HER2 Quantification By Targeted Proteomics Compared To IHC Or ISH In Predicting Clinical Benefit From Anti-HER2 Therapy In HER2-positive Breast Cancer

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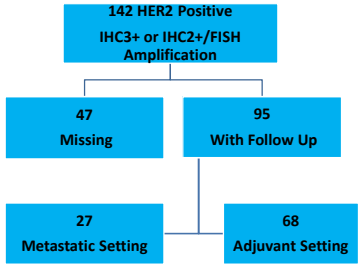
Background

HER2 testing by IHC or ISH is a routine practice to classify the ~20% of breast cancer patients who benefit from anti-HER2 therapy. However, these diagnostic tests are subjective, and a significant number of patients are wrongly classified as HER2 positive (~ 5-10%). Liquid Tissue-Selected reaction monitoring (LT-SRM) a mass spectrometry based targeted proteomics technique is an objective method that is antibody independent and can concurrently quantitate HER2 and other oncogenic proteins from formalin fixed paraffin embedded (FFPE) samples. We compared HER2 SRM with ISH to predict survival benefit after anti-HER2 therapy.

Anti-HER2 treated study population

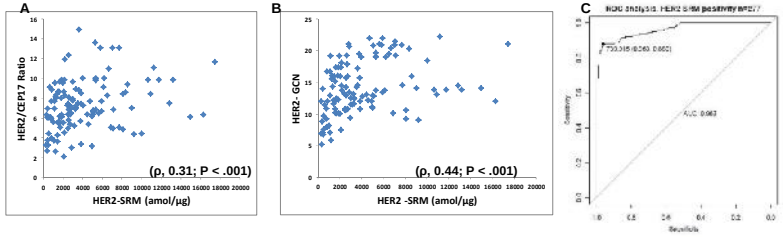


Targeted proteomics workflow

Tumor cell identification | Laser Micro-dissection | Liquid Tissue® solubilizes protein | Mass spec analysis with internal standards | Analysis and reporting

Protein quantitation by mass spectrometry is achieved using Selected Reaction Monitoring (SRM)

Correlation of targeted proteomics and HER2/CEP17 or GCN

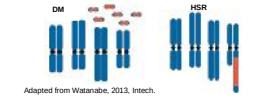


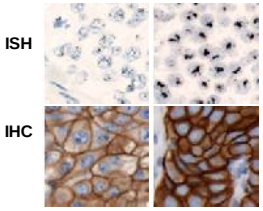
Correlation Between HER2 SRM and FISH Ratio

		HER2 FISH			
		NO AMPL	AMPL	total	pvalue
HER2 amol/µg	low (<740)	53	10	63	<0.001
	high (>740)	5	105	110	
	total	58	115	173	

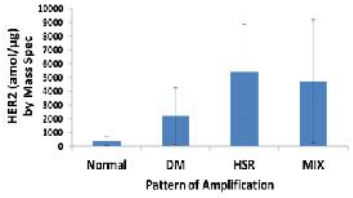
Correlation of HER2 protein expression: (A) SRM-MS and HER2/CEP17 ratio (ρ, 0.31; P < .001) **(B)** SRM and HER2 GCN (ρ, 0.44; P < .001). **(C)** ROC analysis of 277 samples using IHC and/or ISH (Nuciforo et al. SABCS 2014) revealed a HER2 SRM threshold of 740amol/µg for HER2 expression concordant with amplification.

Targeted Proteomics identifies amplification-pattern caused protein expression differences



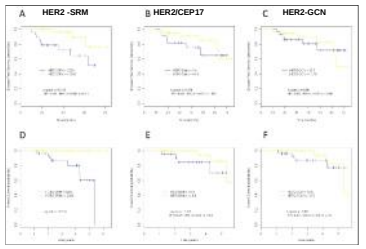


Amplified chromosome regions are present as mini extra chromosome (double minutes- DM) or as repetitive region with in a chromosome (homogenously staining region- HSR)



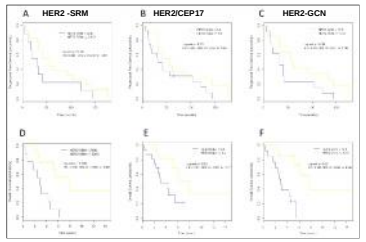
Protein matters: Nearly 38% of the samples were classified as DM by FISH, of which 17% had low levels of HER2 protein (<740 amol/µg), compared with 0% in HSR or 7% in Mix samples. Patients with low levels of HER2 protein may respond less to anti-HER2 therapy

Survival analyses



Adjuvant setting

Kaplan-Meier curves for disease free survival (A-C) and overall survival (D-F) according to HER2 protein expression by SRM (A,D), HER2/CEP17 ratio (B,E) and HER2 gene copy number (GCN) (C,F) in patients treated with anti-HER2 in the adjuvant setting. DFS and OS were superior for patients with high HER2 protein levels (>2200 amol/µg).



Metastatic setting

Kaplan-Meier curves for progression free survival (A-C) and overall survival (D-F) according to HER2 protein expression by SRM (A,D), HER2/CEP17 ratio (B,E) and HER2 GCN (C,F) in patients treated with anti-HER2 in the metastatic setting. OS was superior for patients with high HER2 protein levels (>2200 amol/µg), high HER2 GCN and HER2/CEP17 ratio.

Conclusions

- **HER2 protein levels by SRM correlate with HER2/CEP17 and HER2 GCN determined by FISH.**
- **HER2 protein expression is not only associated with amplification status but also the pattern of amplifcation.**
- **The double minutes (DM) amplification pattern results in lower levels of HER2 protein, which may lower response to anti-HER2 therapy.**
- **High HER2 protein levels (>2200 amol/µg) predict DFS (HR= 0.22; p=0.013) and OS (HR =NA; p=0.001) benefit with HER2 targeted therapy in the adjuvant setting.**
- **Patients who highly express HER2 protein (>2200 amol/µg) also have significant OS benefit (HR=0.20 ; p<0.001) from HER2 targeted therapy in the metastatic setting.**
- **Neither HER2 GCN, HER2/Chr17 ratio nor pattern of amplification correlated with outcome in the adjuvant setting though GCN predicted OS in the mestastic setting.**
- **Upfront multiplex SRM testing of HER2 and other co-expressed targets in tumor biopsies can reveal different biomarkers and can support physicians in making informed treatment decisions.**

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