A specific vascular endothelial growth factor receptor tyrosine kinase inhibitor enhances the antiproliferative effect of trastuzumab in human epidermal growth factor receptor 2 overexpressing breast cancer cell lines.
Background
Background

- Breast cancer is a devastating disease. The recent clinical success of therapies that target specific proteins expressed by tumor cells has shown that growth factor receptors and tyrosine kinase inhibitors (TKIs) have important clinical benefits.

- Two such targets
  - vascular endothelial growth factor receptor (VEGFR)
  - human epidermal growth factor receptor 2 (HER-2)
  have been shown to be mediators of breast cancer pathogenesis.

- Also, overexpression of these receptors correlates strongly with aggressive behavior in breast carcinomas.
Background

- Therapy targeting the HER-2 oncoprotein using monoclonal antibodies is a promising treatment strategy for breast cancer patients with HER-2 positive tumors.

- Trastuzumab (Herceptin) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity to the extracellular domain of the HER-2 protein. It has been shown in vitro and in vivo to inhibit the proliferation of human tumor cells that overexpress HER-2.
Figure 1  Mechanism of HER-2 activation in the presence of trastuzumab.
Although trastuzumab has been heralded as a newfound hope for many breast cancer patients it is not without limitations…

Studies have shown that less than 35% of patients with HER-2–overexpressing metastatic breast cancer will respond to trastuzumab as a single agent.

Furthermore, a significant number of patients who achieve an initial response acquire resistance within 1 year.

in patients with HER-2–positive breast cancer, trastuzumab currently is approved as part of a regimen
Background

- VEGFR is a receptor tyrosine kinase, which plays an important role in promoting tumorigenesis by affecting not only the tumor vasculature, but the cancer cells as well.
- Recent data have indicated a translational up-regulation of the VEGF protein in HER-2–overexpressing breast cancer cells.
- Which suggested that this up-regulation may contribute to the aggressive phenotype.
Background

- VEGF produced by breast cancer cells promotes angiogenesis through a **paracrine effect** on tumor vascular endothelial cells.
- An **autocrine effect** that enables these tumor cells to promote their own growth.
- Blockade of VEGF and VEGFR signaling **can inhibit angiogenesis**, as well as **tumor cell proliferation** and **migration**.
Background

- it has been shown that the overexpression of both HER-2 and VEGFR is associated with **poorer prognosis** than overexpression of either protein alone in breast carcinoma patients

- these studies suggest that targeting both HER-2 and VEGFR may be an **effective therapeutic strategy**

- We chose to examine the antiproliferative and apoptotic effects of this novel reagent in combination VEGFR TKI with trastuzumab in human breast carcinoma cells **in vitro**
Materials and Methods
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- Three human breast carcinoma cell lines from the American Type Culture Collection (Manassas, VA) were studied
- 2 HER-2–overexpressing cell lines **BT474** and **SKBR3**
- BT474 and SKBR3 show a 25- and 30-fold increase, respectively, in HER-2 expression
- 1 HER-2–underexpressing cell lines **MCF-7**
Materials and Methods

- **Growth inhibition** was assessed after 5 days of drug treatment using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric (MTT) colorimetric assay.

- **Apoptosis**, was determined using a caspase 3 fluorometric assay kit, prepared from cells treated for 48 hours with drugs.
MTT colorimetric assay
MTT colorimetric assay

- The MTT assay are colorimetric assay for measuring the activity of enzymes that reduce MTT to formazen dyes giving a purple color.

- A main application allows to assess the viability (cell counting) and the proliferation of cells.

- It can also be used to determine cytotoxicity of potential medical agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth.
A microtiter after an MTT assay.

**Increasing amounts of cells** resulted in **increased purple colouring.**
Data analysis

- **For the MTT assays**, cells were plated in triplicate for each treatment group, and the average of 3 separate growth inhibition experiments was determined.

- **Results for the 4 treatment groups** (control, trastuzumab alone, VEGFR TKI alone, and the combination) were analyzed for statistical significance by one-way analysis of variance (*P* .05 was considered significant).

- **Drug interaction** was examined by the combination index (CI) method of median dose-effect analysis.

- The CI values were calculated at the 50% inhibition level for the BT474 and SKBR3 cell lines treated with trastuzumab and the VEGFR TKI at a fixed molar ratio.
Caspase 3 fluorometric assay
The purpose of this kit is to determine the increased enzymatic activity of the caspase-3 class of proteases in apoptotic cells by fluorometric reaction.

Caspase-3, also known as CPP-32, Yama or Apopain (6), is an intracellular cysteine protease that exists as a proenzyme, becoming activated during the cascade of events associated with apoptosis.
Caspase 3 fluorometric assay

- The cell lysate can be tested for protease activity by the addition of a caspase-specific peptide that is conjugated to the fluorescent reporter molecule 7-amino-4-trifluoromethyl coumarin (AFC).

- The cleavage of the peptide by the caspase releases the fluorochrome that, when excited by light at 400 nm wavelength, emits fluorescence at 505 nm.

- The level of caspase enzymatic activity in the cell lysate is directly proportional to the fluorescence signal detected with a fluorimeter or a fluorescent microplate reader.
Results
A

BT474 (HER-2+)

Proliferation (% of Control)

Trastuzumab (nM), VEGF-R TKI (µM)
- 0.125, 1.25
- 0.25, 2.5
- 0.5, 5
- 1.0, 10
- 2.0, 20

Trastuzumab VEGF-R-TKI Trastuzumab + VEGF-R TKI

B

SKBR3 (HER-2+)

Proliferation (% of C control)

Trastuzumab (nM), VEGF-R TKI (µM)
- 0.125, 1.25
- 0.25, 2.5
- 0.5, 5
- 1.0, 10
- 2.0, 20

Trastuzumab VEGF-R-TKI Trastuzumab + VEGF-R TKI

C

MCF7 (HER-2low)

Proliferation (% of C control)

Trastuzumab (nM), VEGF-R TKI (µM)
- 0.125, 1.25
- 0.25, 2.5
- 0.5, 5
- 1.0, 10
- 2.0, 20

Trastuzumab VEGF-R-TKI Trastuzumab + VEGF-R TKI
Results

- To assess the potential inhibitory effects of trastuzumab and/or the VEGFR TKI, the growth inhibition of cell lines differing in HER-2 expression was tested.

- **Both HER-2– overexpressing cell lines**, BT474 and SKBR3 showed a **dose-dependent inhibition** with trastuzumab or the VEGFR TKI after 5 days of treatment.

- **the combination** of trastuzumab and the VEGFR TKI significantly enhanced growth inhibition at all doses compared with either agent alone (\(P < .001\)).
Results

- **in the MCF-7** the VEGFR TKI also reduced cellular proliferation.
- Little effect was seen with trastuzumab in the low HER-2–expressing MCF-7 breast cancer cell line.
- No increase in growth inhibition was seen with the **combination** of drugs compared with VEGFR TKI inhibition alone.
- The CI values for the combination of trastuzumab and VEGF TKI indicated a **synergistic interaction**.
- At the 50% inhibition level the CI was .62 for the **BT474** cells and .87 for the **SKBR3** cells.
Figure 3  Caspase 3 activation as an indicator of apoptosis in the 3 breast carcinoma cell lines after 48 hours of drug treatment. ■, Control; □, trastuzumab; ▪, VEGF-R TKI; □, trastuzumab + VEGF-R TKI.
Results

- **Trastuzumab**, at the highest dose tested (2 nmol/L) did not induce apoptosis in any of the cell lines examined.
- **BT474** cells showed the highest level of caspase 3 activity (15 U/mg) after treatment with VEGFR TKI (20 mol/L)
- but no difference compared with untreated controls was observed for **SKBR3** or **MCF-7** at this time point
- However, the combination of drugs resulted in a 10.9- and 1.9-fold increase in caspase 3 activity in **BT474** and **SKBR3**, respectively.
This study shows that the combination of trastuzumab and a specific VEGFR TKI can significantly decrease proliferation and increase apoptosis in HER-2–overexpressing breast cancer cell lines.

These findings strongly suggest that simultaneous blockade of different growth factor–driven signal transduction pathways might result in a more significant antitumor effect.
Some men hate it. They make it their enemy. Better to treat it like a friend, make thyself like it. Don't mind because it is hard.

If you thinks about what a good house you build, then who cares if the beams are heavy and it is far from the well to carry the water for the plaster.

THANK YOU FOR YOUR ATTENTION
HAVE YOU ANY QUESTION ? ? ?