

Breast cancer patients with very high tumor HER2 expression levels might not benefit from treatment with trastuzumab plus chemotherapy: A retrospective exploratory analysis of the FinHer trial.

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BACKGROUND

FinHer is one of the several prospective randomized clinical trials that show a clinical benefit from trastuzumab added to adjuvant chemotherapy. We have previously reported that patients with metastatic breast cancer who had very high levels of HER2 protein expression as measured by the HERmark assay and who were treated with trastuzumab have similar time-to-progression (TPP) compared to a group of patients that was FISH-negative and HER2 normal, suggesting reduced efficacy of trastuzumab when tumor HER2 content is very high (Sperinde, ASCO 2009¹). Here we investigate the relationship between clinical benefit from trastuzumab and quantitative HER2 protein expression (H2T) as determined by the HERmark assay (Fig. 1).

METHODS

FFPE Tissue Samples

Formalin-fixed, paraffin-embedded (FFPE) tissue from 899 invasive breast cancer cases of the FinHer study that had adequate invasive tumor tissues for the HERmark assay were included (Fig. 2). 196 of these were HER2-positive by CISH. Clinical data were provided by the FinHer study (Joensuu et al. *N Engl J Med* 2006, *J Clin Oncol* 2009).^{2,4} In this study patients with HER2-positive cancer (n=232) were randomly assigned to receive either trastuzumab administered concomitantly with chemotherapy for 9 weeks or to chemotherapy alone. The median FU time after randomization was 62 months. Women assigned to trastuzumab plus chemotherapy tended to have more favorable distant disease free-survival (Fig. 3).⁴

HERmark Assay: Novel Proximity Based Technology

H2T was detected through the release of a fluorescent tag ("VeraTag reporter") conjugated to a monoclonal antibody directed against the cytoplasmic domain of HER2 (Ab8, LabVision). For the H2T assay, this antibody is paired with a biotinylated second antibody directed against the C-terminus of HER2 (Ab15, LabVision). The "molecular scissors" (streptavidin-conjugated methylene blue) that is subsequently added and bound to the biotinylated antibody liberates singlet O₂ upon irradiation with red light. The release of VeraTag reporter molecules (Pro11, Fig. 1) requires proximity of the VeraTag antibody to a second HER2 "scissors" antibody (proximity based assay). Signal quantified by capillary electrophoresis is normalized to tumor area on the FFPE tissue section.

Statistical analysis

In this study we focused on the patients with HER2-positive cancer who were randomized to trastuzumab treatment or control. Cox proportional hazards analyses, sub-population treatment effect pattern plots (STEP analyses), positional scanning analyses, and Kaplan-Meier analyses were used to identify sub-populations of HER2 over-expressing patients who experienced different clinical outcomes on trastuzumab. Time to distant recurrence (TDR) and overall survival (OS) were used as endpoints.

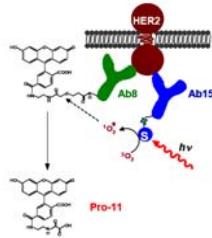
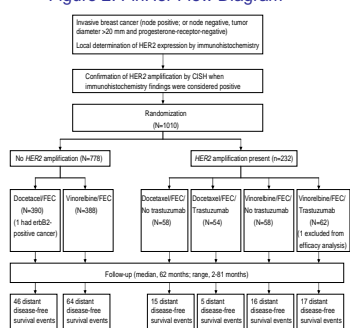


Figure 1. The principle of HERmark assay – novel proximity based technology

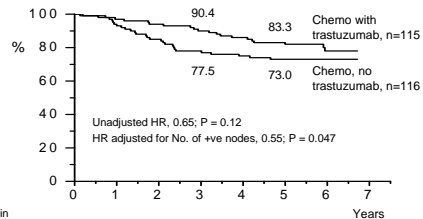
A monoclonal antibody specific for a unique epitope of HER-2 (Ab8) is conjugated to a fluorescent VeraTag reporter (Pro11) or a molecular scissors (S) by means of a cleavable tether (Ab15). The molecular scissors liberates singlet O₂ upon irradiation with red light. The free radicals cleave all thioether bonds in close proximity (within approximately 30-100 nM), releasing the "VeraTag reporter." The signal (Pro11) can then be collected and analyzed on a capillary electrophoresis (CE) array. Each VeraTag reporter is designed with a unique charge-mass ratio and can thus be identified and quantified by comparison to assay standards. The standard unit of VeraTag measurement from tumor samples is relative peak area (RPA) x collection volume (uL)/ tumor area (mm²).

Figure 2. FinHer Flow Diagram



Adapted from Joensuu H et al., *J Clin Oncol* 2009.⁴

Figure 3. FinHer Final Analysis: Distant Disease-free survival*



*Joensuu H et al. *J Clin Oncol* 2009

Figure 5A. Influence of adjuvant trastuzumab on TDR when total HER2 expression is not very high (<2.1)

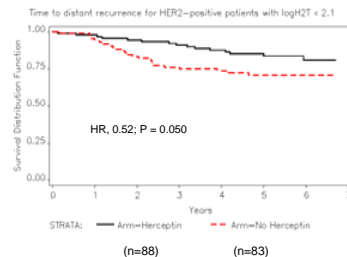
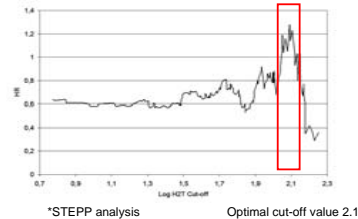
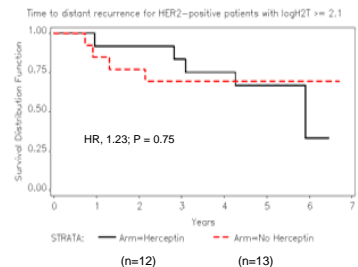


Figure 4. Time to distant recurrence Hazard Ratio (HR) for trastuzumab vs. no trastuzumab*



*STePP analysis Optimal cut-off value 2.1

Figure 5 B. Influence of adjuvant trastuzumab on TDR when total HER2 is very high (>=2.1)



RESULTS and DISCUSSION

• Cox proportional hazards analyses treating H2T as a continuous variable did not show a relationship between HER2 expression levels and clinical benefit from trastuzumab (HR=1, P = ns).

•STePP analyses were performed to look for non-linear relationships between H2T and clinical outcome. At the highest levels of H2T, the hazard ratio comparing trastuzumab treatment to control approached and exceeded 1 (Fig. 4).

•Positional scanning analyses were conducted to identify the optimal cutoff discriminating the very high H2T group. Patients with very high H2T values (log H2T >=2.1; >=125.9 HERmark units) did not benefit from trastuzumab plus chemotherapy treatment relative to controls (HR=1.23, P = 0.75 for TDR, HR=1.05, P = 0.95 for OS), while those with H2T values <125.9 did benefit (HR=0.52, P = 0.05 for TDR, HR=0.48, P=0.1 for OS; Fig. 5).

•The very high H2T group represented 13% of the HER2-positive population compared with 16% in the prior study from Sperinde et al.¹

CONCLUSIONS

In this trial, the 13% of patients with the highest H2T values showed no evidence of clinical benefit from adding trastuzumab to chemotherapy. Potential explanations include a) insufficient trastuzumab dose, b) steric hindrance preventing access of trastuzumab to its epitope target under conditions of HER2 over-crowding, or c) the existence of trastuzumab-resistant forms of HER2 at the highest levels of over-expression (eg. p95/HER2, HER2:HER3 heterodimers).

These results are in agreement with prior observations from the metastatic setting, and will be tested in larger randomized trials of trastuzumab in early breast cancer.

References:

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- Isola J et al. *Clin Cancer Res* 2004;10:4793-8
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- Joensuu H et al. *J Clin Oncol* 2009 Nov 2 [Epub ahead of print]