

Supplementary Table 1. HER2 antibodies and specifications.

Antibody	Vendor	Species-Type	Optimized titer (concentration)	Dynamic range (AU) / R²	Target within HER2
CB11	Biocare Medical	Mouse monoclonal	1:1000 (10.4 ng/ml)	261.8-13302.3 / 0.98	Intracellular domain
A0485	Dako	Rabbit polyclonal	1:10000	119.6-13304.4 / 0.95	Intracellular domain
SP3	Spring Bioscience	Rabbit monoclonal	1:100 (indeterminable as produced)	135.5-6837.70 / 0.91	Extracellular domain
D8F12	Cell Signaling Technology	Rabbit monoclonal	1:5000 (4.36 ng/ml)	89.1-13310.20 / 0.98	Extracellular domain

* AU: arbitrary unit of fluorescence; R²: linear regression coefficient.

Supplementary Table 2. HeCOG 10/05 cohort characteristics.

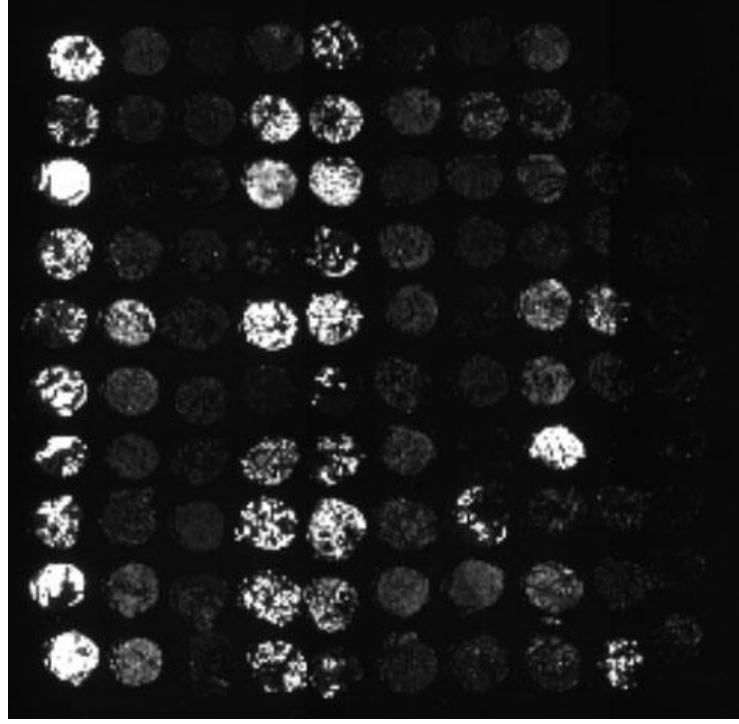
Parameter	N (%)
All patients	705
Age (y) range 21-79	
<50	277 (39.2)
≥50	428 (60.7)
Nodal Status	
Positive	542 (76.9)
Negative	163 (23.1)
Tumor Size	
<2 cm	218 (30.9)
2-5 cm	437 (62.0)
>5 cm	50 (7.1)
ER status	
Positive	545 (77.3)
Negative (0)	159 (22.6)
Unknown	1 (0.1)
PgR status	
Positive (1-3)	479 (67.9)
Negative (0)	226 (32.1)
HER2 status	
Positive (2-3)	189 (26.8)
Negative (0-1)	516 (73.2)
Adjuvant treatment	
Chemotherapy	705 (100)
Hormonotherapy	549 (77.9)
Radiotherapy	521 (73.9)
Trastuzumab	180 (25.5)
Follow-up (m)	
Median (range)	60.3 (0.43 – 78.98)

Supplementary Figure 1. HER2 standardization tissue microarray (YTMA 263). A tissue microarray was built and designed using 80 breast cancer cases with CLIA-certified immunohistochemistry (IHC) and fluorescent *in situ* hybridization (FISH) information, non-tumor breast cancer tissue and breast cancer cell lines with known HER2 gene copy number. Cases with high HER2 protein expression group in the clinically-positive columns, as shown in **A** and **B** for CB11 using QIF and chromogenic IHC, respectively. AMP: amplified, NOT AMP: not amplified.

SUPPLEMENTAL FIGURE 1

HER2 CB11

A



3+, AMP

1+, NOT AMP

0, NOT AMP

2+, AMP

3+, AMP

1+, NOT AMP

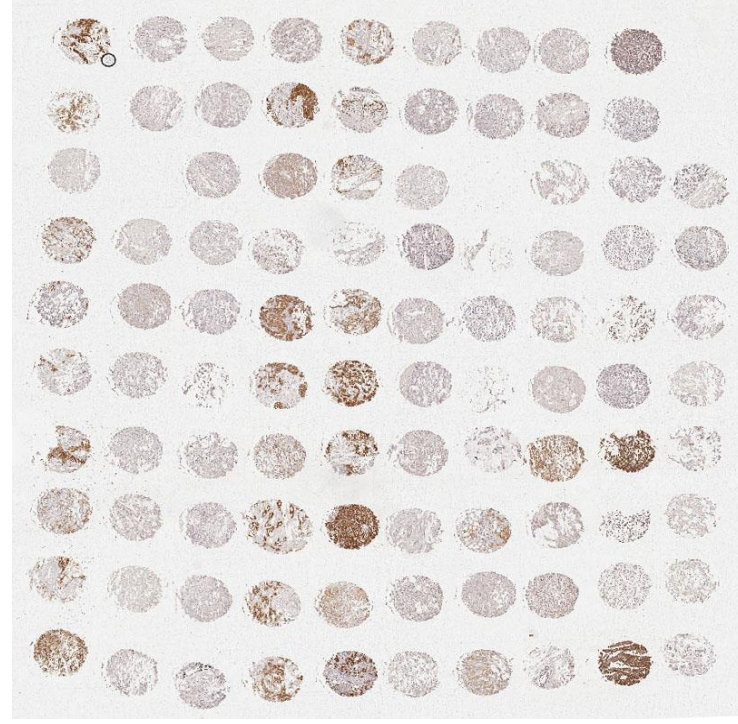
0, NOT AMP

2+, NOT AMP

CELL LINES

NORMAL
BREAST TISSUE

B



3+, AMP

1+, NOT AMP

0, NOT AMP

2+, AMP

3+, AMP

1+, NOT AMP

0, NOT AMP

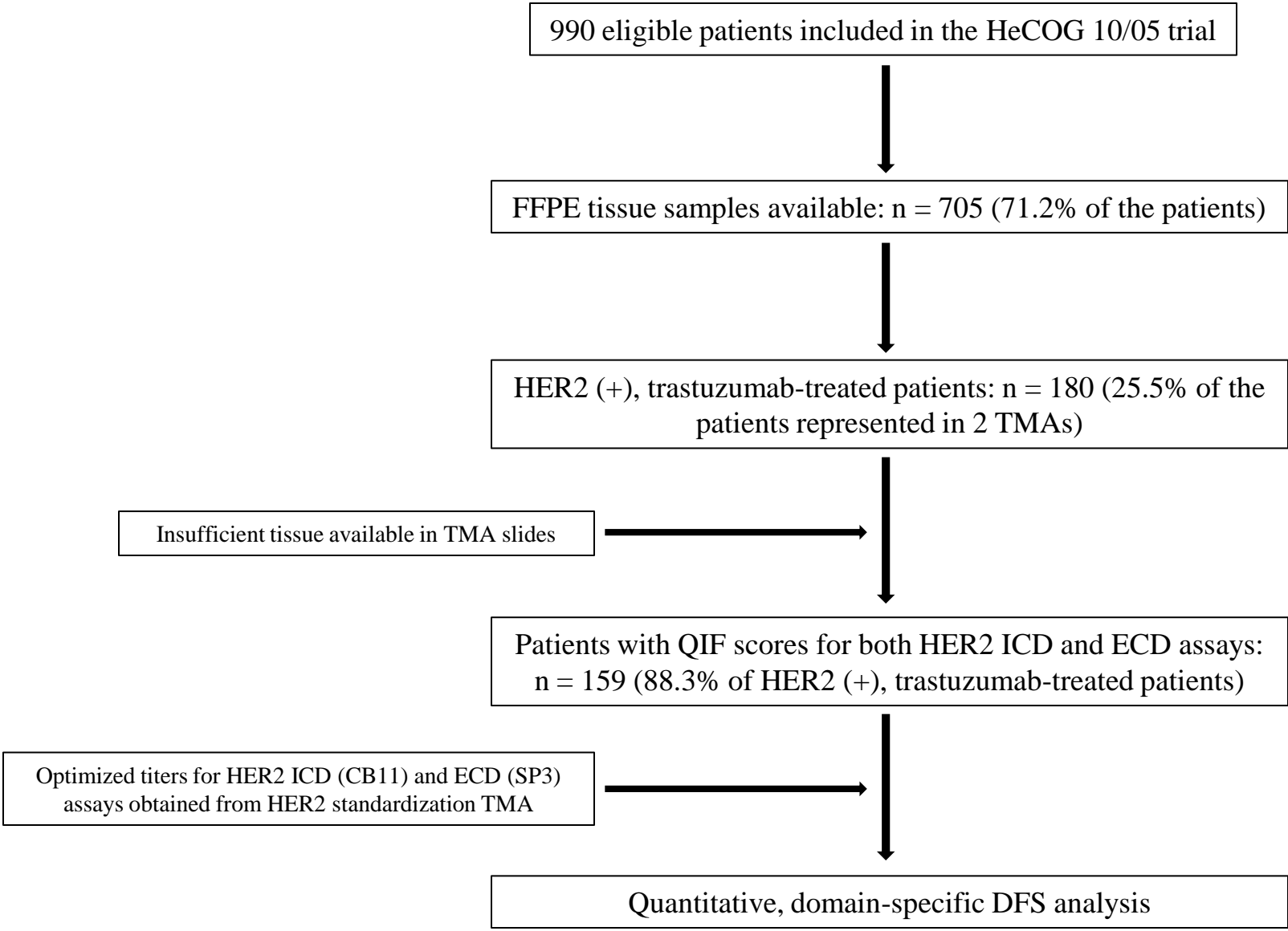
2+, NOT AMP

CELL LINES

NORMAL
BREAST TISSUE

Supplementary Figure 2. REMARK diagram detailing FFPE tissue availability and flow in the present study.

SUPPLEMENTAL FIGURE 2



Supplementary Figure 3. Quantitative analysis of HER2 chromogenic immunohistochemistry in the HER2 standardization tissue microarray. Distribution of HER2 CB11 (A) and SP3 (B) scores obtained using optimized titer analysis with the Aperio algorithm. Joinpoint cut-point for diagnostic test evaluation was generated for each assay (red dotted lines).

SUPPLEMENTAL FIGURE 3

