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SYNERGISTIC MECHANISMS OF ACTION: COMPARISON OF ADCC AND SIGNALING PATHWAYS INHIBITION BY TRASTUZUMAB BIOSIMILAR OR ORIGINATOR TRASTUZUMAB IN COMBINATION WITH PERTUZUMAB IN HER2+ BREAST CANCER CELL LINES

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Objectives: Trastuzumab and pertuzumab bind to HER2 synergistically at different subdomains, improving the clinical benefit of breast cancer treatment. Recently, trastuzumab biosimilars were approved after comparison with the originator, proving high similarity in structure and activity, and equivalent efficacy, safety, and immunogenicity. This study aimed to compare in vitro antibody-dependent cell-mediated cytotoxicity (ADCC) and HER2-signaling inhibition elicited by biosimilar trastuzumab (BS-TZB) or originator trastuzumab (TZB) in combination with pertuzumab (PTZ). **Methodology:** Human breast cancer cell lines BT474 (HER2+/estrogen [ER] and progesterone receptor [PR]-positive) and HCC1954 (HER2+/ER and PR-negative) were used. Anti-HER2 antibodies: TZB (Herceptin[®] 440 mg, Roche, batch: N7194B11-B3064); BS-TZB (Zedora[®] 440 mg, Libbs/Biocon [trastuzumab-dkst], batch: 18F0072); and PTZ (Perjeta[®] 420 mg, Roche, batch: H0260B01) were commercially acquired. ADCC assay: cells were incubated with TZB or BS-TZB, with/without PTZ. To determine the cytotoxicity percentage, the LDH activity using the CytoTox-ONETM was measured. Western blot: HCC1954 cells were incubated with TZB or BS-TZB, with/without PTZ to analyze the HER2 signaling cascade. We analyzed that Akt, ERK, STAT3, and MKK3/6 were the downstream molecules. **Results:** ADCC activity of BS-TZB was higher than that of TZB in both cell lines (EC50 [$\mu\text{g}/\text{mL}$]: 1.01 and 1.32, respectively, for BT474: 0.16 and 0.23, for HCC1954). Combined with PTZ, BS-TZB also showed slightly higher ADCC activity than TZB (EC50 [$\mu\text{g}/\text{mL}$]: 0.90 and 1.15, for BT474: 0.13 and 0.19, for HCC1954). BS-TZB and TZB inhibited the phosphorylation levels of Akt, ERK, STAT3, and MKK3/6 in a dose-dependent manner. PTZ enhanced inhibition effects of BS-TZB and TZB on p-AKT and p-ERK, while it had no effect on the p-STAT3 and p-MKK3/6. **Conclusions:** In association with pertuzumab, trastuzumab biosimilar showed non-inferior biological potency and equivalent signaling inhibition compared with originator trastuzumab. This study explored mechanisms elicited by dual HER2 blockade, showing the same pattern for BS-TZB as well as TZB, combined with PTZ.

Keywords: Biodrugs; Oncology; Targeted Therapy; Biological Drug.