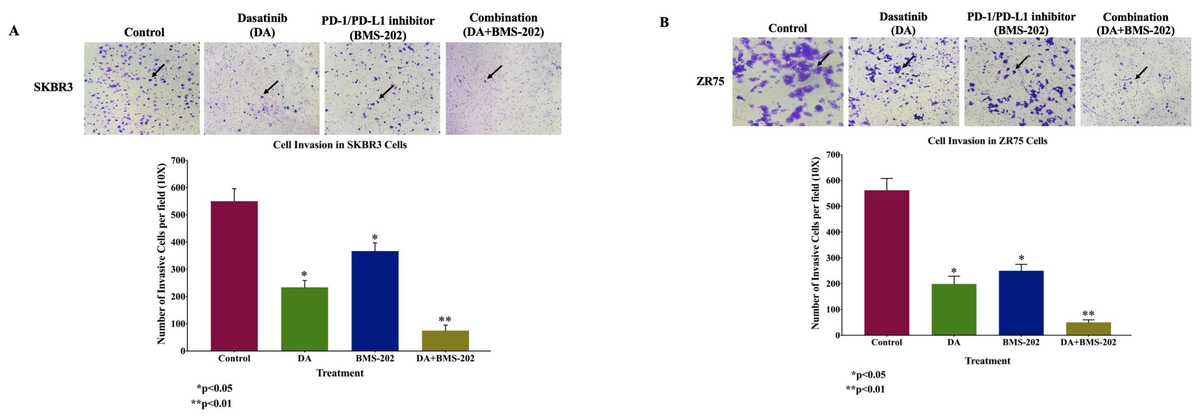

Figure 2 (A and B). Effects of DA and PD-1/PD-L1 inhibitor on cell invasion of human HER2-positive breast cancer cell lines, SKBR3 and ZR75.

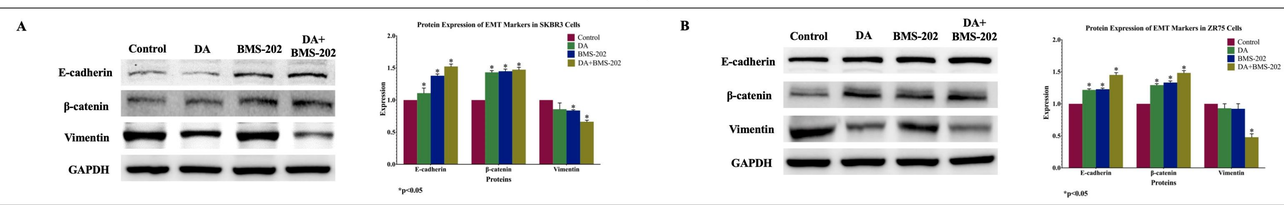

Figure 3. Western blot analysis of E-cadherin, β-catenin, and vimentin expression in (A). SKBR3 and (B). ZR75 under the effect of DA and PD-1/PD-L1 inhibitors.

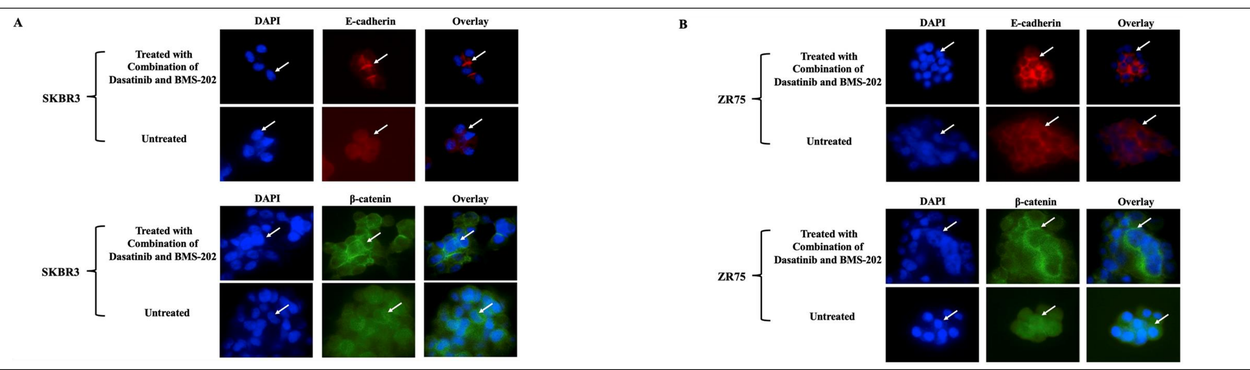

Figure 4 (A and B). Immunofluorescence analysis of E-cadherin and β -catenin expression patterns of (A). SKBR3 and (B). ZR75-1 cells.

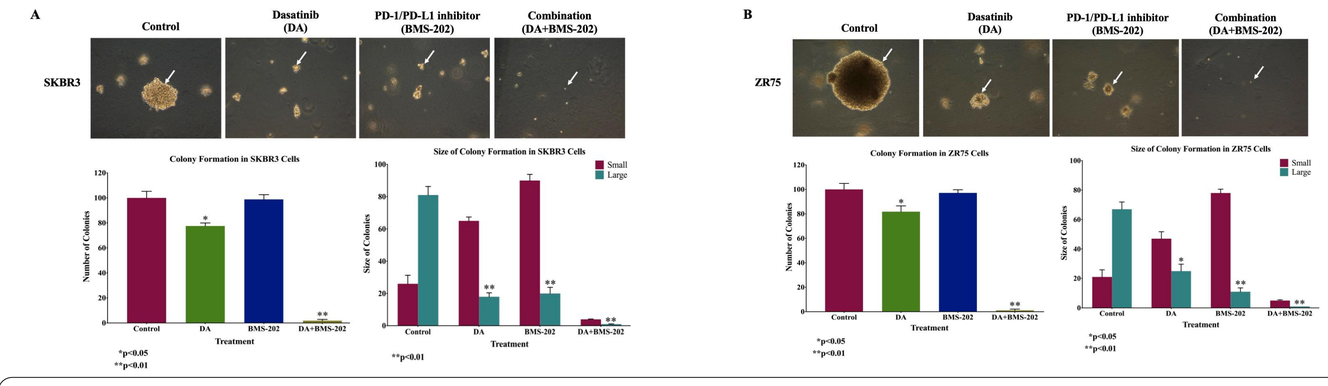

Figure 5 (A and B). The outcome of DA and PD-1/PD-L1 inhibitors on colony formation of (A). SKBR3 and (B). ZR75 cell lines.

Conclusion

This study reports for the first time the synergistic effect of DA and PD-L1 inhibitor on HER2-positive breast cancer and its underlying mechanism. Our findings implicate that, in comparison to monotreatment, combination of DA and BMS-202 could have a significant impact on the management of HER2-positive breast cancer via HER2 inactivation and specifically beta-catenin signaling pathways. Thus, we believe that our study will help pave the way for potential and advanced therapeutic approaches in breast cancer management, especially HER2- positive cases. However, more studies, especially in vivo, preclinical and clinical, are necessary to allow such a combination in cancer patients.