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TITLE
A Novel Proteotoxic Combination Therapy for EGFR+ and HER2+ Cancers

HYPOTHESIS:
Overexpression of EGFR and/or HER2 presents a severe burden to the secretary pathway, as HER-family proteins EGFR, HER2, HER3 share conserved extracellular cysteine-rich and proline-rich repeats that form numerous disulfide bonds and control cis-trans isomerization in the protein folding processes. Thus, cancer cells that overexpress HER-family protein members are hypothesized to be exquisitely sensitive to inhibitors of disulfide bond formation and proline isomerization because disruption of the protein folding process in these cells may further increase the protein folding burden, which in turn elevates the misfolded/unfolded protein levels in the Endoplasmic Reticulum (ER).

BACKGROUND/AIMS:
EGFR and/or HER2 are frequently overexpressed oncoproteins in HER2+ breast cancer, Triple Negative Breast Cancer (TNBC), and Inflammatory Breast Cancer (IBC). However, EGFR and/or HER2 targeting agents have failed to make major advances in improving survival for these cancer patients, largely because these cancers frequently develop resistance to these drugs. The identification of novel treatment strategies is critically needed. In our previous studies, we demonstrated that Disulfide bond Disrupting Agents (DDAs) interfere with disulfide bond formation in EGFR/HER2/HER3, trigger ER stress selectively in HER2 and/or EGFR overexpressing breast cancer cells, and induce AKT dephosphorylation. We propose here that another group of agents termed Proline Isomerization Inhibitors (PIIs), which also disturb the protein folding process by inactivating Peptidyl-prolyl isomerases (PPIases), mimic the effects of DDAs and may be useful for treating breast cancer. We also want to investigate that whether the combination of DDAs and PIIs would be synergistic in treating breast cancer.

METHODS:
The mechanisms of CsA, and the importance of HER-family proteins and ER stress pathways were investigated by constructing EGFR+ or HER2+ breast cancer cell lines and pharmacological inhibition studies. The synergistic effects of drug combinations were evaluated based on the combination index (CI) which was calculated by applying Chou-Talalay method using CalcuSyn software. Cell viability assay and animal model were used to evaluate the anticancer efficiency of DDAs and PIIs. The animal model was created by injecting 5 × 10^6 BT474 cells into the mammary fat pads of female NSG mice. Mice with palpable tumors were randomly assigned to vehicle, PII Cyclosporine A (CsA), DDA tcyDTDO, and the combination of CsA and tcyDTDO for tumor growth study and survival analysis.

RESULTS & CONCLUSIONS
We demonstrated that the PIIs CsA selectively killed EGFR+ and/or HER2+ breast cancer cells in vitro and decreased tumor growth of HER2+ cancer in a xenograft mouse model. PII-dependent anti-cancer actions resulted from downregulation of EGFR/HER2/HER3, induction of AKT dephosphorylation, and activation of ER stress. We also investigated a novel proteotoxic combination therapy employing DDAs and PIIs in treating breast cancers. DDAs potentiated the efficacy of PIIs in downregulating EGFR/HER2/HER3 levels and initiating ER stress, and the combination was highly synergistic in reducing EGFR+ and/or HER2+ breast cancer cell viability. Importantly, a combination of the DDA tcyDTDO and PII CsA overcame single drug resistance, allowed dosages of each drug to be lowered, and significantly decreased tumor volumes and increased the survival of xenograft mice. Evidence that the drug combination was effective against metastatic breast cancer cells is also provided.