Quantitative HER family proteins assessment as prognostic and predictive biomarkers in the EGF30008 clinical trial

Background
Combined targeted strategy with letrozole and lapatinib improves progression-free survival (PFS) in patients with metastatic breast cancer (MBC) expressing HER2 and presents a potential role for human epidermal growth factor receptor positive (HER2+) but not in HER2+/HER2-negative (HER2) disease (Johnston et al., 2009). However, among HER2+ tumors, a broad dynamic range of quantitative levels of HER2 are observed, corresponding to 163.7 to 1744.6 amol/μg as previously reported (Nufloco et al., 2020). In addition, within HER2- tumors, quantitative measurement of HER family proteins may identify patients most likely to benefit from the addition of lapatinib to letrozole. In this retrospective study, we tested the prognostic and predictive ability of HER protein quantification in clinically HER2+ tumor samples from the EGF30008 study.

Objectives
- To quantify HER2 and related family receptors (EGFR and HER3) in clinically HER2+ tumor samples using Selected Reaction Monitoring (SRM) mass spectrometry.
- To determine quantitative levels of HER2 protein according to molecular subtype by PAM50.
- To correlate HER2 protein levels by SRM with ERα and ERBB2 gene expression levels by qPCR.
- To determine association between HER2 protein levels by SRM and outcome independent of treatment (predictive effect).
- To determine association between HER2 protein levels by SRM and lapatinib benefit (predictive effect).

Methods
Formalin-fixed paraffin-embedded (FFPE) tumor tissues sections from HER2+ MBC population were used. HER2 positivity was assessed previously by standard immunohistochemistry (IHC) analysis. Fluorescence in situ hybridization (FISH) after laser microdissection, tissue lysates were prepared for selected reaction monitoring mass spectrometry (SRM) analysis. Absolute quantification was accomplished through simultaneous detection of endogenous target and synthetic labeled heavy peptide identical to analytical targets (EGFR, HER2, HER3). HER2 protein levels were correlated with PAM50 molecular subtypes, ERBB2 and ERα genes by qPCR. PFS and overall survival (OS) were analyzed by Kaplan-Meier and log-rank test. Cox proportional hazard models for PFS and OS was to generate point estimates of hazard ratios and corresponding 95% confidence intervals.

Results
Within the HER2+ study cohort (n=219), 107 had an available tumor block; 84 cases had sufficient material for HER expression measurement by SRM.

HER2 levels were lower in Letrozole + Lapatinib (H, n=45); mean, 1761 amol/μg compared to Letrozole + Placebo (I; F, n=41; mean, 2086 amol/μg) arms, although the difference was not significant (p=0.106). No expression of EGFR and HER3 was observed.

HER2 protein levels were significantly different among PAM50 subtypes with HER2-enriched (HER2+) tumors showing the highest expression followed by Basal-like, Luminal A, Luminal B, and Normal-like (p<0.001).

Conclusions
A subgroup analysis showed that the addition of Lapatinib to Letrozole improved PFS in patients with low HER2 levels (11.2 vs 2.2 months, log-rank p=0.034) and those with high HER2 levels (4.9 vs 2.8 months, log-rank p=0.29).

The clinicopathological characteristics of the SRM study population were similar to those of the original EGF30008 HER2+ population.

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