Several studies suggested that some of the HER2 negative patients may respond to anti-HER2 treatments and that moderately HER2 expressing breast tumours may respond better to tyrosine kinase inhibitors compared to trastuzumab. The aim of this study is to understand the mechanisms of action and resistance of HER2 targeting treatments in HER2 moderate-expressing and low-expressing breast cancer cells. Combining treatments in HER2 moderate-expressing and low-expressing breast cancer cells.

**Methods:**

HER2 expression of a panel of 8 breast cell lines was assessed by IHC, FISH, western blot, and qRT-PCR. Using cell viability studies we found that in comparison with high (HC 3+) and low HER2 expressing (HC 0 or 1+) breast cancer cells, moderately expressing HER2 (2+) cells showed an intermediate response to trastuzumab and neratinib. Three breast cancer cell lines, MDA-MB-361, MDAMB-453 and HCC1569 were more sensitive to neratinib than trastuzumab monotherapy. The response to trastuzumab and neratinib correlated with basal ADAM17 but not ADAM10 expression. ADAM17 inhibition, using a specific anti-ADAM17 antibody or knockdown of the protein, decreased activation of HER members as well as downstream markers, correlating with markedly reduced cell viability in IHC 2+ breast cancer cells.

**RESULTS**

1. **Cell viability studies:** Using cell viability studies we found that in comparison with high (HC 3+) and low HER2 expressing (HC 0 or 1+) breast cancer cells, moderately expressing HER2 (2+) cells showed an intermediate response to trastuzumab and neratinib. Three breast cancer cell lines, MDA-MB-361, MDAMB-453 and HCC1569 were more sensitive to neratinib than trastuzumab monotherapy. The response to trastuzumab and neratinib correlated with basal ADAM17 but not ADAM10 expression. ADAM17 inhibition, using a specific anti-ADAM17 antibody or knockdown of the protein, decreased activation of HER members as well as downstream markers, correlating with markedly reduced cell viability in IHC 2+ breast cancer cells.

2. **Combination of neratinib and trastuzumab:** In addition, the combination of neratinib with trastuzumab +/- pertuzumab was more effective than neratinib alone in downregulating HER2 and decreasing cell viability in IHC 2+ breast cancer cells. The combination of neratinib with trastuzumab was more effective than trastuzumab alone in IHC 2+ breast cancer cells.

3. **Combination of neratinib and/or pertuzumab:** Combination of neratinib with trastuzumab and/or pertuzumab was more effective than any single agent in MDAMB-361 breast cancer cells. The combination of neratinib with trastuzumab and/or pertuzumab was more effective than any single agent in MDAMB-361 breast cancer cells.

**CONCLUSIONS**

Trastuzumab induces upregulation of ADAM17 in IHC 2+ breast cancer cells, resulting in activation of HER receptors. Trastuzumab with or without neratinib was more effective than trastuzumab alone in reducing cell viability in IHC 2+ breast cancer cells.