Quantitative Mass Spectrometry of HER2 Protein Levels Reveals High Variability within HER2 IHC Grades

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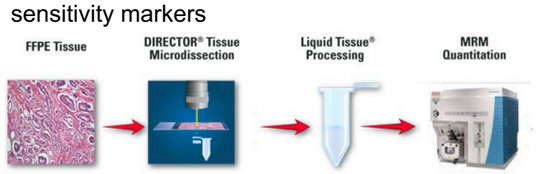
Introduction

- Recently, new HER2-targeting antibody drug conjugates (ADCs) have showed anti-tumor activity, not only in patients with HER2 over-expressing (IHC3+/2+ISH+) breast cancer (BC) but also in HER2 low (IHC1+/2+ ISH neg) expressing tumors in whom to date, there are no effective anti-HER2 therapies indicated (1)
- We propose to evaluate more sensitive and quantitative HER2 expression platforms to identify additional patients, who may benefit from HER2 ADC treatment, such as trastuzumab deruxtecan (DS-8201). DS-8201 is an antibody-drug conjugate consisting of an anti-HER2 antibody, a cleavable tetrapeptide-based linker, and a cytotoxic topoisomerase I (TOPO-1) inhibitor. DS-8201 showed activity in HER2 low tumors.
- Due to the unique mechanism of ADCs, we should investigate not only the presence of the antibody targets, but also markers of response and resistance to the payload (e.g. TOPO-1)

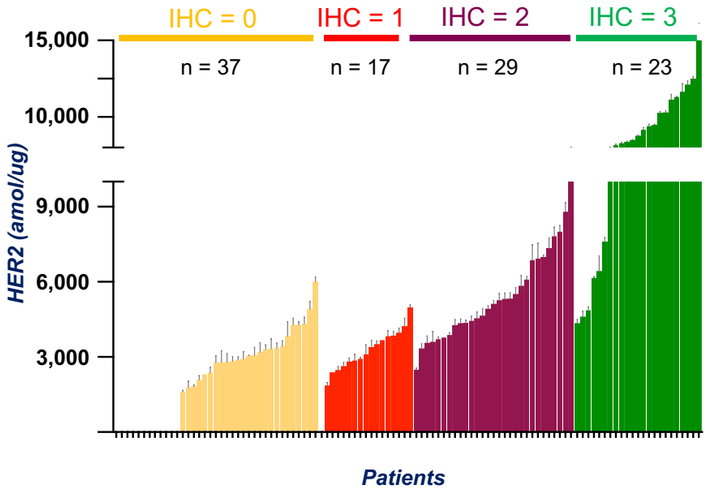
Methods

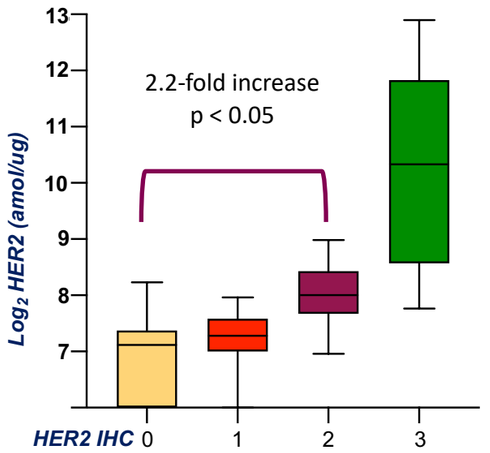
- We have analyzed 107 BC acquired from a vendor (Proteogenix) for ERBB2 RNA and protein expression using, respectively QRT-PCR (Fluidigm) and SRM-MS (mProbe) and compared between patients with HER2 expression levels of IHC 0 (34.6%), 1+ (15.9%), 2+ (27.1 %) or 3+ (22.4%) (ANOVA).
- Fluidigm multi-omic 14 gene expression: ERBB1/ERBB2/ERBB3/ERBB4, SLFN11, Top1, Top2A, ki67, ABCG2, ABCB1, GRB7 FGFR4, TMEM45B, and GPR160.
- Multiplexed Quantitative Mass Spectrometry (MS) has a 72 biomarkers panel that comprises of ErbB family members, drug efflux and other CTX sensitivity markers

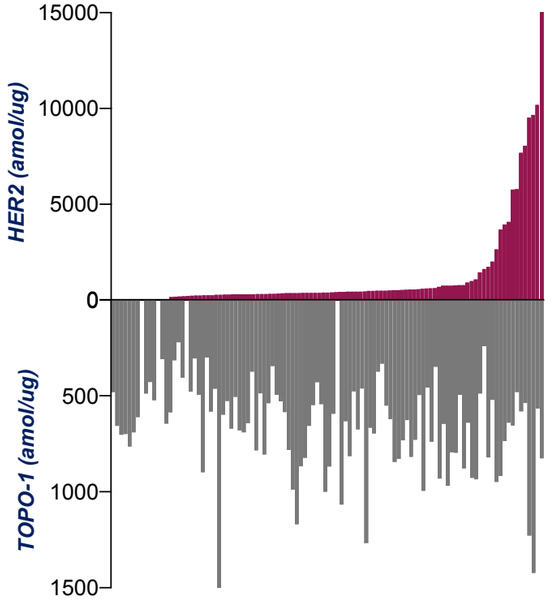


Results

Multiplexed Quantitative MS Allows Parallel Analysis of ErbB family and markers known to sensitize response to payload

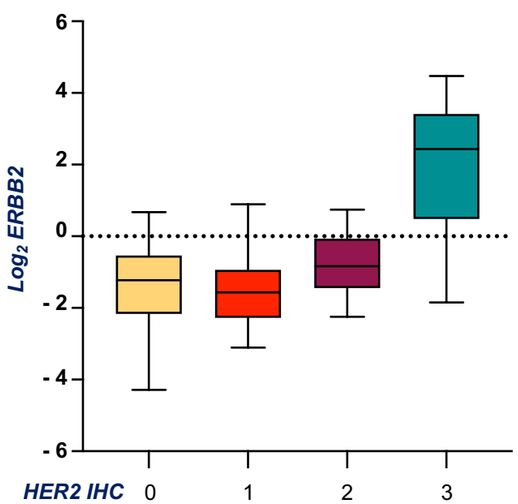


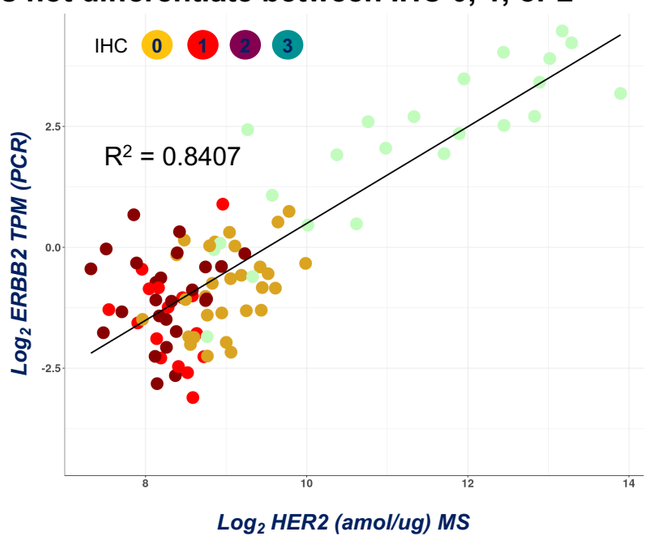




HER2 concentration progressively increased according to HER2 IHC grouping (i.e. lowest concentration in HER2 0 samples, highest in HER2 3+ samples). 73% of samples scored as IHC 0 had detectable HER2 by MS (from 168 to 623 amol/µg) (LEFT). HER2 levels were elevated in 2+ vs. 0 ($p < 0.05$) There is no significant difference in 2+ vs 1+ or 1+ vs 0 (MIDDLE). Additionally multiplexing allows to contextualize HER2 with the payload target expression (e.g. TOPO-1) (RIGHT).

ERBB2 RNA quantification does not differentiate between IHC 0, 1, or 2+





PCR-based method was not sensitive enough to differentiate HER2 Low expressors

Conclusions

- We used an objective multiplex non-antibody-based method to quantify targets from FFPE
- Targeted MS revealed a 100-fold difference in HER2 expression between the HER2 IHC 0 versus IHC 3+. Moreover, it identified patients with detectable HER2 level that were scored IHC 0
- There is variable expression of TOPO-1 and it is independent of HER2 levels
- Multiplexed quantitative proteomics identified both antibody targets (e.g. HER2) and markers of resistance or response to the payload (e.g. TOPO-1) for approved and investigational ADC therapies.

References

1. NCCN Clinical Practical Guidelines in Oncology: Breast Cancer, Version 2.2019

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