23P - Clinical, pathological and gene expression features of HER2-low breast cancer

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BACKGROUND

HER2-positive breast cancer (BC) is currently defined according to the ASCO/CAP guidelines using immunohistochemistry (IHC) and/or *in situ* hybridization (ISH)-based techniques[1, 2]. Following these guidelines, a breast tumor is defined as HER2-positive if there is a complete and intense circumferential HER2 IHC staining in ≥10% of cells (score 3+) and/or the gene is amplified with an HER2/CEP17 ratio ≥ 2.0 and an average HER2 gene (*ERBB2*) copy number ≥ 4.0 signals/cell using ISH-based techniques[1]. Based on this definition, 10-20% of breast tumors are HER2-positive and 80-90% are HER2-negative[3, 4].

Within HER2-negative disease, substantial heterogeneity exists regarding the expression of hormone receptors (HR) and HER2. For example, HER2-negative tumors can express some protein level of HER2 by IHC[5] (i.e. 1+ or 2+ and a negative ISH result) and are identified as HER2-low. Traditionally, patients with HER2-low-expressing tumors do not seem to benefit from HER2-targeted therapies, such as 1-year of adjuvant trastuzumab[6]. However, two HER2-directed antibody-drug conjugates (ADC) with chemotherapeutics, namely trastuzumab deruxtecan (T-DXd) and trastuzumab duocarmazine (SYD985) have shown very promising therapeutic activity in HER2-low BC patients[7–9], and a large pivotal randomized phase III trial of T-DXd in patients with pre-treated HER2-low metastatic breast cancer is underway (i.e. NCT03734029/DESTINY-Breast04). Therefore, there is a need to better understand the clinicopathological and molecular characterization of HER2-negative/HER2-low breast tumors.

METHODS

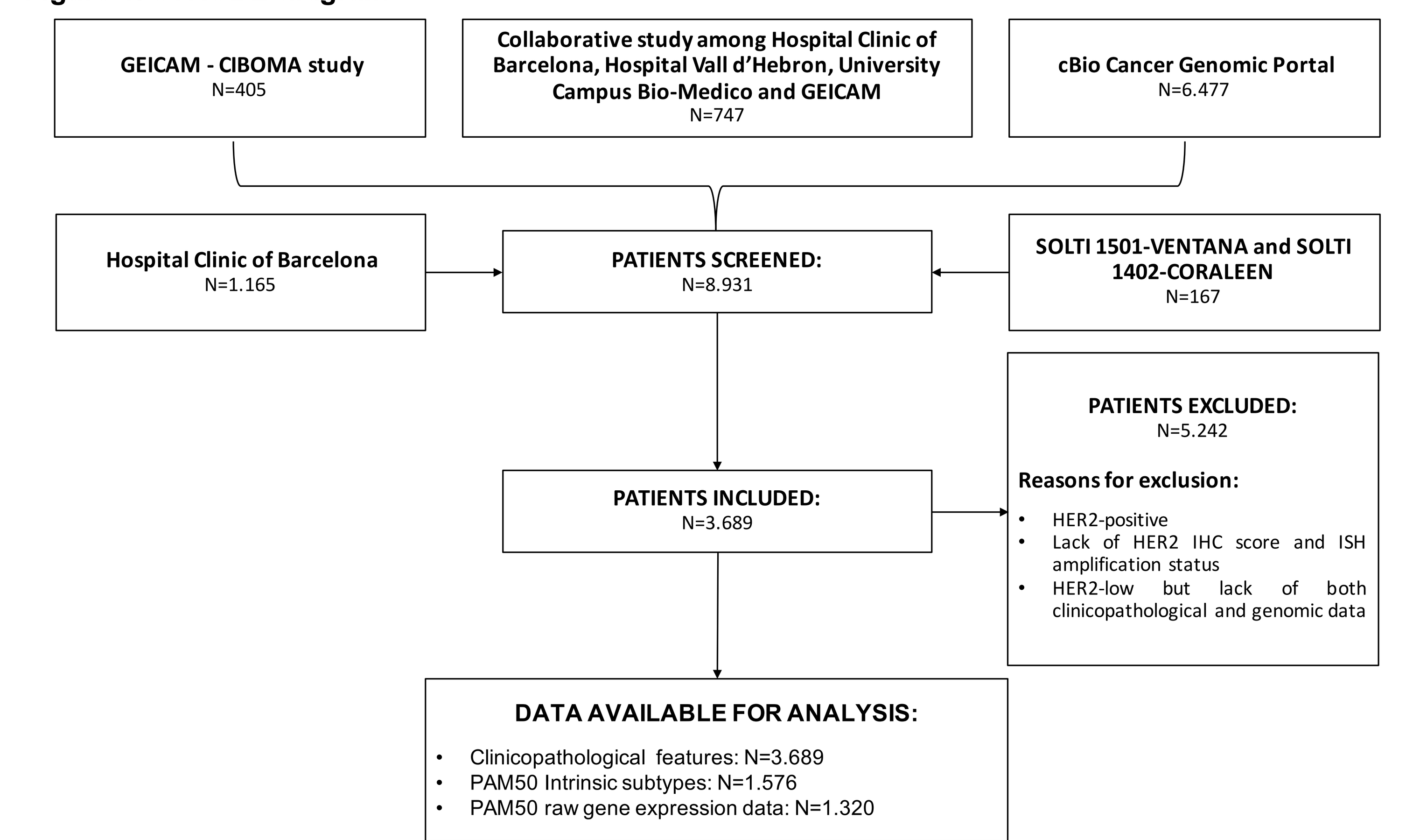
Patients datasets

We collected clinicopathological and gene expression data from several public and internal databases[10-17]. The selection process is resumed in **figure 1.**

Inclusion criteria

Patients were included if they were HER2-negative with known IHC and ISH status and if they had at least one of the following information available: 1) clinicopathological features, 2) PAM50 gene expression data 3) PAM50 intrinsic subtype. The following clinical-pathological features were evaluated, when available: Ki67 IHC, histological grading (G), estrogen receptor (ER) status, progesterone receptor (PgR) status, age at diagnosis, menopausal status, tumor sample origin (primary versus metastatic), histological subtype and tumor infiltrating lymphocytes (TILs).

Figure 1. STROBE diagram



IHC-based classification

Tumors were divided into HR-positive (i.e. ER and/or PgR ≥1%) or triple-negative (TNBC), defined as ER<1% and PgR<1%, and classified into HER2 0 (IHC score of 0) and HER2-low (HER2 IHC of 1+ or 2+ with an ISH-based negative). HER2 IHC 0 and 1+ were considered HER2 0 and HER2-low, respectively, unless ISH-based data was available and reported as HER2-amplified. HER2 status in each cohort was determined using standard FDA-approved antibodies and ISH-techniques and classified according to the ASCO/CAP guidelines[1, 2].

PAM50 subtypes and gene expression data

Raw gene expression data from the PAM50 assay was available from 9 of the 13 cohorts (cBio Cancer genomic portal excluded) and subtype information was obtained independently from the different cohorts. In a majority of samples, intrinsic subtypes were obtained from formalin-fixed paraffin-embedded tumor samples by the research version of the PAM50 assay using the nCounter platform (NanoString Technologies, Seattle WA)[18]. PAM50 gene expression data were processed as previously described[19, 20]. The determination of intrinsic subtypes for TCGA BC data was performed as elsewhere described[11].

Objectives

- *Primary objective:*
  - Compare the clinicopathological and genomic differences between HER2-low and HER2 0 tumors;
- *Secondary objectives:*
  - Compare the genomic differences in HER2-negative disease between HER2 0 and HER2-low tumors within HR-positive (+) disease;
  - Compare the genomic differences in HER2-negative disease between HER2 0 and HER2-low tumors within TNBC;
  - Compare *ERBB2* mRNA levels between HER2 0 and HER2-low tumors in the overall population, in the HR+ tumors, in TNBC and in HR+/HER2-low vs TNBC/HER2-low tumors.

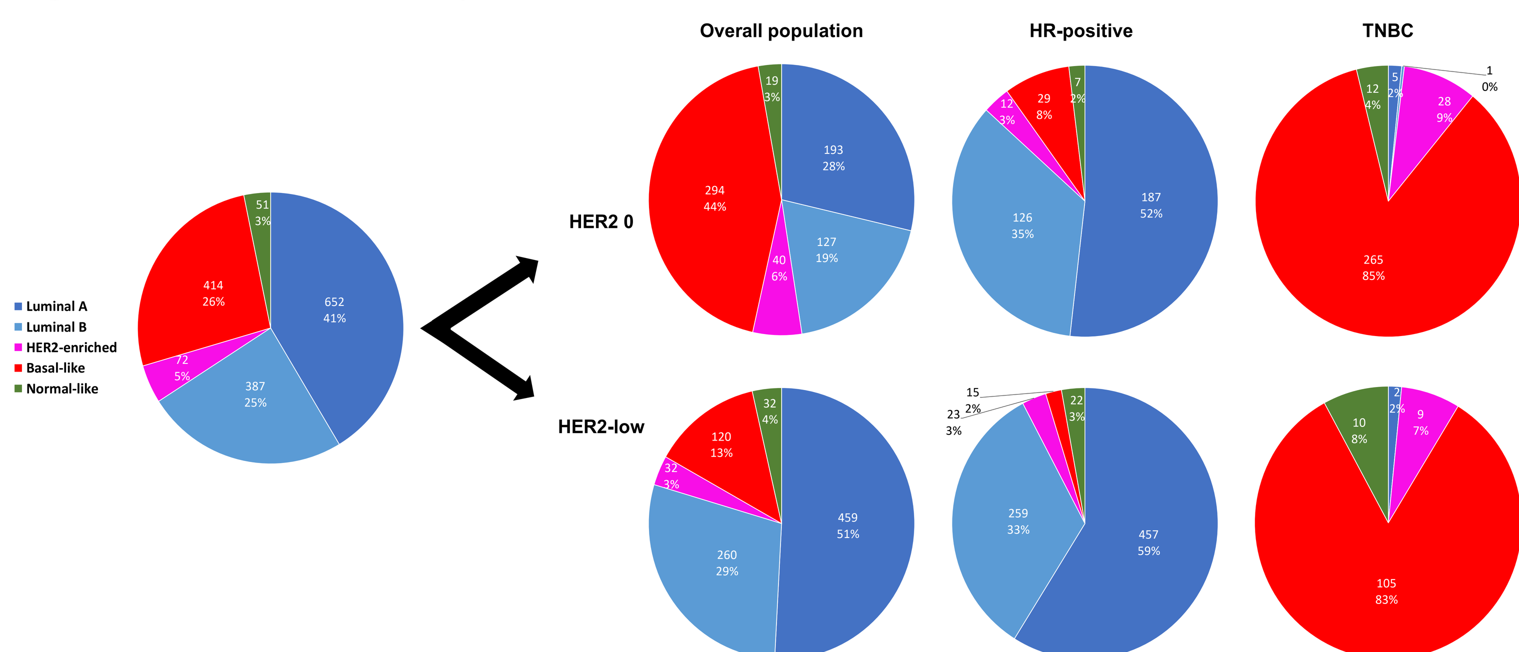
Statistical analysis

Patient and tumor characteristics were analyzed using χ^2 test, Fisher's exact test, Kruskalis-Wallis and Wilcoxon rank-sum test with continuity correction, where appropriate. Differences were considered significant at $p<0.05$. Significance Analysis of Microarray (SAM) for unpaired samples (multiclass and 2 class) was used to compare gene expression profiles between groups[21]. Differences were considered significant at a false discovery rate (FDR)<5%. A list of the genes and PAM50 intrinsic subtypes' signatures evaluated for differential expression analysis in the overall HER2-negative population, as well as in HR-positive and TNBC is fully reported in **table 2**.

RESULTS

Overall 3689 patients were compared for their clinicopathological features. All descriptions and analyses are reported in **table 1**. PAM50 intrinsic subtypes calls were available from 1,576 (42.7%) patients. Intrinsic subtypes were differentially distributed between HER2-low and HER2 0 tumors (p<0.001). Intrinsic subtypes distribution varied also between HR-positive and TNBC (p<0.001). Within HR-positive disease, intrinsic subtypes were differentially distributed between HER2-low and HER2 0 tumors (p<0.001). On the contrary, there was no significant difference in subtype distribution within TNBC according to HER2-low status (p=0.438). All subtypes' distributions are reported in **figure 2.**

Figure 2. PAM50 Intrinsic Subtypes' distributions



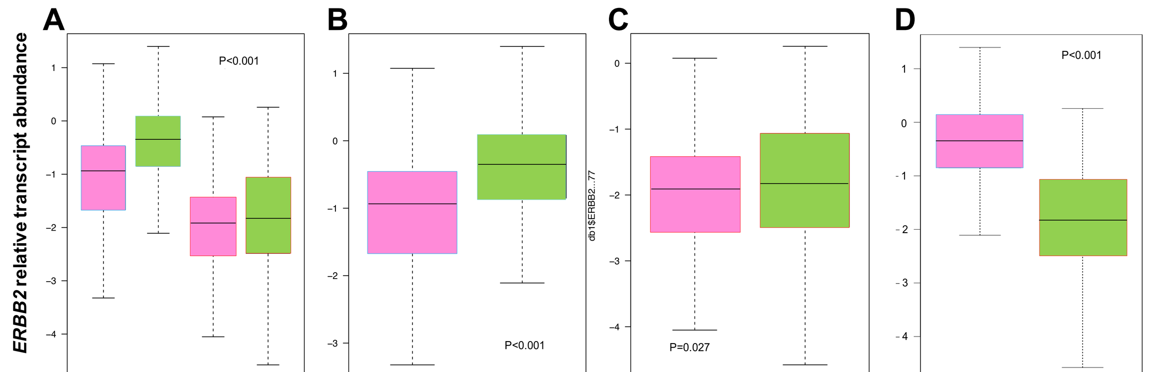
Differences in gene expression for the overall and HR+ populations are reported in **table 3**. No significant differences were observed in TNBC tumors. Finally, *ERBB2* relative transcript abundance was higher in HR+ tumors compared to TNBC (p<0.001; **figure 4A**), in HR+/HER2-low compared to HR+/HER2 0 tumors (p<0.001; **figure 4B**), as well as in TNBC/HER2-low compared TNBC/HER2 0 (p=0.027; **figure 4C**). However, relative transcript abundance was higher in HR+/HER2-low compared to TNBC/HER2-low (p<0.001; **figure 4D**).

Table 1. Population characteristics according to HER2 status

DEMOGRAPHICS	HER2-NEGATIVE HER2 0 N	HER2 0 %	HER2-LOW N	HER2-LOW %	OVERALL POPULATION N	OVERALL POPULATION %	P*
	1486	40.3	2203	59.7	3689	100	
Age at diagnosis (years)							
Median	55		59		58		
IQR	46 - 65		49 - 67		48 - 67		**0.003**
Min - max	24 - 93		26 - 96		24 - 96		
Sex							
Male	0	0	15	0.7	15	0.4	
Female	1486	100	2187	99.3	3673	99.6	**0.001**
Total	1486	40.3	2202	59.7	3688	100	
Menopaual status							
Pre/perimenopausal	385	37.3	660	37.1	1045	37.2	
Postmenopausal	646	62.7	1119	62.9	1765	62.8	0.898
Total	1031	36.7	1779	63.3	2810	100	
Biospecimen							
Primary lesion	1000	73.7	1382	71.1	2382	72.1	
Other lesion	357	34.6	563	28.9	920	27.9	0.096
Total	1357	41.1	1945	58.9	3302	100	
Histotype							
Ductal	639	70.8	1214	74.3	1853	73	
Lobular	194	21.5	314	19.2	508	20	0.175
Other	69	7.6	107	6.5	176	6.9	
Total	902	35.6	1635	64.4	2537	100	
T							
1	509	55.8	807	48.7	1316	51.2	
2	294	32.2	618	37.3	912	35.5	
3	71	7.8	142	8.6	213	8.3	**0.007**
4	38	4.2	89	5.4	127	4.9	
Total	912	35.5	1656	64.5	2568	100	
N							
0	556	58.8	937	55.6	1493	56.8	
1	272	28.8	464	27.6	736	28	
2	71	7.5	148	8.8	219	8.3	**0.010**
3	46	4.9	135	8	181	6.9	
Total	945	35.9	1684	64.1	2629	100	
Metastatic status							
Yes	529	65.6	881	62.2	1410	63.4	
No	278	34.4	536	37.8	814	36.6	0.112
Total	807	36.3	1417	63.7	2224	100	
Ab initio Yes	136	10	231	12	367	11.2	
Ab initio No	1218	90	1687	88	2905	88.8	0.074
Total	1354	41.4	1918	58.6	3272	100	
ER							
Positive	983	67	1894	87.1	2877	79	
Negative	484	33	280	12.9	764	21	**<0.001**
Total	1467	40.3	2174	59.7	3641	100	
PgR							
Positive	789	54.7	1542	71.8	2331	64.9	
Negative	654	45.3	606	28.2	1260	35.1	**<0.001**
Total	1443	40.2	2148	59.8	3591	100	
G							
1	67	8.8	139	10.6	206	9.9	
2	272	35.6	514	39.1	786	37.8	**0.0499**
3	426	55.7	660	50.3	1086	52.3	
Total	765	36.8	1313	63.2	2078	100	
Ki67							
Median	16		18		18		
IQR	9 - 30		10 - 27		10 - 27		0.892
Min - max	0.5 - 95		0.5 - 95		0.5 - 95		
Pts with available data	433	36.4	756	63.6	1189	100	
≤14%	190	43.9	294	38.9	484	40.7	0.092
>14%	243	56.1	462	61.1	705	59.3	
TILs							
Median	1		1		1		
IQR	0 - 5		1 - 5		1 - 5		0.218
Min - max	0 - 80		0 - 80		0 - 80		
Pts with available data	102	37.2	172	62.8	274	100	
IHC subtypes simplified							
HR-positive	1025	69.6	1937	88.2	2962	80.8	
Triple Negative	448	30.4	258	11.8	706	19.2	**<0.001**
Total	1473	40.2	2195	59.8	3668	100	

Legend and footnotes. Pts: patients; HR: hormone receptors; IQR: interquartile range; IHC: immunohistochemical; TILs: tumor-infiltrating lymphocytes; *: Chi square test for differences in proportions, Kruskalis-Wallis and Wilcoxon rank sum test with continuity correction, where appropriate, for continuous variables (median comparisons)

Figure 4. Box plots of relative transcript abundance of *ERBB2*



Legend. Pink boxes with blue borders represent *ERBB2* levels in HR+/HER2 0 BC and green boxes with blue borders in HR+/HER2-low BC. Pink boxes with red borders represent *ERBB2* levels in TNBC/HER2 0 and green boxes with red borders in TNBC/HER2-low

Table 2. Gene expression of HER2-low vs. HER2 0 tumors in overall and HR+ tumors

Gene/Signature	OVERALL Score(d)	OVERALL Fold Change	OVERALL FDR*	HR-POSITIVE Score(d)	HR-POSITIVE Fold Change	HR-POSITIVE FDR*
Basal-like	-5.67277	0.8	0.0	-0.00299	1.0	24.7
HER2-enriched	-0.39253	1.0	0.0	1.18400	1.1	3.2
Luminal A	4.73173	1.3	0.0	3.38402	1.3	0.0
Luminal B	2.06840	1.1	0.0	2.34168	1.2	0.0
Normal-like	-0.33430	1.0	0.0	1.14341	1.1	3.2
ACTR3B	0.72473	1.0	1.8	0.74613	1.1	8.6
ANLN	-7.39622	0.6	0.0	-2.27444	0.8	0.0
AR	10.64278	3.9	0.0	-	-	-
BAG1	-1.38371	0.9	0.0	0.90829	1.2	6.0
BCL2	8.20430	2.0	0.0	2.51291	1.3	0.0
BIRC5	-5.55863	0.6	0.0	-1.40851	0.8	0.9
BLVRA	5.14298	1.4	0.0	1.73118	1.2	0.0
CCNB1	-3.76904	0.8	0.0	-1.84185	0.8	0.0
CCNE1	-9.55346	0.4	0.0	-3.03781	0.8	0.0
CD274 (PD-L1)	2.00646	1.2	0.0	-	-	-
CD4	2.21548	1.1	0.0	-	-	-
CD8A	-0.61556	0.7	0.0	-	-	-
CDC20	-4.47577	0.7	0.0	-0.87565	0.9	7.4
CDC6	-3.37515	0.8	0.0	-0.28853	1.0	21.8
CDCA1	-4.18599	0.7	0.0	-1.11544	0.9	6.0
CDH3	-5.87928	0.6	0.0	-1.06410	0.9	6.0
CENPF	-6.99532	0.6	0.0	-1.83252	0.8	0.0
CEP55	-7.83024	0.5	0.0	-1.28199	0.9	3.2
CXXC5	-3.61475	0.6	0.0	0.24982	1.1	21.8
EGFR	-0.82066	0.9	0.0	0.28270	1.0	21.8
ERBB2	9.97921	2.0	0.0	5.17067	1.6	0.0
ESR1	14.29386	9.9	0.0	4.96165	2.1	0.0
EXO1	-7.12534	0.6	0.0	-2.43602	0.8	0.0
FGFR4	-1.39985	0.9	0.0	-0.07579	1.0	24.7
FOXA1	13.26991	9.8	0.0	4.92557	2.0	0.0
FOXC1	-8.43702	0.4	0.0	-0.73815	0.9	9.7
GPR160	7.74052	2.0	0.0	2.78485	1.4	0.0
GRB7	6.09456	1.5	0.0	2.76279	1.3	0.0
KIF2C	-3.12355	0.8	0.0	-1.39427	0.9	0.9
KNTC2	-7.77839	0.5	0.0	-2.31629	0.8	0.0
KRT14	-1.86137	0.8	0.0	0.61877	1.1	11.4
KRT17	-5.64524	0.4	0.0	0.04017	1.0	27.5
KRT5	-2.51720	0.7	0.0	0.84223	1.2	7.4
MAPT	9.94512	3.4	0.0	2.93645	1.6	0.0
MDM2	0.06691	1.0	5.3	1.12099	1.2	3.2
MELK	-6.64057	0.6	0.0	-1.49359	0.9	0.9
MIA	-5.35993	0.5	0.0	-0.95634	0.9	7.4
MKI67	-8.67347	0.5	0.0	-2.35485	0.8	0.0
MLPH	8.78099	3.6	0.0	2.63999	1.6	0.0
MMP11	6.58603	2.1	0.0	2.60115	1.5	0.0
MYBL2	-3.97998	0.7	0.0	-1.44618	0.8	0.9
MYC	-5.90597	0.6	0.0	-0.88871	0.9	7.4
NAT1	12.27258	5.4	0.0	4.10788	2.0	0.0
ORC6L	-7.61981	0.6	0.0	-2.32482	0.8	0.0
PDCD1 (PD1)	0.26237	1.0	5.3	-	-	-
PGR	11.22998	5.2	0.0	3.17887	1.9	0.0
PHGDH	-8.41385	0.5	0.0	-1.68128	0.8	0.0
PTTG1	-4.82226	0.7	0.0	-1.44424	0.9	0.9
RRM2	-5.93472	0.6	0.0	-1.25960	0.9	3.2
SFRP1	-4.83858	0.6	0.0	-0.33182	1.0	16.4
SLC39A6	11.60247	3.5	0.0	3.96208	1.7	0.0
TMEM45B	7.09375	2.1	0.0	2.02627	1.3	0.0
TYMS	-3.20623	0.8	0.0	-0.56947	0.9	13.6
UBE2C	-7.60564	0.6	0.0	-0.37100	1.0	16.4
UBE2T	-4.54516	1.4	0.0	1.69281	1.1	0.0

Legend and footnotes. HR: hormone receptors; FDR: false discovery rate; *: significant if FDR<5.0. Positive and negative Score(d) represent genes up- and down-regulation in HER2-low vs. HER2 0 BC.

- TNBC were characterized by a predominance of Basal-like (84.7%) and HER2-E (8.5%) subtypes. No significant difference in subtype distribution was observed between HER2 0 and HER2-low tumors.
- HER2-low compared to HER2 0 BC, presented the vast majority of proliferation-related (e.g. *CCNB1, CCNE1, MKI67* etc.), Basal-like-related (e.g. *KRT14, KRT17, KRT5, FOXC1, MYC* etc.), tyrosine-kinase receptors genes (i.e. *EGFR, FGFR4*) and Basal-like molecular signature down-regulated, while Luminal genes (e.g. *FOXA1, ESR1, PGR* and *AR*), as well as Luminal A and B molecular signatures, *ERBB2* and its companion *GRB7* were up-regulated. A similar pattern was observed for HR-positive disease. Within TNBC, no gene expression differences were observed.
- A higher relative transcript abundance of *ERBB2* was observed in HER2-low compared to HER2 0 tumors in the overall, HR+ and TNBC. When comparing HR-positive/HER2-low tumors over TN/HER2-low, *ERBB2* mRNA levels were also higher in the first group.

All these features suggest the presence of biological differences that might go beyond the mere HR+ vs HR-negative dichotomy and that might also explain the differencial response rates observed between HR+ and TN/HER2-low BC with the novel ADC T-DXd and SYD985[9,23]. Furthermore, higher levels of the immune-related genes in HER2-low tumors compared to HER2 0 might suggest a certain degree of immune activation.

Limitations

- Retrospective study and combination of patients deriving from databases pertaining to different studies.
- Pathology was not centralized.
- We were not able to evaluate differences in terms of DNA methylation, chromosomal aberrations, gene mutations and amplification.

Strenghts

- First comprehensive study focusing specifically on HER2-low tumors, dissecting their clinicopathological and genomic features.
- We also provided comparisons based on HR status.
- High number of patients enrolled.

To conclude, HER2-low disease within HER2-negative BC is frequent. However, compared to TN/HER2-low, HR-positive/HER2-low disease is a more distinct biological entity and has higher ERBB2 expression. Our data might provide an explanation for some preliminary results obtained in early phase clinical trials with new ADC directed to HER2[8,24] and be hypothesis-generating for further trials.

CONCLUSIONS

- HER2-low tumors represented the majority (59.7%) of HER2-negative BC, were apparently more frequent in older patients and male, slightly more differentiated but with bigger primary tumor size and more axillary lymph-node involvement compared to HER2 0 BC.
- HER2-low tumors were more frequently HR+ and Luminal than HER2 0 BC (88.2 vs 69.6% and 80% vs 47%, respectively). Within HR+ tumors a lower prevalence of Basal-like and Luminal B and a slightly higher prevalence of Luminal A tumors was observed for HER2-low compared to HER2 0 BC (2% vs 8%, 33% vs 35% and 59% vs 52%, respectively).

REFERENCES

1. Wolff AC, Hammond MEH, Allison KH et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2018; 36(20):2105–2122.
2. Wolff AC, Hammond MEH, Hicks DG et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2013; 31(31):3997–4013.
3. Cronin KA, Harlan LC, Dodd KW et al. Population-based estimate of the prevalence of HER-2 positive breast cancer tumors for early stage patients in the US. Cancer Invest. 2010; 28(9):963–968.
4. Slamon DJ, Clark GM, Wong SG et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235(4785):177–182.
5. Schalper KA, Kumar S, Hui P et al. A retrospective population-based comparison of HER2 immunohistochemistry and fluorescence in situ hybridization in breast carcinomas: impact of 2007 American Society of Clinical Oncology/College of American Pathologists criteria. Arch. Pathol. Lab. Med. 2014; 138(2):213–219.
6. Fehrenbacher L, Cecchini RS, Geyer CE et al. NSABP B-47/NRG Oncology Phase III Randomized Trial Comparing Adjuvant Chemotherapy With or Without Trastuzumab in High-Risk Invasive Breast Cancer Negative for HER2 by FISH and With IHC 1+ or 2. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2020; 38(5):444–453.
7. Iwata H, Tamura K, Doi T et al. Trastuzumab deruxtecan (DS-8201a) in subjects with HER2-expressing solid tumors: Long-term results of a large phase 1 study with multiple expansion cohorts. J. Clin. Oncol. 2018; 36(15_suppl):2501–2501.
8. Rinnerthaler G, Gampenrieder SP, Greil R. HER2 Directed Antibody-Drug-Conjugates beyond T-DM1 in Breast Cancer. Int. J. Mol. Sci. 2019. doi:10.3390/ijms20051115.
9. Modi S, Park H, Murthy RK et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2020:JCO1902318–JCO1902318.
10. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012; 490(7418):61–70.
11. Ciriello G, Gatza ML, Beck AH et al. Comprehensive molecular portraits of invasive lobular breast cancer. Cell 2015; 163(2):506–519.
12. The Metastatic Breast Cancer Project. [https://www.mbcproject.org/].
13. Razavi P, Chang MT, Xu G et al. The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers. Cancer Cell 2018; 34(3):427-438.e6.
14. Adamo B, Bellet M, Paré L et al. Oral metronomic vinorelbine combined with endocrine therapy in hormone receptor-positive HER2-negative breast cancer: SOLTI-1501 VENTANA window of opportunity trial. Breast Cancer Res. BCR 2019; 21(1):108.
15. Prat A, Saura C, Pascual T et al. Ribociclib plus letrozole versus chemotherapy for postmenopausal women with hormone receptor-positive, HER2-negative, luminal B breast cancer (CORALLEEN): an open-label, multicentre, randomised, phase 2 trial. Lancet Oncol. 2020; 21(1):33–43.
16. Lluch A, Barrios CH, Torrecillas L et al. Phase III Trial of Adjuvant Capecitabine After Standard Neo-/Adjuvant Chemotherapy in Patients With Early Triple-Negative Breast Cancer (GEICAM/2003-11_CIBOMA/2004-01). J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2020; 38(3):203–213.
17. Fernandez-Martinez A, Pascual T, Perrone G et al. Limitations in predicting PAM50 intrinsic subtype and risk of relapse score with Ki67 in estrogen receptor-positive HER2-negative breast cancer. Oncotarget 2017; 8(13):21930–21937.
18. Geiss GK, Bumgarner RE, Birditt B et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat. Biotechnol. 2008; 26(3):317–325.
19. Prat A, Cheang MCU, Galván P et al. Prognostic Value of Intrinsic Subtypes in Hormone Receptor-Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib. JAMA Oncol. 2016; 2(10):1287–1294.
20. Prat A, Ellis MJ, Perou CM. Practical implications of gene-expression-based assays for breast oncologists. Nat. Rev. Clin. Oncol. 2011; 9(1):48–57.
21. R: Significance Analysis of Microarray. [http://ugrad.stat.ubc.ca/R/library/siggenes/html/sam.html].
22. Bivin WW, Yergiyev O, Bunker ML et al. GRB7 Expression and Correlation With HER2 Amplification in Invasive Breast Carcinoma. Appl. Immunohistochem. Mol. Morphol. AIMM 2017; 25(8):553–558.
23. Banerji U, van Herpen CML, Saura C et al. Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. Lancet Oncol. 2019; 20(8):1124–1135.

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