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

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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**

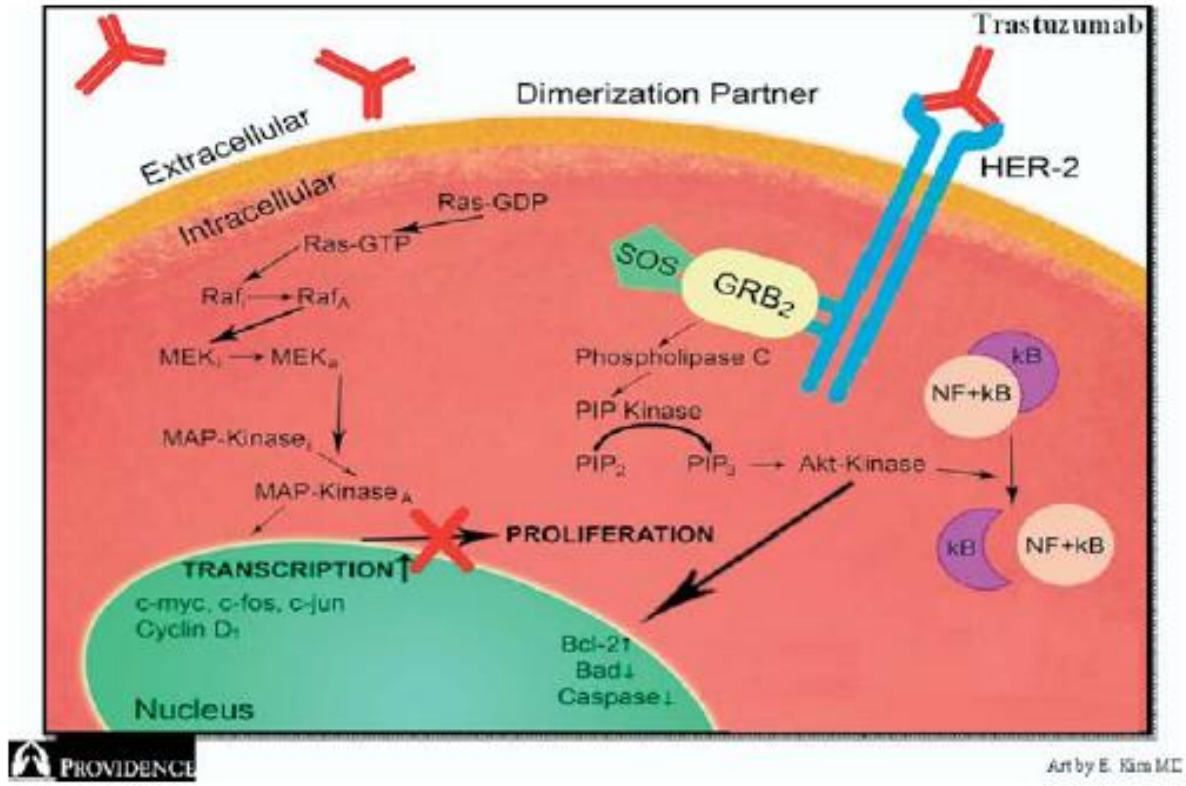

Figure 1 Mechanism of HER-2 activation in the presence of trastuzumab.

Background

- 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**

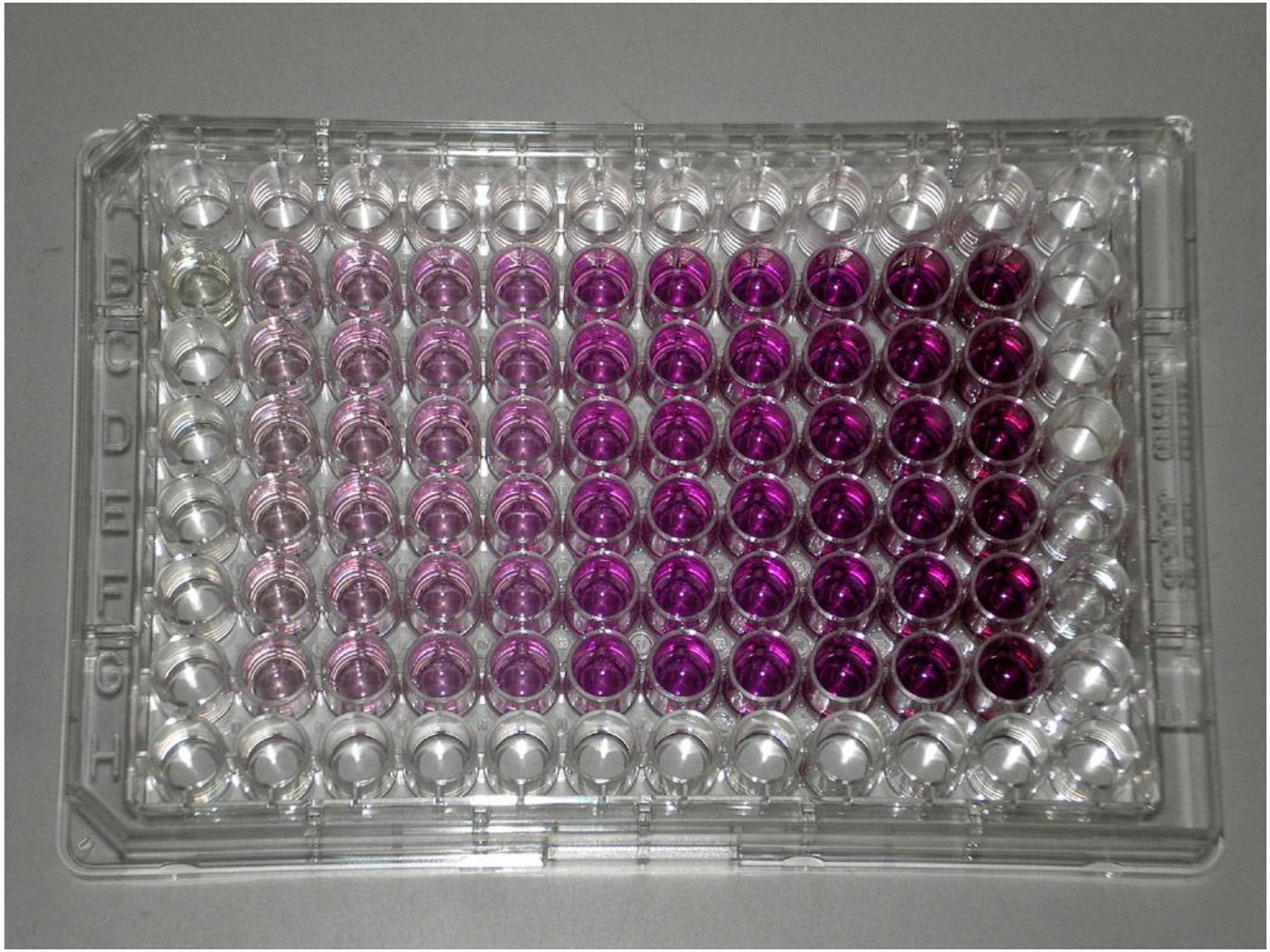
Materials and Methods

- **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.



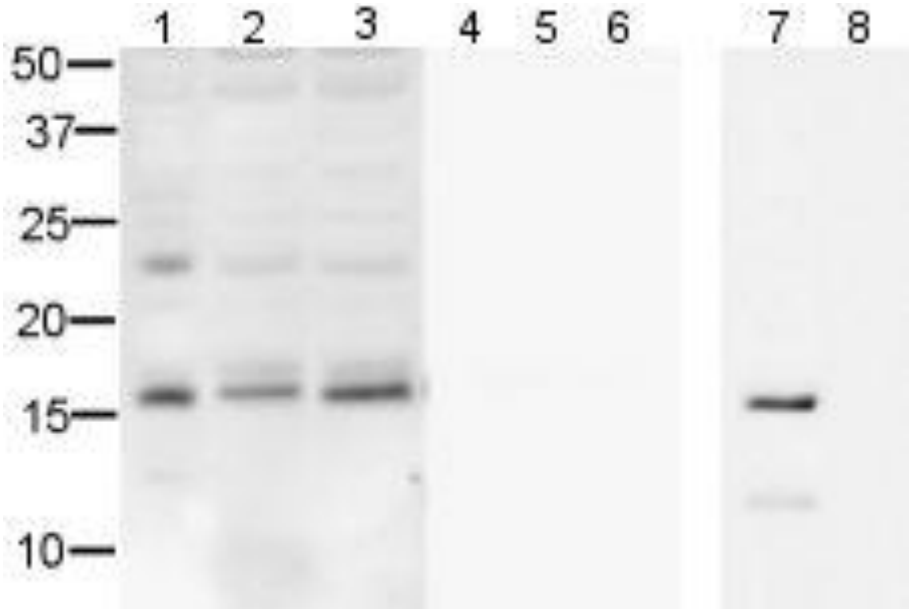
A microtiter after an MTT assay.

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



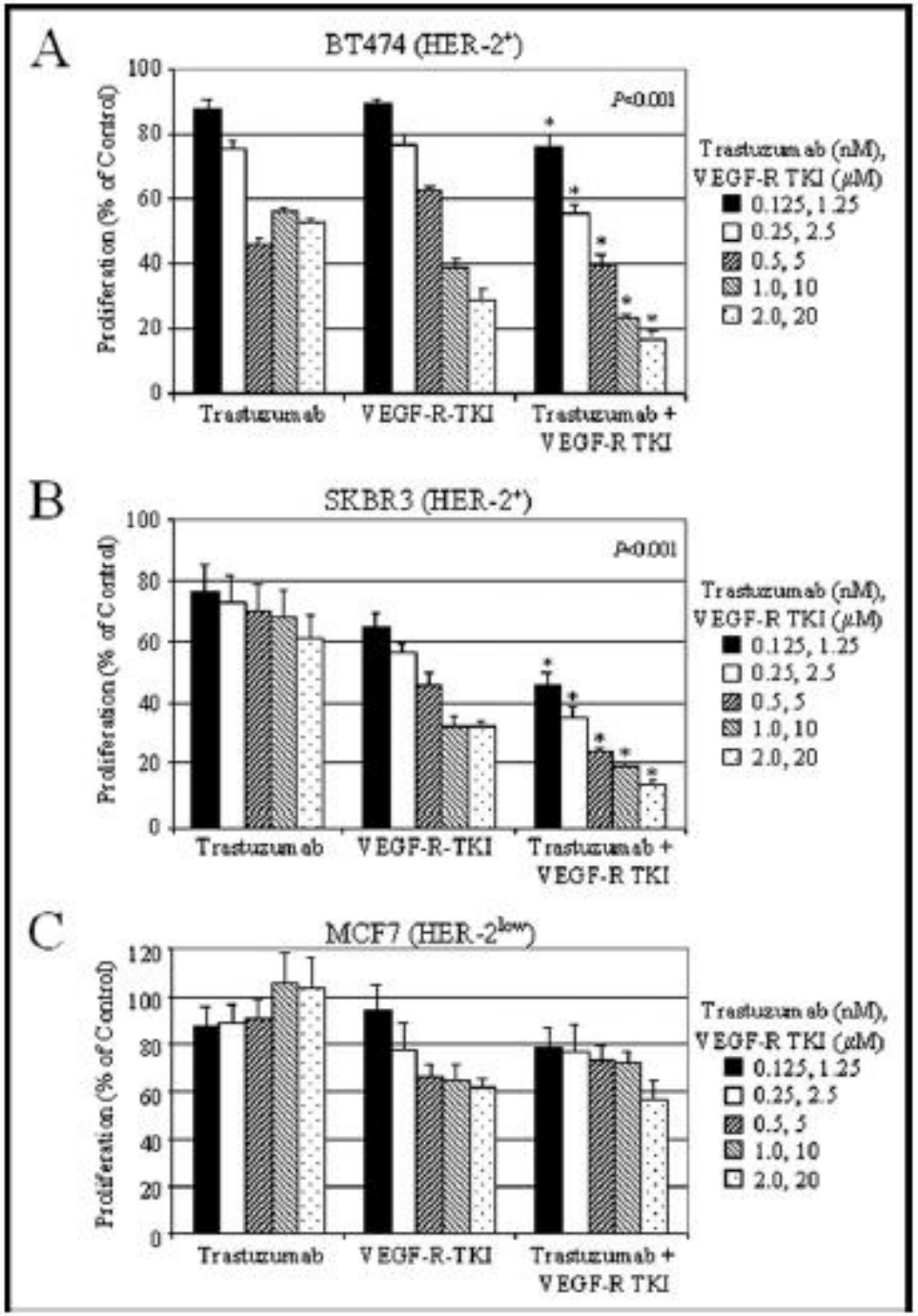
Caspase 3 fluorometric assay

- **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+)
P<0.001
Proliferation (% of Control)
100
80
60
40
20
0
Trastuzumab
VEGF-R-TKI
Trastuzumab + VEGF-R TKI
Trastuzumab (nM), VEGF-R TKI (μM)
0.125, 1.25
0.25, 2.5
0.5, 5
1.0, 10
2.0, 20
B
SKBR3 (HER-2+)
P<0.001
Proliferation (% of Control)
100
80
60
40
20
0
Trastuzumab
VEGF-R-TKI
Trastuzumab + VEGF-R TKI
Trastuzumab (nM), VEGF-R TKI (μM)
0.125, 1.25
0.25, 2.5
0.5, 5
1.0, 10
2.0, 20
C
MCF7 (HER-2low)
Proliferation (% of Control)
120
100
80
60
40
20
0
Trastuzumab
VEGF-R-TKI
Trastuzumab + VEGF-R TKI
Trastuzumab (nM), VEGF-R TKI (μM)
0.125, 1.25
0.25, 2.5
0.5, 5
1.0, 10
2.0, 20

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-dependen 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

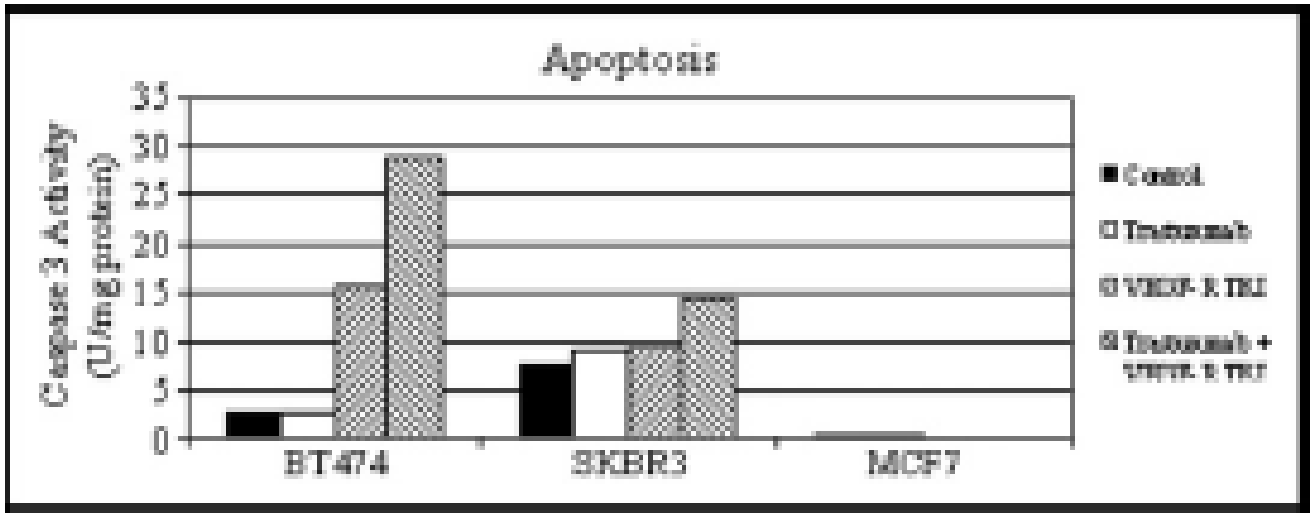


Figure 3 Caspase 3 activation as an indicator of apoptosis in the 3 breast carcinoma cell lines after 48 hours of drug treatment. ■, Control; □, trastuzumab; ▩, VEGF-R TKI; ▨, trastuzumab + VEGF-R TKI.

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.

Comment

comment

- 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.**

THANK YOU FOR YOUR ATTENTION

HAVE YOU ANY QUESTION ? ? ?

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