DOI:10.1093/jnci/dju152

ARTICLE

Molecular Features and Survival Outcomes of the Intrinsic Subtypes Within HER2-Positive Breast Cancer

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Manuscript received November 16, 2013; revised April 18, 2014; accepted May 1, 2014.

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Background The clinical impact of the biological heterogeneity within HER2-positive (HER2+) breast cancer is not fully understood. Here, we evaluated the molecular features and survival outcomes of the intrinsic subtypes within HER2+ breast cancer.

Methods We interrogated The Cancer Genome Atlas (n = 495) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) datasets (n = 1730) of primary breast cancers for molecular data derived from DNA, RNA and protein, and determined intrinsic subtype. Clinical HER2 status was defined according to American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines or DNA copy-number aberration by single nucleotide polymorphism arrays. Cox models tested the prognostic significance of each variable in patients not treated with trastuzumab (n = 1711).

Results Compared with clinically HER2 (cHER2)-negative breast cancer, cHER2+ breast cancer had a higher frequency of the HER2-enriched (HER2E) subtype (47.0% vs 7.1%) and a lower frequency of Luminal A (10.7% vs 39.0%) and Basal-like (14.1% vs 23.4%) subtypes. The likelihood of cHER2-positivity in HER2E, Luminal B, Basal-like and Luminal A subtypes was 64.6%, 20.0%, 14.4% and 7.3%, respectively. Within each subtype, only 0.3% to 3.9% of genes were found differentially expressed between cHER2+ and cHER2-negative tumors. Within cHER2+ tumors, HER2 gene and protein expression was statistically significantly higher in the HER2E and Basal-like subtypes than either luminal subtype. Neither cHER2 status nor the new 10-subtype copy number-based classification system (IntClust) added independent prognostic value to intrinsic subtype.

Conclusions When the intrinsic subtypes are taken into account, cHER2-positivity does not translate into large changes in the expression of downstream signaling pathways, nor does it affect patient survival in the absence of HER2 targeting.

JNCI J Natl Cancer Inst (2014) 106(8): dju152

HER2/ERBB2 is an oncogene coding for a tyrosine kinase receptor that activates critical signal transduction pathways resulting in an aggressive phenotype and poor outcome in breast cancer (1–3). Fortunately, amplification and/or overexpression of HER2 in breast cancer (HER2+) are associated with a high benefit from anti-HER2 therapies in combination with chemotherapy (4–8). In addition, dual HER2 targeting without chemotherapy is showing promising activity in a subset of HER2+ tumors (9,10).

To date, HER2+ breast cancer has been envisioned as a single disease entity, as hormone receptor-positive breast cancer had also been initially considered a uniform disease subtype. While the reason to consider HER2+ breast cancer as a single disease subtype may have been dictated by the dominant role of the HER2 receptor itself as well as the availability of the anti-HER2 agent trastuzumab, it is now increasingly apparent that HER2+ is clinically and biologically heterogeneous (11–17).

Gene expression profiling has identified four main intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2 enriched [HER2E], and Basal-like) with different outcomes and responses to therapy (18–30). Among the different subtypes, the HER2E subtype is characterized by the high expression of HER2-regulated genes and low expression of luminal-related (12–15).

Although the HER2E subtype largely overlaps with cHER2-positivity as determined by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH), all of the intrinsic subtypes can be identified within cHER2+ breast cancer (12–14). Conversely, HER2E tumors are also identified within cHER2-negative tumors (12–14); therefore, the apparent similar classifications are sufficiently different that each should be considered unique.

Recently, a combined analysis of gene expression and DNA copy-number data has identified 10 different subtypes (known as IntClust 1–10) with different survival outcomes (31). Among them,

the IntClust-5 subtype is characterized by the amplification of the HER2 chromosomal amplicon (31). However, similar to cHER2+ heterogeneity, all of the intrinsic subtypes can be identified within the IntClust-5 subtype (31).

Surrogate pathology-based definitions of the intrinsic subtypes are an integral part of the St. Gallen Expert Consensus Guidelines for the recommendation of chemotherapy, endocrine therapy, and/or anti-HER2 therapy in early breast cancer (32). However, St. Gallen´s criteria that divide HER2+ disease into two groups (i.e. HER2+/ER+ and HER2+/ER-) are driven by treatment considerations mostly based on the necessity to recommend endocrine therapy for patients with hormone receptor-positive tumors, while chemotherapy and anti-HER2 therapy is recommended for both (32). The purpose of this work is to review the molecular features and the behaviors of the different subgroups of HER2+ breast cancer.

Methods

Patients, Samples, and Clinical Data

Two independent publicly available breast cancer datasets (The Cancer Genome Atlas Project [TCGA] and Molecular Taxonomy of Breast Cancer International Consortium [METABRIC]) were evaluated (12,31). TCGA includes multiple molecular data-types in 825 primary breast tumors: mRNA expression (17 814 unique genes), DNA copy-number changes (19 780 genes), protein expression (171 proteins and phosphoproteins), DNA methylation status (21 986 CpG sites of 14 475 genes), miRNA expression (306 transcripts) and whole exome somatic mutations. All data is publicly available at TCGA website (http://cancergenome.nih.gov/) (12). The METABRIC dataset (31) includes breast cancer-specific survival data as well as whole gene expression and DNA copy-number data of 1992 resected primary breast tumors. No patient with cHER2+ disease received anti-HER2 therapy. All clinical and genomic data is publicly available at the European Genome-phenome Archive (EGAS00000000083) (31).

HER2 Clinical Status

cHER2 status in the TCGA dataset (12) was determined using the 2007 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines (33), and for those cases that lacked IHC/FISH data, DNA copy-number data was used to determine HER2 amplification status. In the METABRIC data set, cHER2 status was based on DNA copy-number changes (31). Of note, classifying cHER2 based only on gene amplification may miss 0.5%–7.3% of tumors that are overexpressing the HER2 protein but are HER2 non-amplified (33). Tumor samples without cHER2 status were excluded from the analysis.

PAM50 Subtyping

Intrinsic subtyping was performed using the research-based 50-gene prediction analysis of microarray (PAM50) subtype predictor, which classifies tumors into the following groups: Luminal A, Luminal B, HER2E, Basal-like and Normal-like (13). Samples without PAM50 data, or those identified as Normal-like (which often represent inadequate tumor cellularity), were excluded from the analysis. From TCGA, we used the subtype calls as previously reported (12). From the METABRIC dataset, we applied the PAM50 predictor in the normalized gene expression data obtained from the EGA website after performing median centering of the PAM50 genes and column standardization.

Statistical Analysis

Statistically significant differences in molecular features between groups were evaluated using an unpaired two class Significance Analysis of Microarrays (SAM) (34). Differences in the distribution of subtypes in cHER2+ vs cHER2-negative disease were analyzed by the chi-square (χ^2) test or the Fisher´s exact test. Estimates of breast cancer-specific survival were from the Kaplan-Meier curves and tests of differences by the log-rank test. Univariate and multivariable Cox-models were used to test the independent prognostic significance of each variable. The assumption of proportionality was verified by estimating the slope of the Schoenfeld residuals.

To test the prognostic contribution of cHER2 status and the PAM50 subtypes, we estimated the log likelihood ratio statistic of each variable as an addition to a model containing the following clinical variables in the METABRIC dataset: cohort (discovery vs validation), tumor size, nodal status, systemic treatment (none, endocrine therapy only, endocrine and cytotoxic therapies) and menopausal status (31). In addition, we estimated the log likelihood ratio statistic of each variable as an addition to a model containing clinical variables, and the other variable being evaluated (cHER2 status or PAM50 subtypes). Finally, we repeated the same analysis using the METABRIC 10-subtype classification (IntClust) and the PAM50 subtypes in the validation cohort only (13,31). The performance of each variable was also compared using receiver operating characteristic (ROC) curve analysis. All statistical tests were two sided, and the statistical significance level was set to less than 0.05.

Results

Distribution of the Intrinsic Subtypes Based on cHER2 Status

Clinical-pathological features, subtype distribution, and key molecular data-types for 481 clinically HER2+ (cHER2+) tumors were evaluated from two independent datasets of primary breast cancer (Table 1). In a combined analysis of both datasets, all intrinsic subtypes were identified regardless of cHER2 status (Figure 1), although HER2E tumors were far more frequent among cHER2+ disease (47.0% vs 7.1%). Overall, cHER2-positivity enriched 6.62-fold for the HER2E subtype ($P < .001$, χ^2 test) and diminished 3.65-fold and 1.66-fold for the Luminal A (10.7% vs 39.0%) and Basal-like (14.1% vs 23.4%) subtypes, respectively ($P < .001$ for both, χ^2 test). The proportion of Luminal B tumors based on cHER2 status (28.2% in cHER2+ and 30.4% in cHER2-negative) was not statistically significantly different ($P = .39$, χ^2 test). Counter to common perception, while there were more Luminal B (28.2%) than Luminal A (10.7%) tumors, both luminal subtypes were represented within cHER2+ disease (32).

Gene Expression Features of the Intrinsic Subtypes Based on cHER2 Status

Similar distributions of cHER2-positivity were identified in each subtype across both datasets (Table 2), except for the Basal-like

Table 1. Clinical-pathological features of the 2 breast cancer datasets evaluated in this study

Clinical-pathological features	TCGA*	METABRIC*
Patients, No. (%)	495 (100)	1730 (100)
Mean age (range), y	58.0 (26.0–90.0)	61.7 (22.0–96.3)
Tumor size, No. (%)		
T0-T1	123 (24.8)	732 (42.7)
T2-T3-T4-TX	372 (75.2)	982 (57.3)
Nodal status, No. (%)		
N0	246 (49.7)	875 (51.1)
N1-N3-NX	249 (50.3)	839 (48.9)
cHER2 status, No. (%)		
cHER2-negative	420 (84.8)	1324 (76.5)
cHER2-positive	75 (15.2)	406 (23.5)
PAM50 subtype, No. (%)		
Luminal A	223 (45.1)	512 (29.6)
Luminal B	122 (24.6)	539 (31.2)
HER2-enriched	55 (11.1)	295 (17.1)
Basal-like	95 (19.2)	384 (22.2)
Available genomic data-types		
Gene Expression	Yes	Yes
DNA Copy-number	Yes	Yes
Protein Expression	Yes	No
DNA Methylation	Yes	No
miRNA Expression	Yes	No
Breast cancer survival data	No	Yes

* Inclusion criteria: primary tumors with cHER2 and PAM50 data. Normal-like tumor samples were excluded. T = tumor stage; N = nodal stage; cHER2 = clinical HER2 status; TCGA = the cancer genome atlas; METABRIC = molecular taxonomy of breast cancer international consortium; PAM50 = 50-gene prediction analysis of microarray.

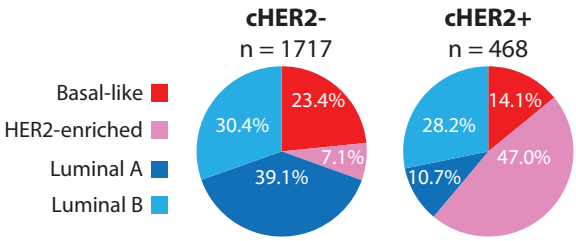

Figure 1. Distribution of the intrinsic molecular subtypes of breast cancer in clinical HER2 status (cHER2)-negative and cHER2+ disease in the combined the cancer genome atlas and molecular taxonomy of breast cancer international consortium dataset. cHER2 = clinical HER2 status.

subtype (2.1% of cHER2+ in TCGA vs 17.4% of cHER2+ in METABRIC, $P < .001$, Fisher´s exact test). In both datasets combined, the incidence of cHER2-positivity was 64.6%, 20.0%, 14.4%, and 7.3% in HER2E, Luminal B, Basal-like, and Luminal A subtypes, respectively.

Next, we determined in each subtype of the METABRIC dataset how many genes were differentially expressed between cHER2+ and cHER2-negative tumors (31). Interestingly, only 0.3%, 1.2%, 1.2%, and 3.9% of all genes evaluated (n = 25,186) were found differentially expressed (False Discovery Rate of 0%) within Luminal A, Luminal B, Basal-like, and HER2E subtypes, respectively (Table 2). In each subtype, the top 100 genes statistically significantly up-regulated in cHER2+ tumors were found enriched for genes located in the 17q12 and 17q21 DNA amplicons, such as HER2, GRB7, ORMLD3, PNMT, and STARD3. Interestingly, among cHER2+ tumors, HER2 gene expression (and the expression of other 17q12 amplicon genes) was statistically significantly higher in cHER2+/HER2E, or cHER2+/Basal-like tumors than in cHER2+/Luminal A or cHER2+/Luminal B tumors (Figure 2A).

A similar analysis in the TCGA dataset, which has a lower number of samples compared to METABRIC, revealed a lower number of differentially expressed genes (13 to 44) between cHER2+ and cHER2-negative tumors within each subtype (12). However, most of the up-regulated genes (72.7%–95.2%) in cHER2+ tumors of each subtype were also found located in the 17q12 and 17q21 amplicon regions.

To further explore the degree of impact of HER2 amplification on global gene expression in each subtype, we performed a clustering analysis in the METABRIC dataset of the most variable genes (n = 13 497) across the four subtypes based on cHER2 status (Figure 2B; Supplementary Figure 1, available online) (31). The result revealed that despite the higher expression of 17q genes in cHER2+ tumors compared to cHER2-negative tumors, the overall profile of the subtypes is largely maintained regardless of cHER2 status, except for the HER2E subtype, in which we found HER2E/cHER2+ tumors to be more similar to Basal-like tumors than HER2E/cHER2-negative tumors, although these HER2E/cHER2+ tumors were still far away from being Basal-like. Similar clustering results were obtained with the ~1900 intrinsic gene list (13) (data not shown).

Additional Molecular Features of the Intrinsic Subtype Based on HER2 Status

The TCGA dataset offers the opportunity to interrogate molecular data-types such as protein expression, miRNA expression, and DNA methylation status (Supplementary Table 1, available online) (12). Similar to gene expression, minor molecular differences between cHER2+ and cHER2-negative tumors within each subtype were identified. Indeed, only 6 to 12 proteins or phosphoproteins (representing 3.5% to 7.0% of all proteins evaluated) were found differentially expressed in each subtype according to cHER2 status, and the vast majority of proteins up-regulated in cHER2+ tumors, such as HER2, RPS6KB1 and ACACA, originate from genes located in the 17q DNA region. Regarding DNA methylation and miRNA expression, less than 0.3% CpG islands or miRNAs were found statistically significantly methylated or expressed in cHER2+ tumors compared to cHER2-negative tumors within each subtype.

Survival Outcomes of the Intrinsic Subtypes Based on cHER2 Status

To evaluate the impact of HER2 amplification on survival either alone or in the context of the subtypes, we used the METABRIC dataset of 1711 patients with resected primary tumors and long-term clinical follow up (31). Of note, all this dataset is from patients with newly diagnosed breast cancer and no patient-received adjuvant trastuzumab (31). When evaluated alone, cHER2+ status was found statistically significantly associated with poorer breast cancer-specific survival (BCSS) compared to cHER2-negative status (hazard ratio [HR] = 1.53; 95% confidence interval [CI] = 1.26 to

Table 2. Differentially expressed genes between clinical HER2 status (cHER2)-positive vs cHER2-negative disease within each intrinsic subtype of breast cancer

		METABRIC (n = 1730)				TCGA (n = 495)			
Subtype	HER2 status*	N	%	No. genes up-regulated	%†	N	%	No. genes up-regulated	%‡
Luminal A	cHER2+	40	7.8	72	0.3%	14	6.3%	21	0.1
	cHER2-neg	472	92.2	3	0%	209	93.7%	0	0
Luminal B	cHER2+	112	20.8	245	1.0%	20	16.4%	44	0.2
	cHER2-neg	427	79.2	51	0.2%	102	83.6%	0	0
HER2-enriched	cHER2+	187	63.4	505	2.0%	39	70.9%	13	0.1
	cHER2-neg	108	36.6	467	1.9%	16	29.1%	0	0
Basal-like	cHER2+	67	17.4	279	1.1%	2	2.1%	NA	NA
	cHER2-neg	317	82.6	30	0.1%	93	97.9%	NA	NA

* cHER2 status was determined by single nucleotide polymorphism (SNP) arrays in the molecular taxonomy of breast cancer international consortium (METABRIC) dataset and immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH) in the cancer genome atlas (TCGA) (except for samples with missing IHC/FISH data where SNP data was used). cHER2 = clinical HER2 status; TCGA = The Cancer Genome Atlas Project; METABRIC = molecular taxonomy of breast cancer international consortium.

† Proportion of genes statistically significantly up-regulated of the 25 186 unique genes evaluated in the Illumina microarray dataset.

‡ Proportion of genes statistically significantly up-regulated of the 17 814 unique genes evaluated in the 244K Agilent microarray dataset.

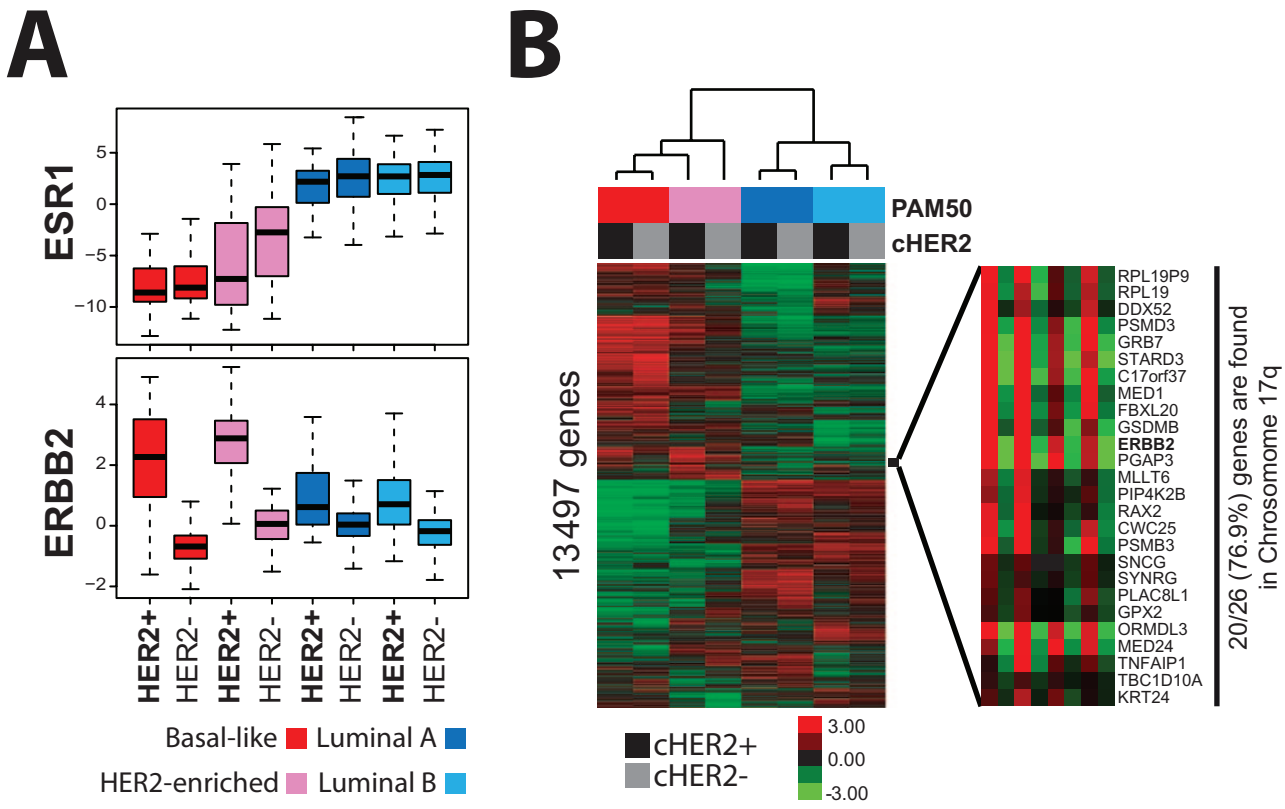

Figure 2. Gene expression patterns of the intrinsic molecular subtypes of breast cancer based on clinical HER2 status (cHER2) status in the molecular taxonomy of breast cancer international consortium (METABRIC) dataset. **A**) Relative transcript abundance of estrogen receptor gene (ESR1) and HER2 gene (ERBB2) across the intrinsic subtypes and based on HER2 status. The boxes represent the interquartile range (25th and 75th percentiles), and the horizontal line in the box represents the median value. The whiskers show the range of largest and smallest values. **B**) Hierarchical clustering of the cHER2+ and cHER2- negative intrinsic subtypes (total of 8 classes) with 13 497 most variable genes in the METABRIC dataset. Sample and gene expression data from tumor samples of the same subtype, and of the same cHER2 status, have been combined into a single category. For each gene in a class, we calculated the standardized mean difference between the gene´s expression in that class, vs its overall mean expression in the dataset using an 8-class Significance Analyses of Microarrays on the METABRIC dataset. On the right panel, a heatmap of genes located in a selected gene cluster is shown. For both heatmaps, red color represents relative high gene expression, green represents relative low gene expression, and black represents median gene expression.

1.86; $P < .001$) (Figure 3A). In addition, cHER2 status provided independent prognostic information beyond that provided by clinical-pathological variables (Supplementary Figure 2, available online). However, the prognostic value of cHER2 status disappeared when subtype was taken into consideration, whereas subtype did provide independent prognostic information beyond

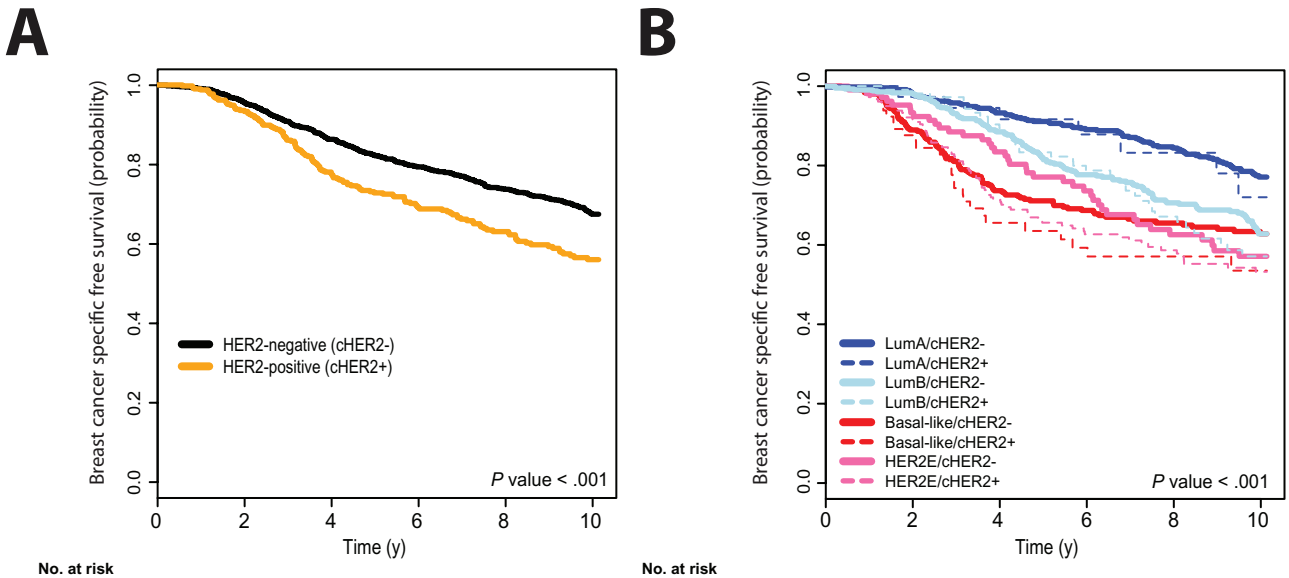

Figure 3. Kaplan-Meier 10-year breast cancer-specific survival analyses in the molecular taxonomy of breast cancer international consortium dataset (all patients). **A**) Based on cHER2 status. **B**) Based on intrinsic subtype and cHER2 status. Tables of the numbers of patients at risk in each group at various time points are below each graph. Statistically significant differences were determined by two-sided log-rank test.

cHER2 status. There was no statistically significant impact of HER2 amplification on BCSS within any subtype (Supplementary Figure 3, available online). For example, patients with Luminal A/cHER2+ tumors showed similar survival outcomes as patients with Luminal A/cHER2-negative tumors (HR = 1.34; 95% CI = 0.62 to 2.90; P = .46), and both of these groups showed a better outcome compared to the other subtypes regardless of their cHER2 status (Figure 3B; Supplementary Table 2, available online).

Finally, we compared the prognostic value of the recently reported 10-subtype classification (IntClust) vs intrinsic subtypes (31). The results revealed that the 10-subtypes do not provide additional prognostic value when subtype is taken into consideration, whereas intrinsic subtype does provide independent prognostic information beyond the one provided by the 10-subtypes (Table 3; Supplementary Figure 2, available online). In multivariable analysis, variables that independently contributed to poor prognosis included clinical-pathologic factors (tumor size and nodal status), intrinsic subtype, and the IntClust-2 subtype (31). Similar results were obtained by comparing the performance of each variable using ROC curve analysis (Supplementary Figure 4, available online).

Discussion

Our study provides new insights into the biological and clinical heterogeneity of cHER2+ breast cancers. Our results suggest that: 1) all the intrinsic molecular subtypes can be identified within cHER2+ disease; 2) cHER2+ tumors of a given subtype are largely undistinguishable from cHER2-negative tumors of the same subtype except for the high expression of genes in or near the HER2 amplicon on 17q in cHER2+ tumors; 3) within cHER2+ tumors, HER2 is more highly expressed in the HER2E or Basal-like tumors than Luminal A or B tumors, and all cHER2+ tumors demonstrate more HER2 mRNA expression than cHER2-negative tumors, regardless of intrinsic subtype; 4) HER2 amplification *per se* does not statistically significantly affect survival outcomes once intrinsic subtype is taken into account; and 5) clinical HER2 status, or the recently reported 10-subtype classes, do not provide additional prognostic information beyond that provided by the intrinsic subtypes.

At first glance, the observation that HER2 amplification does not translate into large changes in the expression of intracellular signaling pathways (as assessed by gene or reverse phase protein array protein expression) within a given subtype might seem counterintuitive since HER2 is considered an activating oncogene of the MAP-Kinase and PI3K/AKT/mTOR signaling pathways leading to cell growth and increased proliferation (1, 35–37). However, it is important to note that our results do not preclude a potential role of HER2 within each subtype, but rather highlight that the magnitude of the impact of HER2 amplification as a single entity is small compared to the magnitude of genome expression as a whole, and that the biologically distinct intrinsic subtypes appear to retain their individual characteristics and behavior regardless of cHER2 status. In any case, the levels of HER2 amplification/overexpression are higher in the HER2E than in the rest of subtypes. Overall, these data suggest that, although HER2 does not induce large molecular changes in the HER2E subtype, HER2 is still likely to be the main driver of the cHER2+/HER2E subtype. On the other hand, the molecular impact of HER2 amplification in the other subtypes seems to be minor.

Table 3. Cox model disease 10-year breast cancer specific-survival analysis in the molecular taxonomy of breast cancer international consortium validation set (n = 818)

	Univariate analysis		Multivariable analysis	
Variables	**HR (95% CI)**	***P****	**HR (95% CI)**	***P****
Tumor size				
T2 vs T0-1	2.08 (1.51 to 2.87)	<.001	1.61 (1.16 to 2.25)	.005
T3 vs T0-1	3.78 (2.19 to 6.52)	<.001	1.92 (1.07 to 3.45)	.03
Nodal status				
1–3 vs 0	2.22 (1.56 to 3.15)	<.001	2.07 (1.34 to 3.22)	.001
>3 vs 0	5.43 (3.79 to 7.79)	<.001	4.44 (2.82 to 6.99)	<.001
Postmenopausal vs Premen.	0.74 (0.53 to 1.02)	.07	1.01 (0.68 to 1.50)	.96
PAM50 Subtype				
(Luminal A as reference)				
Luminal B	3.34 (2.04 to 5.42)	<.001	2.51 (1.47 to 4.28)	.001
Basal-like	4.03 (2.47 to 6.58)	<.001	3.35 (1.73 to 6.49)	<.001
HER2E	4.92 (2.99 to 8.08)	<.001	3.33 (1.81 to 6.14)	<.001
Systemic treatment				
Chemo vs None	3.87 (2.46 to 6.09)	<.001	1.15 (0.61 to 2.17)	.66
Chemo/HT vs None	1.63 (1.09 to 2.43)	.02	1.02 (0.61 to 1.70)	.95
HT vs None	3.93 (2.33 to 6.65)	<.001	1.23 (0.63 to 2.38)	.55
Curtis et al. Subtypes				
(IntClustMemb3 as reference)				
IntClustMemb1	3.43 (1.56 to 7.56)	.002	1.56 (0.68 to 3.56)	.29
IntClustMemb2	5.26 (2.19 to 12.64)	<.001	3.24 (1.28 to 8.16)	.01
IntClustMemb4	2.55 (1.19 to 5.44)	.02	1.40 (0.63 to 3.13)	.42
IntClustMemb5	5.65 (2.81 to 11.37)	<.001	2.03 (0.93 to 4.45)	.08
IntClustMemb6	4.09 (1.77 to 9.46)	<.001	2.31 (0.98 to 5.47)	.06
IntClustMemb7	1.33 (0.54 to 3.27)	.54	1.33 (0.54 to 3.28)	.54
IntClustMemb8	1.75 (0.84 to 3.67)	.14	1.39 (0.66 to 2.96)	.39
IntClustMemb9	3.53 (1.67 to 7.46)	.001	1.58 (0.72 to 3.51)	.26
IntClustMemb10	3.70 (1.84 to 7.46)	<.001	1.32 (0.57 to 3.06)	.52

* Calculated using Cox proportional hazards two-sided test. HR = hazard ratio; CI = confidence interval; HT = hormone therapy; Chemo = chemotherapy; T = tumor stage; METABRIC = molecular taxonomy of breast cancer international consortium; PAM50 = 50-gene prediction analysis of microarray.

The clinical relevance of the luminal vs non-luminal distinction within cHER2+ breast cancer is not new. Two studies have shown that hormone receptor status determined by IHC identifies two main groups of cHER2+ tumors with different survival outcomes (38,39). In the 4-year follow-up of the N9831 and National Surgical Adjuvant Breast and Bowel Project B-31 adjuvant trials of trastuzumab in HER2+ disease (n = 4045), hormone receptor-positive disease was found statistically significantly associated with approximately 40% increased disease-free survival and overall survival, compared to hormone receptor-negative disease (38). This association of hormone receptor status with survival was found to be independent of the main clinical-pathological variables, including trastuzumab administration (38). Similar data was observed in a prospective cohort study of 3394 patients with stage I to III cHER2+ breast cancer from National Comprehensive Cancer Network centers (39). In both studies, hormone receptor-negative disease experienced more cancer relapse in the first 5 years than hormone receptor-positive (38,39). Interestingly, patients with hormone receptor-negative tumors were less likely to experience first recurrence in bone and more likely to recur in brain, compared to patients with hormone receptor-positive tumors (39).

Hormone receptor status in cHER2+ breast cancer is also predictive of pathological complete response (pCR) after neoadjuvant anti-HER2 therapy in combination with chemotherapy across multiple clinical trials (10,40-43). In Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (NeoALTTO), the pCR rates after paclitaxel in combination with one of the three anti-HER2 regimens (lapatinib, trastuzumab, and lapatinib combined with trastuzumab) were higher in hormone receptor-negative disease compared to hormone receptor-positive disease (42). Although not statistically significant, similar response data has been reported in the Cancer and Leukemia Group B (CALGB) 40601 neoadjuvant trial, which has a similar design to NeoALTTO (42,44). Interestingly, PAM50 data in a subset of samples of the CALGB40601 trial (n = 160) identified all the intrinsic subtypes within HER2+ disease and global pCR rates as 75%, 36%, 35%, and 29% in the HER2E, Basal-like, Luminal A, and Luminal B subtypes, respectively (44). Similar results with the PAM50 predictor were observed in patients with cHER2+ disease treated with neoadjuvant trastuzumab and anthracycline/taxane-based chemotherapy in the neoadjuvant trastuzumab in locally advanced breast cancer trial (45). Overall, these data suggest that within cHER2+ tumors, the HER2E subtype benefits the most from anti-HER2-based chemotherapy.

There are limitations to this study that must be acknowledged. The survival data is derived from one dataset, METABRIC, which is a very large cohort of nearly 2000 tumors that were heterogeneously treated. None received HER2-targeted therapy so the prognostic implications detected represent historical behavior. There is no doubt that modern HER2-targeting changes the natural history of HER2-positive breast cancer. Additional datasets that have RNA-based assays and survival will be needed to confirm the findings related to

outcome and will extend this to the impact of HER2 targeting by subtype. Furthermore, the identification of genes that are up- and down-regulated among cHER2-positive breast cancer of various subtypes is intriguing but must be considered exploratory. Fortunately, there are over 1000 patients treated on large cooperative group neoadjuvant studies with RNA-based assays planned that can be used to further and confirm our findings. Although central confirmation of clinical assay status could not be performed in these large merged datasets, it is reassuring that the primary findings by mRNA regarding expression of HER2-related genes, regardless of intrinsic subtype, corresponded with the clinical HER2 status. Finally, it is important to point out that classification of HER2 status based on gene amplification only might miss 5%–8% of tumors that are overexpressing HER2 and that might benefit from anti-HER2 therapies.

To conclude, cHER2+ disease appears as heterogeneous as the other clinical subsets of breast cancer. When the intrinsic subtypes are taken into account, HER2 amplification does not translate into large changes in the expression of downstream signaling pathways or differences in patient survival outcomes. Thus, the mechanisms of action and the effectiveness of anti-HER2 therapies on cHER2+ tumors might depend in part upon their intrinsic tumor profile, with the cHER2+/HER2E subtype tumors showing the likely greatest impact for outcomes and HER2-targeting. Finally, subtyping might help identify patients with cHER2+ disease that need less intensive systemic therapy.

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Funding

This work was supported by funds from a Susan G. Komen Foundation Career Catalyst Grant (to AP), the National Cancer Institute Breast SPORE program (to CMP) (P50-CA58223), the Banco Bilbao Vizcaya Argentaria (BBVA) Foundation (to AP), and the Breast Cancer Research Foundation (to CMP, LAC, JB).

Notes

The funders had no role in the design of the study, the collection, analysis, or interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication.

CMP is an equity stock holder and Board of Directors member of BioClassifier LLC and University Genomics. CMP is also listed as an inventor on a patent application on the PAM50 molecular assay. Uncompensated advisory role of AP for Nanostring Technologies.

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DOI:10.1093/jnci/dju212

EDITORIAL

HER2-Positive Breast Cancer, Intrinsic Subtypes, and Tailoring Therapy

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Successful efforts to target the HER2 oncogene have driven marked improvements in breast cancer outcomes in both the early and advanced disease settings (1). However, controversies persist in the adjuvant setting concerning the use of carboplatin vs anthacycline-based regimens (2) and the role of pertuzumab (3). The introduction of the HER2 dimerization inhibitor pertuzumab is a particularly interesting development, because the provisional US Food and Drug Administration approval is based on an increase in the pathological complete response rate (pCR) after neoadjuvant treatment rather than survival (4). With such complex and expensive regimens in play, there is increasing concern that patients are at risk of overtreatment. What tools do we have to more effectively tailor regimens in order to achieve optimal risk/benefit and cost/benefit ratios? A traditional direction for tailoring therapy is to use pathological stage to guide regimen intensity. For example, the results of a phase II adjuvant trial that examined a simple regimen of 12 weeks of weekly paclitaxel and a year trastuzumab for stage I/IIA, HER2-positive breast cancer has been completed (5). If a very low event rate is confirmed, this simple and cost effective approach could become a standard for patients with small cancers. It is also logical to restrict the use of pertuzumab to locally advanced or pathological stage III disease until survival benefits in lower stage disease has been established.

A complementary approach, widely investigated for tailoring the treatment of ER+, HER2- disease, is to tailor therapy based on biological and prognostic tumor characteristics revealed by gene expression profiling (6). The underexplored potential of this approach in HER2+ disease is nicely illustrated by the analysis provided by Prat et al. in this issue (7), which focused on the prognostic effect of HER2 amplification within the context of an intrinsic subtype assignment. The intrinsic subtypes, defined by the PAM50 model, include Luminal A, Luminal B, HER2-enriched, and Basal-like (8). Importantly, HER2 amplification occurs in all four subtypes with varying frequencies, with the lowest incidence in luminal A disease (7.3%) and highest in the aptly named HER2-enriched subtype (HER2-E) (64.6%). HER2 positivity rates in luminal B and basal-like were 20.0% and 14.4%, respectively. Importantly, HER2 positivity did not influence prognosis in patients treated with a pre-trastuzumab standard of care once the effect of intrinsic subtype was taken into account. For example, HER2+, Luminal A disease does not have an obviously different prognosis from HER2-, Luminal A disease in the absence of HER2-directed therapy (although the relatively small numbers of Luminal A, HER2+ tumors means that small effects cannot be excluded). Furthermore, the presence of HER2 amplification does not have a strong effect on global gene expression outside of coamplified genes on Chromosome 17q. Thus, the authors reasonably conclude that HER2+ status does not uniquely define a subset of breast cancers, but rather HER2 amplification is an oncogenic driver that functions within the biological context of any of the four intrinsic subtypes.

Consideration of intrinsic subtype when treating HER2+ disease is therefore rational and raises some testable hypotheses. For example, if Luminal A, HER2+ tumors were substantially represented in a trial of HER2-directed therapy, event rates will be lower than expected and power diluted. This could be part of an explanation for the recently reported negative results of the Adjuvant Lapatinib And/Or Trastuzumab Treatment Optimisation (ALTTO) adjuvant trial, which failed to show benefits for laptinib when added to trastuzumab (9). Future study designs might consider excluding these cases (as well as stage I disease). Another idea would be to optimize the endocrine approach, as tamoxifen has been long been suspected to be inadequate treatment for ER+, HER2+ disease (10). The HER2-E, HER2+ subset (almost all ER low/negative) is emerging as a chemotherapy-sensitive subgroup that may exhibit differential sensitivity to anthacyclines (11) and to combine HER2 targeting with lapatinib and trastuzumab (12). As Pratt et al. demonstrate, an explanation may be that HER-E tumors exhibit the highest levels of HER2 expression. Because HER2-E, HER2+ tumors often present with rapidly progressing breast masses, neoadjuvant investigation would be the optimal setting to investigate tailoring treatments. Salvage regimens for HER2-E patients without pCR are a particularly important consideration, thus the efficacy of postsurgical adotrastuzumab emtansine is of considerable interest. Basal-like and Luminal B, HER2+ disease represent clinical conundrums, with lower pCR rates in comparison with HER2-E, HER2+ disease. Sequencing studies and gene copy analysis have revealed contrasting molecular characteristics, which drive different therapeutic hypotheses (13). For example, basal-like, HER2+ tumors might benefit from studies that establish the addition of carboplatin or perhaps poly ADP ribose polymerase inhibition to the standard triplet of anthacycline, cyclophosphamide, and taxane, mirroring approaches for homologous recombination-defective, basal-like, HER2- disease (14). For Luminal B, HER2+ tumors, the role of targeting

recurrent mutation characteristics of this disease subset, including PI3 kinase pathway inhibitors, is a logical consideration (15). A final issue raised by this paper is the awkwardly named HER2-E, HER2-group. Interestingly these tumors can express ER, but unfortunately are probably not sensitive to endocrine treatment (16).

Prat et al. rejected the hypothesis that further genomic classification, based on an extensive study of copy number in breast cancer by Curtis et al. (17), could contribute to PAM50-based prognostic models. However, any conclusion does not extend to patients treated with trastuzumab. It is quite possible that copy number could drive the expression of genes that modify trastuzumab efficacy. Clearly the way forward is to apply a validated assay for the intrinsic subtypes to standard formalin-fixed tissues so that prospectively planned retrospective analyses of completed clinical trials of HER2-targeted therapy can be undertaken. Given the results of the ALTTO trial, it will be of particular interest to determine the degree of benefit from pertuzumab when added to trastuzumab by stage and by intrinsic subtype. In conclusion, Prat and colleagues have provoked an important conversation regarding how intrinsic subtype information might prove to be a useful tool to rationalize treatment for HER2+ breast cancer.

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Funding

MJE receives funding from the National Cancer Institute, Susan G. Komen for the Cure, the AVON Foundation, the Breast Cancer Research Foundation, and the Department of Defense Breast Cancer Research Program. These agencies had no role in the drafting or approval of this editorial.

Notes

MJE is an equity stock holder and Board of Director Member of BioClassifier, LLC and University Genomics. MJE is also listed as an inventor on a patent application on the PAM50 molecular assay. He has also received ad hoc consulting fees from Genentech.

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