Comprehensive characterization of HER2-low breast cancers: implications in prognosis and treatment

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Summary

Background HER2-low cancers are heterogeneous with different degrees of HER2 expression and hormone receptor (HR) status. Currently, its analysis is mostly focused on the standard clinic-pathologic features or common biomarkers expression, without considering the heterogeneity within the category. A further characterization and understanding of this cancer subgroup will facilitate its management.

Methods A large cohort of HER2-negative cancers (N = 1464) was included. The HER2-low (N = 412) and HER2-zero cancers (N = 1052) were compared and correlated with a comprehensive panel of clinico-pathologic features and biomarker expression according to different HER2 expressions and HR statuses. The prognostic values of these features in HER2-low cancers were also evaluated.

Findings The characteristics of HER2-low breast cancers, as compared to HER2-zero, varied with the HR status. HER2-low luminal cancers were associated with younger age, larger tumor, high pAKT and high HLA expression. Among TNBCs, opposite trends in age and tumor size were found. Additionally, HER2-low TNBC showed less necrosis, higher pN, lower c-kit and CK14 than HER2-zero cancers. Nonetheless, regardless of HR status, HER2-low status was associated with increased COX2 and AR expression, implicated in the biology of HER2-low cancers. HER2-low cancers showed high expression of HLAs in tumors and PD-L1 in immune cells. In particular, the co-expression of HLAs was found to be associated with better survival in HER2-low cancers.

Interpretation This study revealed further characteristic of HER2-low breast cancers as compared to HER2-zero cancers, provided further insights into its prognostication and therapeutic strategies.

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Keywords: HER2-low; Breast cancer; Clinic-pathological features; Survival

Introduction

The current anti-HER2 treatments have been well established for *HER2* gene amplification/HER2 protein overexpressed breast cancers and achieved a high response rate. The conventional HER2 diagnosis mainly focused on the binary classification into HER2-positive and negative cancers. Patients with tumors that are IHC-0 to 1+ and IHC2+ without gene amplification are all diagnosed as HER2-negative as they do not benefit from the conventional anti-HER2 treatments. Recent developments in anti-HER2 antibody–drug conjugates (ADCs) open new therapeutic options for breast cancers. Recent trials of metastatic breast cancers showed that the anti-HER2 ADC, Trastuzumab Deruxtecan (T-Dxd), was effective in HER2-positive breast cancers as well as those with HER2-low expression.[1,2] The distinction of IHC-0 from IHC1+, which once has no clinical relevancy, has now sparked intense interest, leading to the

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Research in context

Evidence before this study

Existing data mainly examined all HER2-low cancers as a single entity. However, they constitute a spectrum of cancers with different degrees of HER2 expression and HR status. Currently its analysis is mostly focused on the standard clinico-pathologic features or common biomarkers expression. A further characterization and understanding of this cancer subgroup will facilitate its management.

Added value of this study

Here, we have revealed further characteristics of HER2-low breast cancers. These features varied with the HR status. Moreover, the existence of AR and COX2 signaling in HER2-low cancers, providing further insights into the biology of this cancer. HER2-low cancers showed high expression of HLAs in tumors and PD-L1 in immune cells. In particular, the co-expression of HLAs was found to be associated with better survival in HER2-low cancers. These immune features may imply potential immunotherapeutic targeting strategies in HER2-low cancers.

Implications of all the available evidence

This study reported further characteristics of HER2-low cancers depending on the HR status, deepening the understanding of their underlying biology. The immune features may provide valuable information for treatment strategies in this cancer subgroup.

reclassification of HER2-negative cancers into HER2-low and HER2-zero cancers.

HER2-low breast cancers accounted for up to 55% of all breast cancers[3,4] and a higher proportion was found in luminal cancers than TNBC.[3,5–7] Moreover, HER2-low cancers were associated with ER positivity, lower grade and lower Ki67. There was also a higher proportion of invasive lobular cancers among them.[5,7] Nonetheless, results from these studies were not all consistent. One study reported the association of HER2-low with older age and higher pT,[3] but such associations were not found by others.[6,7] The distribution of PAM50 subtypes between HER2-low and HER2-zero were reported differently.[3,8] In fact, HER2-low cancers could be heterogeneous, constituting a spectrum of cancers with different degrees of HER2 expression and hormone receptor (HR) status. Existing data examined all HER2-low cancers as a single entity, i.e., IHC 1+ and 2+ without amplification as one group. However, these two groups could be different.[9] In general, a better survival of HER2-low cancers was observed.[4,5,10] However, cancers with IHC2+ without amplification may have a poorer prognosis compared to those with IHC 0/1+, regardless of HR status.[11] IHC1+ cancers had better survival than IHC-0 cancers, but not for IHC2+ cancers.[12] The differences can be found also among HER2-low cancers with different HR status. HR-positive HER2-low cancers had a higher expression of *ERBB2* and luminal-related genes compared to HER2-zero cancers, but no differential gene expression was found among the triple negative breast cancers (TNBC).[3] The HER2-low status was associated with fewer T4 tumors, a higher histologic grade and a negative lymphatic invasion in HR + cancers, but a positive lymphatic invasion in TNBC.[7] Therefore, it would be of interest to refine further the characteristics of HER2-low cancers. So far, only the prognostic significance of HER2-low cancers, as compared to HER2-zero and HER2+ cancers, has been evaluated. Factors associated with patients' outcomes in the group itself have yet to be explored.

In this study, we aimed to examine the clinic-pathologic features of HER2-low cancers in comparison with HER2-zero according to different HER2 expressions and HR statuses. The features that affected the prognosis of HER2-low cancers were also evaluated.

Methods

Study cohort

The current cohort study included all consecutive cases diagnosed with breast cancer for seven (PWH: 2002–2008), seven (KWH: 2003–2009) and eight (TMH: 2003–2010) years in three of the involved institutions. The following clinical and pathologic parameters were retrieved from the medical records: basic demographic characteristics (age and sex), pathologic parameters (tumor size, lymph node involvement, pN and pT stages), and outcome data (date of death, cause of death, date of relapse and type of relapse). Histopathologic features, including histologic types (WHO criteria), grade (Bloom and Richardson grading), lympho-vascular invasion (LVI), phenotypic apocrine feature, fibrotic focus (FF), necrosis and stromal tumor infiltrating lymphocytes (sTILs), for each case were assessed by reviewing the archival haematoxylin and eosin (H&E) stained slides as previously reported.[13] Disease-free survival (DFS) time was calculated from the date of the surgery to the date of the first relapse or death. Overall survival (OS) was calculated from the date of surgery to the date of death from any cause. Breast cancer-specific survival (BCSS) was calculated from the date of surgery to the date of death caused by breast cancer. Data on histological and IHC analysis could be missing due to either insufficient tumor materials for evaluation or loss of tissue section during staining. However, it was generally less than 5% of the population and would not affect the overall conclusion.

Tissue microarray (TMA) construction and immunohistochemistry (IHC)

TMA was prepared as previously described for IHC analysis.[14] Representative tumor areas were selected and two 0.6 mm cores were taken from each case for TMA construction. H&E stained slides were reviewed to confirm the presence of tumor tissues. Immunohistochemical (IHC) staining was carried out on TMA sections with the selected antibodies with BenchMark XT automated slide-staining instrument (Ventana, Arizona, USA) and Ultraview Universal DAB Detection Kit (Ventana, Arizona, USA) after deparaffinization, rehydration and antigen retrieval. All slides were counterstained with hematoxylin. The IHC staining was evaluated based on staining intensity (graded from 0 to 3) and the percentage of positively stained cells in the corresponding cellular location according to different antibodies. HER2 IHC analysis was performed with PATHWAY anti-HER2 antibody (clone 4B5) (Ventana) according to manufacturer's instruction. The interpretation of IHC results were carried out blindly by two of the authors without any clinical information and the staining results of other markers. Any discrepancies were resolved by discussion to reach a consensus. Expression data on TMA for hormonal receptors (androgen receptor (AR), oestrogen receptor (ER) and progesterone receptor (PR)), Ki67, EGFR, basal markers (c-kit, p63, CK5/6 and CK14), immune markers (HLA-A, HLA-B, HLA-C and PD-L1) and markers on cell signaling (COX2 and pAKT) were retrieved from our database. Details of staining and assessment of all markers involved in the study were shown in Supplementary Table S1.

IHC surrogates for molecular subtype classification are as follows[15]: luminal A (ER/and PR+, HER2, Ki67-), luminal B (ER/and PR+, HER2+/and Ki67+), HER2-overexpressed (HER2-OE) (ER-, PR-, HER2+), Basal like breast cancer (BLBC) (ER-, PR-, HER2-, CK5/6+/and EGFR+) and unclassified (5NP) (ER-, PR, HER2-, CK5/6-, EGFR-).

HER2 evaluation

The HER2 test were evaluated according to the latest ASCO guideline.[16,17] For IHC analysis, IHC 0 was defined by no staining or incomplete weak membrane staining in ≤10% tumor cells. IHC 1+ was defined by incomplete weak staining in >10% of tumor cells. Tumor with weak to moderate complete membrane staining in ≥10% of tumor cells was scored as IHC 2+ and was considered equivocal. A homogeneous intense membrane staining of >10% tumor cells was considered IHC 3+. For HER2 IHC 2+ cases, reflex testing by IHC or FISH was performed to confirm HER2-low status. FISH analysis was performed using the PathVysion HER2 DNA probe kit (Vysis) following the manufacturer's protocol.

Statistical analysis

All analyses were performed using SPSS software (version 26). Chi-square analysis or Fisher's exact test was used to test for the association of categorical variables. Survival curves were estimated with the Kaplan–Meier method and the comparisons between groups were performed using a log-rank test. Multivariate Cox proportional hazards model with the backward Wald model was used to identify variables that were independently associated with survival. All statistical tests were two-sided, and p-value of <0.05 was considered statistically significant.

Ethics

The study was approved by Joint Chinese University of Hong Kong—New Territories East Cluster clinical research ethics committee (2014.500). Tissue from patients was acquired with informed consent following the local institutional review and the Declaration of Helsinki.

Role of funders

The Funders made it possible to conduct the study. They supported the cost of research reagents and salaries of supporting staffs. However, they did not have any role in study design, data collection, data analyses, interpretation, or writing of report.

Results

A total of 1464 HER2-negative cases were included. Among them, 1052 cases were HER2-zero and 412 cases were HER2-low (377 cases of IHC1+ and 35 cases of IHC2+ without amplification). The mean patients' age at diagnosis was 54.6 ± 12.9 years (range 22–101 years) and the mean tumor size was 2.56 ± 1.42 cm (range 0.1–10.2 cm). There were 237 (16.2%) grade I cases, 657 (44.9%) grade II cases and 570 (38.9%) grade III cases. One thousand three hundred and three cases (89.0%) were infiltrating duct carcinoma-not otherwise specified (IDC-NOS), 46 cases (3.1%) were ILC, 26 cases (1.8%) were mucinous carcinoma, 13 cases (0.9%) were metaplastic cancers, 33 cases (2.3%) were IDC with medullary phenotype and 43 cases (2.9%) were of other special histotypes. ER and PR positivity were found in 77.6% and 73.2% of the cases respectively; while 17.5% of cases were TNBC. Treatment data on chemotherapy (CT) and hormonal therapy (HT) were available for 1163 and 1171 patients respectively. Overall, there were 65.1% of patients received chemotherapy (CT) and 76.3% of them received hormonal therapy (HT). In HER2-low cancers, a similar proportion of patients received CT (66.2%) and a slightly higher proportion received HT (79.6%). No significant differences were found between the treatments received with HER2 expression.

Clinico-pathologic and biomarker association

Table 1 showed the clinico-pathologic association of HER2-low compared to HER2-zero cancers. Apart from the association with non-IDC (p = .045) and a trend with the absence of necrosis (p = .071), no differences were found in patients' age, tumor size, grade, apocrine features, fibrotic focus, sTIL, LVI, pT and pN stage. Similar results were found when comparing HER2-zero to IHC1+ cancers. Of note, IHC2+ cancers were significantly associated with the absence of fibrotic focus (p = .005* and .002*, compared with HER2-zero and IHC1+ respectively). When compared to HER2-zero caners, HER2-low status was positively associated with the expression of ER (p = .001#), AR (p < .001#), COX2 (p = .004#), pAKT (p < .001#), HLAb (p = .004#), HLAc (p = .022#), and the co-expression of HLA (p = .022#), but negatively with Ki67 (p = .028#) and CK14 (p = .012#). Despite its correlation with ER expression, there were no significant association with PR and no differential distribution in molecular subtypes by IHC. Additionally, the expression of EGFR, c-Kit, p63, CK5/6, tumoral PDL-1, PDL-1 positivity in immune cells and HLAa were also not associated with HER2-low cancers. Although PDL-1 did not appear to be enriched in HER2-low cancers, a remarkable population (32% of HER2-low cancers) showed PDL-1 expression in immune cells. The comparison between HER2-zero and IHC1+ cancers showed similar observations. For IHC2+ cases, we noted a trend of a negative association with COX2 expression (p = .089#) (Table 2).

Tables 3 and 4 showed the clinic-pathologic and biomarker association of HER2-low cancers according to HR status. The HER2-low luminal cancers were significantly correlated with a younger age (p = .016#) and a trend with large tumors (p = .083#). However, an opposite trend was observed in TNBC, being associated with older age (p = .035#) and smaller tumors (p = .027#). A significant differential distribution in histotypes was found only in luminal HER2-low cancers (p = .016*). By contrast, HER2-low TNBCs were associated with the absence of necrosis (p = .015) and a higher pN stage (p = .033#) (Table 3). Regarding biomarkers, HER2-low luminal cancers showed positive associations with ER (p = .016#), AR (p = .002#), pAKT (p < .001#), HLAa (p = .001#), HLAb (p = .001#), HLAc (p = .002#), and HLA co-expression (p = .001#), as well as a marginal association with c-kit (p = .082#) and COX2 (p = .054#). HER2-low TNBC, likewise, was positively associated with AR (p = .004#) and COX2 (p = .042#), but negatively with c-Kit (p = .018#) and CK14 (p = .022#). However, there was no association of HER2-low status with Ki67 when analysed based on HR status (Table 4). Intriguingly, different from IHC1+ luminal cancers, the IHC2+ cancers had a negative association with fibrotic focus (p = .027* and .008*, compared with HER2-zero and IHC1+ respectively) (Table 3) and were negatively associated with COX2 (p = .020#) (Table 4).

("*" indicated Fisher's exact test and "#" indicated chi-square analysis).

Survival

Follow-up data were available for 1282 patients. The median follow-up time was 73 months (range, 1–210 months). During the follow-up period, 192 cases of relapse/death were noted, with 135 patients died of breast cancer. There were no significant differences in DFS and BCSS between HER2-low and HER2-zero groups in the overall population and according to HR status (Fig. 1). The results were similar when compared according to HER2 IHC level (Fig. S1). Next, we characterised the prognostic features in HER2-low cancers and compared them to HER2-zero cancers. Conventional pathological features, including grade. LVI, pT, pN, ER, PR and Ki67 expression, demonstrated prognostic significance in both HER2-low and HER2-zero cancers (data not shown). Regarding immune features, sTIL and individual HLA were not associated with survival in the overall HER2-low cancers (Fig. 2 and Fig. S2). Interestingly, the HER2-low cases with co-expression of HLAs in all loci demonstrated a trend of better survival. Particularly in those with high sTIL, significantly better survival was found (Log-rank test-DFS: chi-sq = 6.945, p = .031; BCSS: chi-sq = 6.110, p = .047). Multivariate cox hazard analysis including age, grade, sTIL, LVI, pT stage, pN stage, ER, PR and Ki67 expression demonstrated that HLA co-expression status was an independent factor of DFS (Multivariate cox regression- HR = .173, p = 0.026, 95%CI = 0.037–0.814) in patients with HER2-low cancers (Table 5). By contrast, the HLA co-expression status was associated with the worst survival, particularly in those cases with low sTIL (Fig. 2).

Discussion

With the promising efficacy of novel anti-HER2 ADC,[1,2] HER2-low cancers have gained a lot of attention in recent research studies. Despite that, the characteristic of this cancer has not been fully examined. Currently its analysis is mostly focused on the standard clinico-pathologic features or common biomarkers expression and without considering the heterogeneity within the category. Here, we have characterised HER2-low cancers in comparison to HER2-zero cancers according to the different HER2-low subgroups, with a comprehensive panel of clinico-pathologic features and biomarkers. Similar to the earlier reports,[3,5–7] we found more HER2-low cancers that were HR-positive than TNBC and were associated with high ER expression as well as lower Ki67. Further, we found its positive correlations with AR, COX2, HLAs and pAKT, but a negative relationship with CK14. Notably, the significant correlations of AR and COX2 remain regardless of HR status. However, differential associations among HR subgroups were

Features	HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
Age							
≤50	469	183	19	.143	.195	.260	.515
>50	581	194	16				
Missing	2	0	0				
Tumor size							
≤2	480	164	13	.583	.693	.446	.549
>2	561	202	20				
Missing	11	11	12				
Grade							
1	163	64	10	.265	.307	.424	.672
2	472	175	10				
3	415	138	16				
Missing	2	1	0				
Apocrine							
Neg	970	342	31	.561	.601	.683	.835
Pos	73	29	3				
Missing	9	6	1				
Necrosis							
Neg	821	308	28	.071	.089	.658	.916
Pos	204	58	5				
Missing	27	11	2				
FF							
Neg	764	261	32	.717	.267	**.005**	**.002**
Pos	275	109	2				
Missing	13	7	1				
sTIL							
<20	735	206	19	.937	.954	.872	.896
≥20	215	61	6				
Missing	102	110	10				
LVI							
Neg	784	258	22	.317	.518	.105	.191
Pos	238	86	12				
Missing	30	33	1				
pT							
1	478	164	15	.671	.738	.551	.638
2	488	172	14				
3	43	21	3				
4	24	6	1				
Missing	19	14	2				
pN							
0	532	181	18	.247	.362	.294	.486
1	292	105	7				
2	106	52	6				
3	72	23	4				
Missing	50	16	0				
Histotype							
IDC-NOS	947	324	31	.066	.081	.620	.861
(Non-IDC NOS)	(105)	(52)	(4)	**(.045)**	**(.044)**	.773	1.00
ILC	30	15	1				
Mucinous	20	6	0				
Metaplastic	11	2	0				
MDL	20	11	2				
Others	24	18	1				
Missing	0	1	0				

(Table 1 continues on next page)

Features	HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
(Continued from previous page)							
Subtype							
Lum A	498	201	15	.122	.117	.466	.375
Lum B	345	124	15				
BLBC	91	25	1				
5NP	106	27	4				
Missing	12	0	0				
Chemotherapy							
No	312	87	7	.660	.862	.317	.362
Yes	573	164	20				
Missing	167	126	9				
Hormonal therapy							
No	220	48	9	.146	.063	.305	.080
Yes	672	204	18				
Missing	160	125	9				

Bold indicates p value <.05. FF, fibrotic focus; sTIL, stromal tumor infiltrating lymphocyte; LVI, lymphovascular invasion; IDC-NOS, invasive ductal carcinoma-not otherwise specified; ILC, invasive lobular cancer; MDL, IDC with medullary phenotype; Lum, luminal; BLBC, basal like breast cancer; 5NP, quintuple negative breast cancer.

Table 1: **Clinico-pathological features of HER2 negative breast cancer.**

found in other features. The HER2-low luminal cancers were associated with a younger age, higher expression of HLAs and pAKT, as well as a trend with a smaller tumor size and higher c-kit expression. By contrast, in the TNBC counterpart, opposite associations were found with patients' age, tumor size, and expression of c-kit. Also, HER2-low TNBC had association with an absence of necrosis, a higher pN stage and lower CK14. The differential clincio–pathological associations of HER2-low in cancers among the HR subgroups has also been recently reported.[7] Our data reaffirmed that characteristics of HER2-low cancers were determined by the HR status. This should be take into account in the management of HER2-low cancers.

Regardless of HR status, HER2-low status was associated with increased COX2 and AR expression. COX2, an enzyme catalysing the conversion of arachidonic acid to prostaglandin E2 (PGE2), has been shown to promote breast cancer development.[18] Its expression has been reported to correlate with HER2 overexpression.[19] On one hand, HER2 upregulation in breast cancers was found to be driven by COX2 expression through RAS/MAPK pathway.[20] On the other hand, COX2 and its product PGE2 can lead to the induction of HER2 expression.[21] In our findings, a significant positive correlation with COX2 was found when comparing IHC 0 to IHC1+, but an opposite trend was found when comparing IHC1+ to 2+. The differential correlation of IHC1+ and 2+ HER2-low cases was also observed with fibrotic focus. These may suggest differences could exist between the two groups. Nonetheless, a positive feedback loop of COX2 and HER2 pathways could be at least operated among the IHC1+ cases. Notably, a recent study suggested detrimental effects on anti-tumor immunity by the upregulation of COX2 in breast cancer cells with pre-existing COX2 activity after chemotherapy. The pharmacological inhibition of COX2 alongside immune checkpoint blockade and chemotherapy showed a better tumor control.[22] In the HER2-low setting, one could speculate that a similar situation could also be implicated. In TNBCs, the molecular apocrine phenotype (defined by co-expression of AR and FOXA1) was demonstrated to be more frequent in HER2-low than HER2-zero cancers.[23] Supporting this result, we showed the positive association of HER2-low status with AR in TNBC. Such relationship was also demonstrated in luminal cancers. Mechanistically, a direct upregulation of the *HER2* gene by AR and an upregulation of *AR* gene by cAMP response element binding protein 1 as a result of HER2-stimulated ERK activity were reported,[24] explaining their positive correlation in our data. The co-expression of AR and HER2-low status, together with the reported functional interaction between the two pathways, could also have clinical implications. AR targeting strategies have been evaluated in breast cancers, including HER2+ tumors and TNBC. Results from a recent phase II trial on the efficacy of combining enzalutamide and trastuzumab in patients with metastatic advanced HER2+AR + breast cancer previously progressed on trastuzumab showed durable disease control on a subset of patients.[25] Notably, 33.3% of anthracycline-insensitive patients with AR + TNBC achieved pCR or RCB-I with enzalutamide and paclitaxel treatment.[26] The AR-associated

	HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
ER							
0	256	64	6	**.001**	**.001**	.334	.895
1–10	25	5	1				
>10	764	307	28				
Missing	7	1	0				
PR							
0	289	88	11	.378	.221	.407	.206
1–20	85	39	4				
>20	668	249	19				
Missing	10	1	1				
AR							
Neg	315	125	8	**<.001**	**<.001**	**.021**	.358
Pos	253	177	17				
Missing							
Ki67							
Low	675	268	22	**.028**	**.017**	.785	.262
High	362	105	13				
Missing	15	4	0				
EGFR							
Neg	995	363	33	.248	.179	.659	.275
Pos	44	10	2				
Missing	13	4	0				
c-Kit							
Neg	897	328	30	.741	.694	.871	.766
Pos	138	47	5				
Missing	17	2	1				
P63							
Neg	1000	361	34	.998	.949	1.00	1.00
Pos	38	14	1				
Missing	14	2	1				
CK5/6							
Neg	898	327	29	.441	.402	.883	.658
Pos	144	45	5				
Missing	10	5	1				
CK14							
Neg	955	356	35	**.012**	**.032**	.078	.385
Pos	85	18	0				
Missing	12	3	0				
PDL-1 (tumor)							
Neg	883	309	31	.134	.228	.357	.493
Pos	98	26	1				
Missing	71	42	3				
PDL-1 (immune cell)							
Neg	714	237	20	.371	.526	.201	.317
Pos	293	106	13				
Missing	53	37	2				
HLAa							
Lo	441	205	18	.138	.174	.381	.727
Hi	134	78	8				
Missing	477	94	10				
HLAb							
Lo	411	174	16	**.004**	**.004**	.315	.961
Hi	172	111	10				
Missing	471	92	9				

(Table 2 continues on next page)

	HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
(Continued from previous page)							
HLAc							
Lo	370	159	15	**.032**	**.030**	.674	.741
Hi	206	122	10				
Missing	476	96	11				
HLA co-expression							
All low	285	116	11	**.022**	**.029**	.478	.927
Mixed	169	93	8				
(Mixed/low)	(454)	(209)	(19)	(.062)	**(.046)**	(.253)	(.738)
All hi	83	56	6				
Missing							
COX2							
Lo	317	131	15	**.004**	**.001**	.521	.089
Hi	225	149	8				
Missing	510	97	13				
pAKT							
Lo	191	41	2	**<.001**	**<.001**	**.007**	.549
Hi	351	236	22				
Missing	510	100	12				

Bold indicates p value <.05. ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; EGFR, epidermal growth factor receptor; CK, cytokeratin; PDL-1, programmed death ligand 1; HLA, human leucocyte antigen; COX2, cyclooxygenase 2.

Table 2: **Biomarker association of HER2 negative breast cancer.**

protein, FOXA1 which functionally co-operated and co-expressed with AR in breast cancer, has been shown to be a determinant of chemotherapy resistance in breast cancer cells.[27] All these point towards the possibility of AR inhibition may also work together with the novel anti-HER2 ADC and increase its clinical benefit in breast cancer treatment.

Another interesting finding was the prognostic role of immune features in HER2-low cancers. Although the subject of investigation, the prognostic factors in HER2-low cancers were not well characterized. We demonstrated the association of immune features, namely the co-expression of HLAs, with better survival in HER2-low cancers, specifically in those tumors with high TILs. Similar results were found in HER2+ cancers in our earlier study.[14] On the contrary, HER2-zero cancers with HLA co-expression showed the worst survival. The data revealed further differences between HER2-low and HER2-zero cancers. The high TIL level may suggest the presence of pre-existing immune surveillance. However, to elicit the effector functions, antigen presentation by HLA expressed on tumor cells is also an important prerequisite. Recent evidence from ICB treatment has also demonstrated that patients with HLA loss was resistant to ICB treatment, even in tumor having an inflammatory microenvironment[28] or high TIL infiltration.[29] In fact, the prognostic impact of CD8+ T cells was found only in HLA intact microsatellite unstable gastric cancer.[30] HLA expression in HER2-low cancers, having an intact antigen presentation in the presence of high TIL, favors anti-tumor immunity for controlling tumor progression. Multiple mechanisms are involved in immune evasion. Tumors can lose their HLA expression via reversible or irreversible processes.[31] Similarly, their intrinsic properties could govern TIL activity against tumor and dynamic of TIL level.[32] It is conceivable that the different intrinsic properties of HER2-low and HER2-zero cancers could have impact on anti-tumor immunity. With the emergence of anti-HER2-ADC treatment on HER2-low cancers, understanding the features that associated with the cancer outcome could be important. The therapeutic actions of anti-HER2 ADC are expected to act in coordination with immune responses. As with Trastuzumab, it could activate antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis.[33,34] Similar to conventional chemotherapy, tumor cell killing by the payload could increase tumor antigen presentation in the tumor microenvironment, consequently activation of anti-tumor immunity. As shown in patients treated with T-DM1 (the other anti-HER2 ADC), an increased TIL level was found after the treatment.[35] Preclinical models have shown that T-Dxd enhanced antitumor immunity. Interestingly, the study also found that T-Dxd combined with PD-1 antibody was more effective than monotherapy with either agent alone.[36] Moreover, a robust

Features	Lum							TNBC						
	HER2			p-value				HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
Age														
≤50	377	166	19	**.016**	**.040**	**.041**	.199	90	17	0	**.035**	.098	.067	.308
>50	472	159	11					108	35	5				
Tumor size														
≤2	419	141	11	.083	.111	.270	.586	59	23	2	**.027**	**.028**	.636	.797
>2	421	175	17					140	27	3				
Grade														
1	156	63	9	.916	.941	.932	.917	6	1	1	.118	.240	.070	.218
2	442	162	9					28	13	1				
3	250	100	13					165	38	3				
Apocrine														
Neg	798	301	28	.941	.838	1.00	1.00	169	41	3	.121	.282	.160	.319
Pos	45	18	1					28	11	2				
Necrosis														
Neg	719	271	26	.900	.705	.564	.557	99	37	2	**.015**	**.007**	1.00	.312
Pos	106	43	2					98	15	3				
FF														
Neg	622	225	27	.565	.222	**.027**	**.008**	139	36	5	.762	.931	.324	.308
Pos	215	93	2					60	16	0				
sTIL														
<20	636	183	17	.336	.332	.761	.773	97	23	2	.607	.432	.660	.379
≥20	136	47	3					79	14	3				
LVI														
Neg	623	225	18	.751	.934	.100	.106	159	33	4	.101	.085	1.00	1.00
Pos	202	72	11					36	14	1				
pT														
1	417	141	13	.134	.160	.377	.698	59	23	2	.160	.155	.826	.797
2	374	150	12					114	22	2				
3	27	17	2					16	4	1				
4	15	4	1					9	2	0				
pN														
0	419	159	14	.803	.996	.255	.257	111	22	4	**.033**	**.022**	.923	.453
1	237	92	7					55	13	0				
2	91	43	6					15	9	0				
3	58	17	3					13	6	1				
Histotype														
IDC	772	280	27	**.016**	**.019**	.717	.919	173	44	4	.611	.551	.896	.613
(Non-IDC NOS)	(78)	(44)	(3)	**(.038)**	**(.032)**	(.751)	(.781)	(26)	(8)	(1)	(.662)	(.653)	(.512)	(1.00)
ILC	26	12	1					3	3	0				
Mucinous	19	6	0					1	0	0				
Metaplastic	4	0	0					7	2	0				
MDL	7	8	1					13	3	1				
Others	22	18	1					2	0	0				
Subtype														
Lum A	498	201	15	.544	.387	.321	.203	–	–	–	.914	.832	.378	.362
Lum B	345	124	15					–	–	–				
BLBC	–	–	–					91	25	1				
5NP	–	–	–					105	27	4				
Chemotherapy														
No	264	77	6	.462	.638	.282	.856	43	10	1	.596	.588	1.00	1.00
Yes	448	141	17					121	22	3				

(Table 3 continues on next page)

Features	Lum							TNBC						
	HER2			p-value				HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
(Continued from previous page)														
Hormonal therapy														
No	77	19	7	.591	.387	.097	.169	–	–	–				
Yes	641	201	36					–	–	–				

Bold indicates p value <.05.

Table 3: **Clinico-pathological features of HER2 negative breast cancer according to HR status.**

Features	Lum							TNBC						
	HER2			p-value				HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
ER														
0	57	12	1	**.016**	**.020**	.504	.889	198	52	5	–	–	–	–
1–10	25	5	1											
>10	764	307	28											
PR														
0	91	36	6	.255	.543	.061	.115	198	52	5	–	–	–	–
1–20	85	39	4											
>20	668	249	19											
AR														
Neg	224	100	6	**.002**	**.004**	**.048**	.310	91	25	2	**.004**	**.006**	.415	1.00
Pos	235	162	19					17	15	1				
Ki67														
Low	587	239	19	.209	.131	.437	.187	88	29	3	.119	.145	.659	1.00
High	252	82	11					110	23	2				
EGFR														
Neg	821	314	28	.855	.842	.173	.175	173	49	5	.220	.220	1.00	1.00
Pos	20	7	2					24	3	0				
c-Kit														
Neg	761	286	25	.082	.138	.145	.428	134	42	5	**.018**	**.040**	.328	.580
Pos	74	38	5					64	9	0				
P63														
Neg	818	313	29	.804	.824	.588	1.00	182	48	5	1.00	1.00	1.00	1.00
Pos	24	10	1					14	4	0				
CK5/6														
Neg	779	301	25	.870	.614	.269	.140	117	26	4	.572	.425	.650	.382
Pos	62	21	4					82	24	1				
CK14														
Neg	808	311	30	.558	.751	.622	.609	144	45	5	**.022**	**.044**	.329	1.00
Pos	32	11	0					53	7	0				
PDL-1 (tumor)														
Neg	714	271	26	.084	.130	.504	1.00	166	38	5	.767	.587	1.00	1.00
Pos	78	20	1					20	6	0				
PDL-1 (immune cell)														
Neg	623	215	18	.117	.193	.138	.347	88	22	2	.958	.976	1.00	1.00
Pos	192	81	10					101	25	3				
HLAa														
Lo	376	177	16	**.008**	**.012**	.175	.737	65	28	2	.199	.202	1.00	1.00
Hi	88	66	7					46	12	1				

(Table 4 continues on next page)

Features	Lum							TNBC						
	HER2			p-value				HER2			p-value			
	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+	0	1+	2+	0 vs low	0 vs 1+	0 vs 2+	1+ vs 2+
(Continued from previous page)														
HLAb														
Lo	353	154	15	**.001**	**.001**	.296	1.00	58	20	1	.763	.877	.605	1.00
Hi	118	92	8					52	19	2				
HLAc														
Lo	317	139	13	**.002**	**.002**	.325	1.00	51	20	2	.372	.453	.587	1.00
Hi	142	103	9					64	19	1				
HLA co-expression														
All low	247	102	10	**.001**	**.001**	.215	.852	38	14	1	.562	.542	.993	.957
Mixed	137	78	7					32	15	1				
(Mixed/low)	(384)	(180)	(17)	**(.001)**	.090	.521	.793	(70)	(29)	(2)	(.360)	(.340)	(1.00)	(1.00)
All high	47	46	5					36	10	1				
COX2														
Lo	230	104	14	.054	**.017**	.130	**.020**	87	27	1	**.042**	.094	.094	.254
Hi	206	137	6					19	12	2				
pAKT														
Lo	156	32	2	**<.001**	**<.001**	**.010**	.749	35	9	0	.235	.308	1.00	1.00
Hi	281	208	20					70	28	2				

Bold indicates p value <.05.

Table 4: **Biomarker association of HER2 negative breast cancer according to HR status.**

treatment benefit of PD-L1 blockade was demonstrated for previously untreated non-metastatic TNBC. The benefit appeared to be consistent across HER2 IHC2+ and HER2 IHC 0–1 subgroups.[37] Currently, combination therapies of anti-HER2 ADC and immune checkpoint blockade are being evaluated in clinical trials

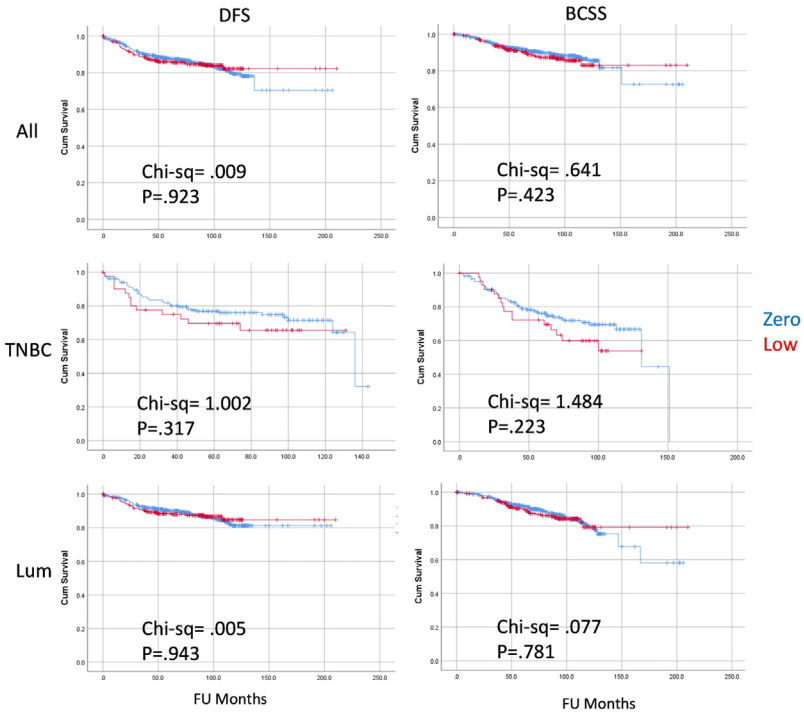

Fig. 1: Impact of HER2-low status on survival in HER2 negative cases.

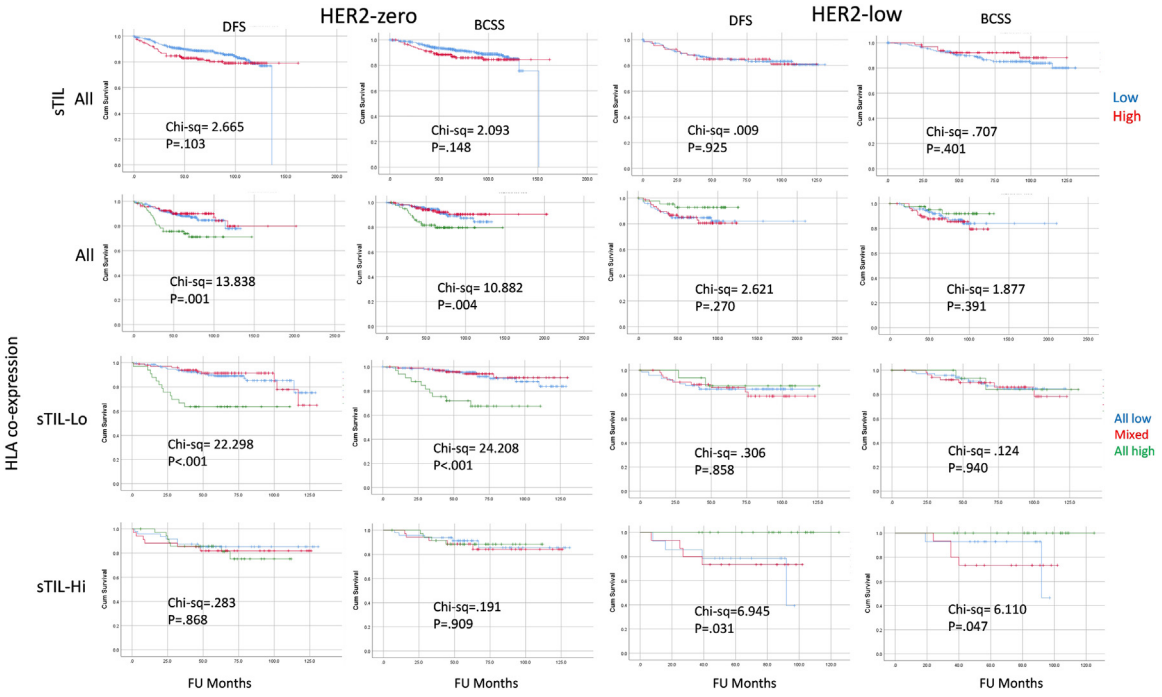

Fig. 2: Impact of sTIL and HLA co-expression status on survival in HER2-zero and HER2-low population.

(NCT02318901, NCT04042701). Immune features have been suggested to be predictive for ICB response, not only in TNBC, but also in luminal B cancers.[38] Apart from high TIL and PD-L1 expression, it has become increasingly evident that HLA-I phenotype could also determine the clinical success of immune checkpoint blockade.[29,39] In our cohort, a substantial proportion of HER2-low cancers was PD-L1 positive (32% with PD-L1 expression in immune cell) and high level of HLA expression further suggesting the validity of the combined treatment. It will be interesting to assess the predictive value of HLA expression and other immune features for anti-HER2 ADC response and also the combination therapy.

Limitations of study included the limited number of cases particularly in the HER2 IHC 2+ group for

	HR	Lower 95% CI	Upper 95% CI	P-value
DFS				
LVI	2.900	1.149	7.320	**.024**
pT	2.235	1.037	4.816	**.040**
pN	1.577	1.027	2.423	**.038**
Ki67	2.207	1.003	4.857	**.049**
PR	0.576	0.371	0.895	**.014**
HLA co-expression				
All low	(ref)			.061
Mixed low/high	1.136	0.507	2.547	.757
All high	0.173	0.037	0.814	**.026**
BCSS				
Age	1.035	0.999	1.072	.054
LVI	4.947	2.930	12.680	**.001**
pT	3.485	1.561	7.782	**.002**
PR	0.627	0.392	1.003	.052

Initial factors: Age, Grade, pT, pN, LVI, sTIL, Ki67, ER, PR status, HLA co-expression status (All low, mixed low/high, All high). Bold indicates p value <.05.

Table 5: **Multivariate Cox regression analysis on HER2-low population.**

subgroup analysis and the use of TMA for IHC analysis. The limited number of cases will affect the power of statistical analysis and further studies are required to validate the findings. Although analysis on TMA has high concordance with that on whole section, they may not completely agree in a minority of heterogeneous tumors. Bias could be introduced by loss to follow-up. However, the loss mainly related to random events, thus would have minimal impact on the final conclusion.

In summary, our study revealed further characteristics of HER2-low breast cancers. These features varied with the HR status. A high heterogeneity could exist within the HER2-low category. Research studies are in progress to dissect the heterogeneity within the category in genomic level.[40] Moreover, the existence of AR and COX2 signaling in HER2-low cancers, providing further insights into the biology of this cancer. The clinical relevance of their expression in HER2-low merits further investigations. Immune feature, namely co-expression of HLAs, was found to be associated with better survival in HER2-low cancers. HER2 It will be interesting to explore if it can be predictive for the novel anti-HER2 ADC. Ongoing clinical trials have been extended from evaluating the efficacy of T-DxD alone to its combined treatment with immunotherapy, endocrine therapies, cyclin-dependent kinase inhibitors, among others. Further characterization of the HER2-low category in light of predictive factors for other treatments and those beyond HER2-low expression are required.

Contributors

YL: data acquisition, data verification, drafting of manuscript; JT: data verification, data analysis and interpretation, drafting of manuscript; FT, TL: data acquisition: GT: study concept and design, study supervision, drafting of manuscript. All authors read and approved the final version to be published.

Data sharing statement

Data are available upon reasonable request.

Declaration of interests

All authors declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104571.

References

1 Nakada T, Sugihara K, Jikoh T, Abe Y, Agatsuma T. The latest research and development into the antibody-drug conjugate, [fam-] trastuzumab deruxtecan (DS-8201a), for HER2 cancer therapy. *Chem Pharm Bull (Tokyo)*. 2019;67(3):173–185.
2 Modi S, Saura C, Yamashita T, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. *N Engl J Med*. 2020;382(7):610–621.
3 Schettini F, Chic N, Braso-Maristany F, et al. Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. *NPJ Breast Cancer*. 2021;7(1):1.
4 Tarantino P, Hamilton E, Tolaney SM, et al. HER2-Low breast cancer: pathological and clinical landscape. *J Clin Oncol*. 2020;38(17):1951–1962.
5 Denkert C, Seither F, Schneeweiss A, et al. Clinical and molecular characteristics of HER2-low-positive breast cancer: pooled analysis of individual patient data from four prospective, neoadjuvant clinical trials. *Lancet Oncol*. 2021;22(8):1151–1161.
6 Zhang H, Katerji H, Turner BM, Audeh W, Hicks DG. HER2-low breast cancers: incidence, HER2 staining patterns, clinicopathologic features, MammaPrint and BluePrint genomic profiles. *Mod Pathol*. 2022;35(8):1075–1082.
7 Won HS, Ahn J, Kim Y, et al. Clinical significance of HER2-low expression in early breast cancer: a nationwide study from the Korean Breast Cancer Society. *Breast Cancer Res*. 2022;24(1):22.
8 Agostinetto E, Rediti M, Fimereli D, et al. HER2-Low breast cancer: molecular characteristics and prognosis. *Cancers (Basel)*. 2021;13(11).
9 Menard S, Balsari A, Tagliabue E, et al. Biology, prognosis and response to therapy of breast carcinomas according to HER2 score. *Ann Oncol*. 2008;19(10):1706–1712.
10 Almstedt K, Heimes AS, Kappenberg F, et al. Long-term prognostic significance of HER2-low and HER2-zero in node-negative breast cancer. *Eur J Cancer*. 2022;173:10–19.
11 Rossi V, Sarotto I, Maggiorotto F, et al. Moderate immunohistochemical expression of HER-2 (2+) without HER-2 gene amplification is a negative prognostic factor in early breast cancer. *Oncologist*. 2012;17(11):1418–1425.
12 Tan R, Ong WS, Lee KH, et al. HER2 expression, copy number variation and survival outcomes in HER2-low non-metastatic breast cancer: an international multicentre cohort study and TCGA-METABRIC analysis. *BMC Med*. 2022;20(1):105.
13 Ni Y, Tsang JY, Shao Y, et al. Combining analysis of tumor-infiltrating lymphocytes (TIL) and PD-L1 refined the prognostication of breast cancer subtypes. *Oncologist*. 2022;27(4):e313–e327.
14 Tsang JY, Ho CS, Ni YB, et al. Co-expression of HLA-I loci improved prognostication in HER2+ breast cancers. *Cancer Immunol Immunother*. 2020;69(5):799–811.
15 Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the st. Gallen international expert consensus on the primary therapy of early breast cancer 2011. *Ann Oncol*. 2011;22(8):1736–1747.
16 Wang C, Tsang JY, Poon IK, et al. An evaluation of clinicopathological correlation and outcome of human epidermal growth factor receptor 2 subgroups reclassified according to the latest ASCO/CAP guideline. *Clin Breast Cancer*. 2022;22(1):e114–e122.
17 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*. 2018;36(20):2105–2122.
18 Hugo HJ, Saunders C, Ramsay RG, Thompson EW. New insights on COX-2 in chronic inflammation driving breast cancer growth and metastasis. *J Mammary Gland Biol Neoplasia*. 2015;20(3–4):109–119.
19 Nassar A, Radhakrishnan A, Cabrero IA, Cotsonis G, Cohen C. COX-2 expression in invasive breast cancer: correlation with prognostic parameters and outcome. *Appl Immunohistochem Mol Morphol*. 2007;15(3):255–259.
20 Subbaramaiah K, Norton L, Gerald W, Dannenberg AJ. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer: evidence for involvement of AP-1 and PEA3. *J Biol Chem*. 2002;277(21):18649–18657.
21 Benoit V, Relic B, Leval Xd X, Chariot A, Merville MP, Bours V. Regulation of HER-2 oncogene expression by cyclooxygenase-2 and prostaglandin E2. *Oncogene*. 2004;23(8):1631–1635.
22 Bell CR, Pelly VS, Moeini A, et al. Chemotherapy-induced COX-2 upregulation by cancer cells defines their inflammatory properties and limits the efficacy of chemoimmunotherapy combinations. *Nat Commun*. 2022;13(1):2063.
23 Jacot W, Maran-Gonzalez A, Massol O, et al. Prognostic value of HER2-low expression in non-metastatic triple-negative breast cancer and correlation with other biomarkers. *Cancers*. 2021;13(23).
24 Naderi A, Hughes-Davies L. A functionally significant cross-talk between androgen receptor and ErbB2 pathways in estrogen receptor negative breast cancer. *Neoplasia*. 2008;10(6):542–548.

25 Wardley A, Cortes J, Provencher L, et al. The efficacy and safety of enzalutamide with trastuzumab in patients with HER2+ and androgen receptor-positive metastatic or locally advanced breast cancer. *Breast Cancer Res Treat*. 2021;187(1):155–165.
26 Lim B, Seth S, Huo L, et al. Comprehensive profiling of androgen receptor-positive (AR+) triple-negative breast cancer (TNBC) patients (pts) treated with standard neoadjuvant therapy (NAT) +/- enzalutamide. *J Clin Oncol*. 2020;38:517.
27 Kumar U, Ardasheva A, Mahmud Z, Coombes RC, Yague E. FOXA1 is a determinant of drug resistance in breast cancer cells. *Breast Cancer Res Treat*. 2021;186(2):317–326.
28 Gettinger S, Choi J, Hastings K, et al. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. *Cancer Discov*. 2017;7(12):1420–1435.
29 Zaretsky JM, Garcia-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med*. 2016;375(9):819–829.
30 Kwak Y, Koh J, Park Y, et al. Differential prognostic impact of CD8(+) T cells based on human leucocyte antigen I and PD-L1 expression in microsatellite-unstable gastric cancer. *Br J Cancer*. 2020;122(9):1399–1408.
31 Hazini A, Fisher K, Seymour L. Deregulation of HLA-I in cancer and its central importance for immunotherapy. *J Immunother Cancer*. 2021;9(8).
32 Lanitis E, Dangaj D, Irving M, Coukos G. Mechanisms regulating T-cell infiltration and activity in solid tumors. *Ann Oncol*. 2017;28(suppl_12):xii18–xii32.
33 Tai YT, Mayes PA, Acharya C, et al. Novel anti-B-cell maturation antigen antibody-drug conjugate (GSK2857916) selectively induces killing of multiple myeloma. *Blood*. 2014;123(20):3128–3138.
34 Griguolo G, Pascual T, Dieci MV, Guarneri V, Prat A. Interaction of host immunity with HER2-targeted treatment and tumor heterogeneity in HER2-positive breast cancer. *J Immunother Cancer*. 2019;7(1):90.
35 Muller P, Kreuzaler M, Khan T, et al. Trastuzumab emtansine (T-DM1) renders HER2+ breast cancer highly susceptible to CTLA-4/PD-1 blockade. *Sci Transl Med*. 2015;7(315):315ra188.
36 Iwata TN, Ishii C, Ishida S, Ogitani Y, Wada T, Agatsuma T. A HER2-targeting antibody-drug conjugate, trastuzumab deruxtecan (DS-8201a), enhances antitumor immunity in a mouse model. *Mol Cancer Ther*. 2018;17(7):1494–1503.
37 Schmid P, Cortes J, Dent R, et al. KEYNOTE-522 study of neoadjuvant pembrolizumab + chemotherapy vs placebo + chemotherapy, followed by adjuvant pembrolizumab vs placebo for early-stage TNBC: event-free survival sensitivity and subgroup analyses. *Cancer Res*. 2022;82:GS1-01.
38 Dieci MV, Guarneri V, Tosi A, et al. Neoadjuvant chemotherapy and immunotherapy in luminal B-like breast cancer: results of the phase II GIADA trial. *Clin Cancer Res*. 2022;28(2):308–317.
39 Chowell D, Morris LGT, Grigg CM, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science*. 2018;359(6375):582–587.
40 Berrino E, Annaratone L, Bellomo SE, et al. Integrative genomic and transcriptomic analyses illuminate the ontology of HER2-low breast carcinomas. *Genome Med*. 2022;14(1):98.