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

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Background

Combined targeted strategy with letrozole and lapatinib improves progression-free survival (PFS) in patients with metastatic breast cancer (MBC) co-expressing hormone receptor-positive (HR+) and human epidermal growth factor receptor positive (HER2+) but not in HR+/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 17446.7 amol/µg as previously reported (Nuciforo et al, 2015). 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 proteins quantification in clinically HER2+ tumor samples from the EGF30008 study.

Objectives

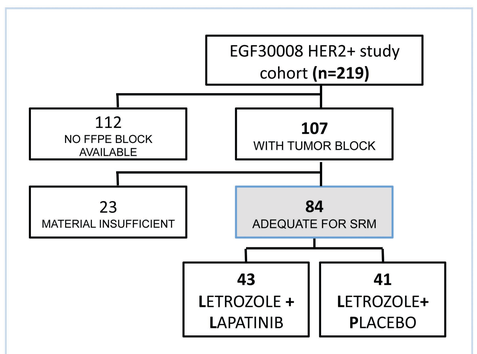
- To quantify HER2 and related family receptors (EGFR and HER3) in clinically HER2+ tumor samples using Selected Reaction Monitoring (SRM) mass spectrometry.
- To determine quantitative levels of HER2 protein according to molecular subtype by PAM50.
- To correlate HER2 protein levels by SRM with *ESR1* and *ERBB2* gene expression levels by nCounter.
- To determine association between HER2 protein levels by SRM and outcome independent of treatment (prognostic 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) and/or Fluorescence in situ hybridization (FISH). After laser microdissection, tissue lysates were prepared for selected reaction monitoring mass spectrometry (SRM) analysis. Absolute quantitation 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 *ESR1* genes by nCounter. PFS and overall survival (OS) were analyzed by Kaplan–Meier and log-rank test. Cox proportional hazard models for PFS and OS was used 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.

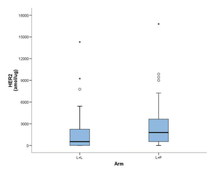


The clinicopathological characteristics of the SRM study population were similar to those of the original EGF30008 HER2+ population.

	HER2+ SRM population		HER2+ Original population		
	N	%	N	%	
N	84		219		
Age (median, range)	59 (45-87)		60 (44-87)		
PS					*p-value*
0	41	48.81	110	50.46	*0.898*
≥1	43	51.19	108	49.54	
Nº metastatic sites					
<3	49	58.33	130	59.36	*0.912*
≥3	35	41.67	89	40.64	
Prior adjuvant therapy					
<6 months	33	39.29	79	36.07	*0.7*
≥6 months	51	60.71	140	63.93	
Visceral disease					
Visceral	71	84.52	185	84.47	*0.989*
Bone	13	15.48	34	15.53	
Treatment					
L+P	41	48.81	108	49.32	*0.989*
L+L	43	51.19	111	50.68	

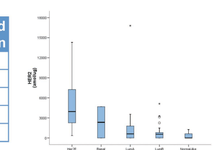
HER2 levels were lower in Letrozole + Lapatinib (L+L, n=43; mean, 1761 amol/µg) compared to Letrozole + Placebo (L+P, n=41; mean, 2908 amol/µg) arms, although the difference was non-significant (p=0.106). No expression of EGFR and HER3 was observed.

HER2 (amol/µg)	ALL	L+L	L+P
N	84	43	41
Mean	2321,1	1761,0	2908,6
Median	817,6	533,1	1790,3
Standard deviation	3246,8	2905,8	3509,7
Min	0,0	0,0	0,0
Max	16795,0	14301,7	16795,0

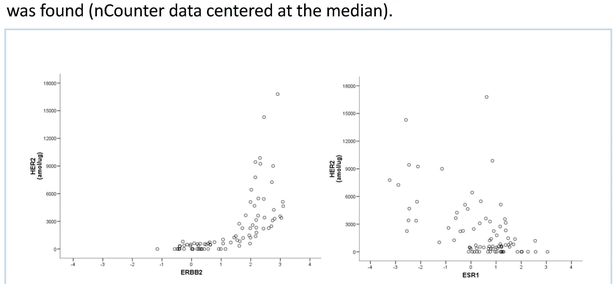


HER2 protein levels were significantly different among PAM50 subtypes with HER2-enriched (HER2E) tumors showing the highest expression followed by Basal-like, Luminal A, Luminal B, and Normal-like (p<0.001).

Subtype	n	Mean	Median	Min	Max	Standard deviation
Her2E	26	4869,6	3948,5	356,1	14301,7	3465,7
Basal	2	2340,5	2340,5	0,0	4681,0	3310,0
LumA	22	1652,9	609,5	0,0	16795,0	3551,8
LumB	30	869,0	558,8	0,0	5117,7	1168,2
Normal-like	4	312,9	0,0	0,0	1251,5	625,8

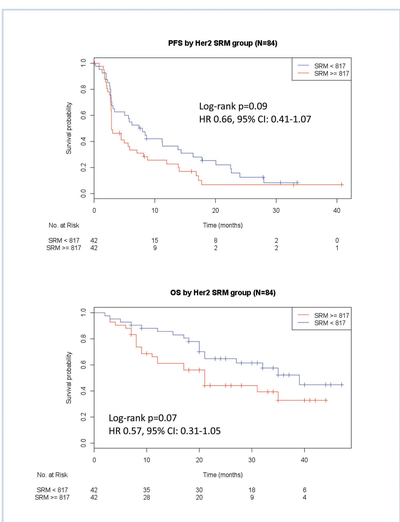


A correlation between HER2 protein by SRM, *ERBB2* (Spearman rho=0.86, p<0.001) and *ESR1* (Spearman rho= -0.48, p=0.001) gene expression by nCounter was found (nCounter data centered at the median).



Survival analysis

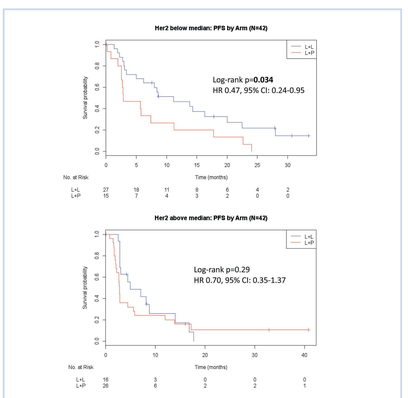
In metastatic breast cancer patients that expresses HER2 protein levels above the median (817 amol/µg), trends towards worse PFS (2.9 vs 7.7 months, log-rank p=0.092) and OS (21 vs 39 months, log-rank p=0.071) were observed



Cox Model PFS analysis indicated that HER2 by SRM was an independent prognostic factor together with treatment arm, number of metastases and performance status. HER2 by SRM was not an independent factor in the Cox model for OS.

	UNIVARIATE			MULTIVARIATE		
	P-value	HR	95% CI	P-values	HR	95% CI
Age	0,774	0,996	0,968 1,024	0,153	0,980	0,952 1,008
mets <3 vs. ≥3	**0,020**	0,569	0,354 0,914	**0,002**	0,417	0,240 0,724
PS ≥1 vs. 0	**0,039**	1,646	1,025 2,645	**0,023**	1,867	1,089 3,199
No Visceral vs. Visceral	0,368	0,712	0,341 1,490	0,586	0,791	0,339 1,843
Prior endocrine therapy <6 vs. ≥6	0,721	1,093	0,672 1,777	0,390	1,260	0,744 2,131
Lapatinib vs. Placebo	**0,024**	0,580	0,362 0,929	**0,009**	0,493	0,291 0,836
HER2 SRM low vs. high	0,095	0,667	0,414 1,074	**0,030**	0,547	0,317 0,944

A subgroup analysis showed that the addition of Lapatinib to Letrozole improved PFS in patients with low HER2 levels (11.2 vs 2.9 months, log-rank p=0.034) compared to those with high HER2 (4.9 vs 2.8 months, log-rank p=0.29).



Conclusions

- **Levels of HER2 protein in HER2+ MBC were extremely heterogeneous ranging from ND (not detectable) to 16795 amol/µg.**
- **HER2 protein levels were significantly different among PAM50 subtypes with HER2E tumors showing the highest levels.**
- **HER2 protein by SRM was positively correlated with ERBB2 and negatively correlated with ESR1 genes expression by nCounter.**
- **HER2 protein by SRM was an independent prognostic factor of PFS in HER2+ MBC.**
- **Surprisingly, patients with high HER2 protein levels did not seem to benefit from lapatinib. Further validation of this finding is warranted.**

Bibliography

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Lapatinib (Tykerb/Tyverb) is an asset of Novartis Pharma AG as of March 02, 2015