**Molecular characterization of HER2 low breast cancers reveals a biologically and clinically distinct entity with heterozygous loss of ERBB2**

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**Purpose**

Breast cancer stratification heavily relies on HER2 status, primarily assessed through immunohistochemistry (IHC) to identify HER2 positive BCs (HER2 3+/ERBB2 amplified). The recent approval of trastuzumab deruxtecan (T-DXd) has instigated a paradigm shift, prompting an evaluation of HER2 levels in HER2 negative (non-amplified) BCs, culminating in the recognition of a new BC category within the HER2 negative category termed HER2 low (IHC 1+ and 2+) versus HER2 non-expressing (IHC 0). To better understand the biological significance of ERBB2 expression levels in HER2 negative BCs through a more quantitative method, we now investigated the impact of ERBB2 mRNA levels on the genetic landscape and transcriptome.

**Materials and Methods**

The study's extensive scope included the analysis of multiple datasets, encompassing Molecular Taxonomy of BC International Consortium (METABRIC, N=2509), The Cancer Genome Atlas (TCGA, N=1097), a new unpublished Dana Farber Cancer Institute breast cancer cohort of primary BC(N=971), the DFCI metastatic BC cohort(N=1063) and MSK met tropism cohort (N=1018). We interrogated the genomic, global transcriptomic and clinical features of primary HER2- BCs expressing higher (enhanced) versus lower (minimal) levels of ERBB2 mRNA.

**Results**

All HER2- (clinical definition) tumors had some degree of ERBB2 expression based on the mRNA level. In METABRIC (microarray), TCGA (RNA-seq) and a new unpublished DFCI BC primary (RT-qPCR) cohort the distribution of logged ERBB2 mRNA levels resembles a normal distribution in HER2- tumors. By categorizing HER2- BCs into ERBB2 mRNA minimal, moderate, and enhanced subgroups, we further investigate variations in mutational landscapes and transcriptional profiles. ERBB2-mRNA enhanced tumors exhibited a higher prevalence of *PIK3CA* mutations and estrogen receptor signaling. Conversely, ERBB2-mRNA minimal tumors had increased expression of proliferation and immune related genes.

Importantly, we identified a biologically distinct subgroup of BCs defined by chromosome 17q12 deletion with heterozygous loss of *ERBB2.* These BCs have very low ERBB2-mRNA and HER2 protein levels, and frequent heterozygous losses of TP53. Importantly, we show in two independent cohorts of patients with HR+/HER2- metastatic BC, patients with 17q12del metastatic disease have decreased overall survival. In the DFCI cohort of ER+/HER2- MBC with clinical NGS (n=749) a significant difference in overall survival (OS) was observed between patients with ERBB2del versus non-ERBB2del tumors (33.8 vs 47.8 months, p=0.037). In the MSK MetTropism cohort (n=883), patients with ER+/HER2- MBC and an ERBB2del had worse OS (16.4 vs 30.8 months, p<0.0001).

**Conclusion**

This study led to the identification of a biologically and clinically distinct subgroup of BCs characterized by a large deletion in chromosome 17q12 (17q12del) with heterozygous loss of ERBB2. This breast cancer subtype is enriched in a heterozygous loss of TP53, extremely low ERBB2 mRNA and HER2 protein expression, for which resistance to T-DXd has been suggested. Validated by large real-world cohorts of patients with HER2-negative metastatic BC, we found that this subgroup of patients had decreased overall survival, underscoring the clinical significance of this genomic entity.