Molecular and immunological characterization of HER2-low, HER2 ultra-low, and HER2-null male breast cancer

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BACKGROUND

- Human epidermal growth factor receptor 2 (HER2) gene expression is an important predictive and prognostic biomarker in breast cancer (BC), which also guides treatment recommendations.
- The expression of HER2 is a continuum from null to positive and includes HER2-low and ultra-low as targets for anti-HER2 antibody–drug conjugates (ADC).
- However, HER2-low and ultra-low have not been studied in male BC.
- Here, we analyze whether there are any differences in molecular and immunological features between HER2-low, ultra-low and HER2-null/negative expression in males with BC.

METHODS

- 183 male breast tumor samples were included in this study.
- HER2-null expression was defined as infiltrating cancer cells completely free of HER2 immunohistochemistry (IHC) staining.
- HER2 ultra-low expression was defined as ≤10% cancer cell showing incomplete and faint/weak membrane staining.
- HER2-low expression was defined as HER2 (IHC) 1+ or 2+ with negative chromogenic *in situ* hybridization (CISH) assay.
- HER2-positive expression was defined as HER2 IHC 3+ staining or 2+ with positive CISH assay.
- Mutations and gene expression were detected by next-generation sequencing (592, NextSeq; WES, NovaSeq) and Whole Transcriptome Sequencing (WTS; NovaSeq) (Caris Life Sciences, Phoenix, AZ), respectively.
- Tumor mutational burden (TMB) totaled somatic mutations per tumor (high>10 mt/MB). Immune cell fractions were calculated by deconvolution of WTS: Quantiseq.
- Statistical significance was determined using chi-square and Mann-Whitney U test and p-value <0.05 was considered significant.

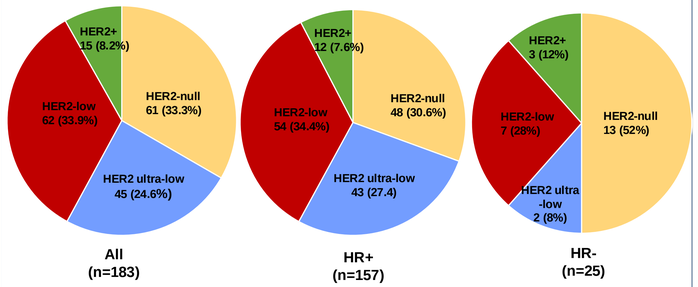
Table 1: Sample demographic information

Variables	Her2-null	Her2 Ultra-low	Her2 Low	Her2 positive
Count (N)	61	45	62	15
Median age (range)	66 (35->89)	69 (39->89)	66.5 (39->89)	64 (29->85)
Race (count, N)				
White	64.7% (33/51)	77.8% (28/36)	69.2% (36/52)	72.7% (8/11)
Black	23.5% (12/51)	8.3% (3/36)	23.1% (12/52)	27.3% (3/11)
Asian or Pacific Islander	5.2% (3/51)	13.9% (5/36)	3.8% (2/52)	0.0% (0/11)
Other	5.9% (3/51)	0.0% (0/36)	3.8% (2/52)	0.0% (0/11)
Ethnicity (count, N)				
Not Hispanic or Latino	89.4% (42/47)	88.9% (33/36)	97.8% (44/45)	90.9% (10/11)
Hispanic or Latino	10.6% (5/47)	11.1% (4/36)	2.2% (1/45)	9.1% (1/11)
Tumor site (count, N)				
Primary	29.5% (18/61)	53.3% (24/45)	41.9% (26/62)	46.7% (7/15)
Metastatic	70.5% (43/61)	46.7% (21/45)	58.1% (36/62)	53.3% (8/15)

Race/ethnicity data is self-reported

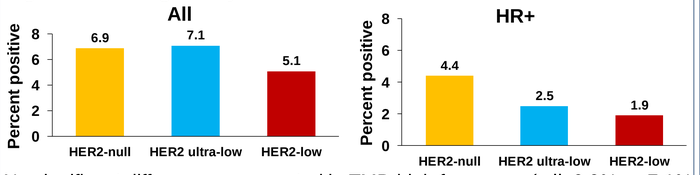
RESULTS

Figure 1. Frequencies of the different expression levels of HER2



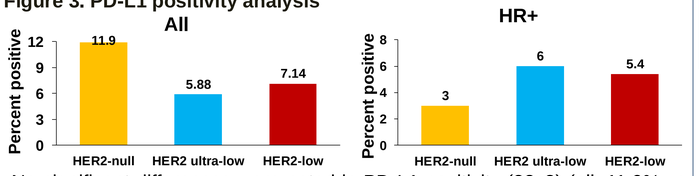
Of 183 samples, there were 33.3% HER2-null tumors, 24.6% HER2 ultra-low tumors, 33.9% HER2-low tumors, and 8.2% HER2-positive. The proportion of HR+ was 30.6% in HER2-null, 27.4% in HER2 ultra-low, 34.4% in HER2-low and 7.6% in HER2-positive tumors. The proportion of HR- was 52% in HER2-null, 8% in HER2 ultra-low, 28% in HER2-low and 12% in HER2-positive tumors.

Figure 2. TMB-high analysis



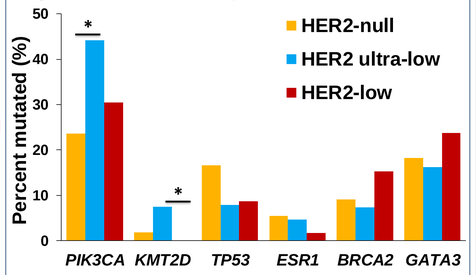
No significant differences were noted in TMB-high frequency (all: 6.9% vs 7.1% vs 5.1%, HR+: 4.4% vs 2.5% vs 1.9%) between HER2-null, HER2 ultra-low and HER2-low. There were not enough samples to perform analysis in HR- tumors.

Figure 3. PD-L1 positivity analysis



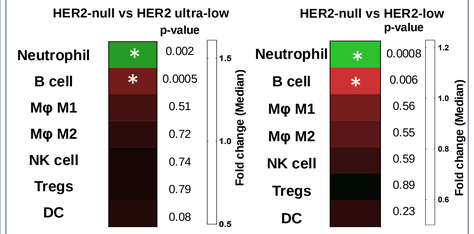
No significant differences were noted in PD-L1 positivity (22c3) (all: 11.9% vs 5.8% vs 7.1%, HR+: 3% vs 6% vs 5.4%) between HER2-null, HER2 ultra-low and HER2-low. There were not enough samples to perform analysis in HR- tumors.

Figure 4. Mutation analysis



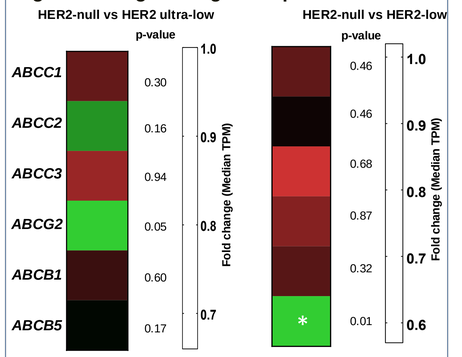
HER2 ultra-low male BC had higher frequency of *PIK3CA* (44.19% vs 23.64%) compared to HER2-null and *KMT2D* (7.5% vs 0%) compared to HER2-low, all p<0.05. HER2-low male BC had numerically lower frequency of *TP53* (8.7% vs 16.6%, p=0.2) and *ESR1* (1.6% vs 5.4%, p=0.2) compared to HER2-null. *p<0.05.

Figure 5. Immune cell infiltration



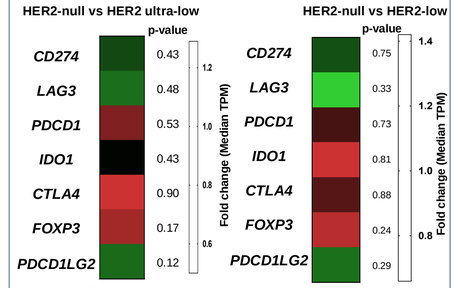
HER2 ultra-low and HER2-low male BC had higher infiltration of B cells (fold change (FC): 1.3 and 1.2) but lower infiltration of neutrophil (FC: 1.6 and 1.8) compared to HER2-null. *p<0.05.

Figure 6. Drug efflux gene expression



HER2-low had lower expression of drug-efflux gene *ABCB5* (FC: 1.7) compared to HER2-null tumors. *p<0.05.

Figure 7. Checkpoint gene expression



No significant differences were noted in immune checkpoint gene expression.

CONCLUSIONS

Our findings add valuable information to the current understanding of the HER2 spectrum in the male breast cancer, including frequency distribution and molecular characterization. With some exceptions, HER2-low, ultra-low and null breast cancer in men shared genomic features, suggesting that the disease biology may not be different across the spectrum of what historically has been considered HER2-negative disease. Interestingly, HER2 ultra-low, HER2-low and HER2-null had differential tumor immune microenvironment that warrant further exploration.