Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes

Hiroko Masuda[1,2,*], **Keith A. Baggerly**[3,*], **Ying Wang**[3], **Ya Zhang**[4], **Ana Maria Gonzalez-Angulo**[2], **Funda Meric-Bernstam**[6], **Vicente Valero**[1,2], **Brian D. Lehmann**[5], **Jennifer A. Pietenpol**[5], **Gabriel N. Hortobagyi**[2], **W. Fraser Symmans**[3], and **Naoto T. Ueno**[1,2]

[1]Morgan Welch Inflammatory Breast Cancer Research Program and Clinic, The University of Texas MD Anderson Cancer Center, Houston, TX

[2]Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

[3]Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX

[4]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX

[5]Department of Biochemistry, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN

[6]Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

Purpose—The clinical relevancy of the 7-subtype classification of triple-negative breast cancer (TNBC) reported by Lehmann and Bauer et al is unknown. We investigated the clinical relevancy of TNBC heterogeneity by determining pathological complete response (pCR) rates after neoadjuvant chemotherapy, based on TNBC subtypes.

Experimental Design—We revalidated the Lehmann and Bauer et al. experiments using Affymetrix CEL files from public datasets. We applied these methods to 146 TNBC patients with gene expression microarrays obtained from June 2000 to March 2010 at our institution. Of those, 130 had received standard neoadjuvant chemotherapy and had evaluable pathological response data. We classified the TNBC samples by subtype, then correlated subtype and pCR status using Fisher's exact test and a logistic regression model. We also assessed survival and compared the subtypes to PAM50 intrinsic subtypes and residual cancer burden (RCB) index.

Results—TNBC subtype and pCR status were significantly associated *(P=0.04379).* The basal-like 1 (BL1) subtype had the highest pCR rate (52%); basal-like 2 (BL2) and luminal androgen receptor (LAR) had the lowest (0% and 10%, respectively). TNBC subtype was an independent

predictor of pCR status *(P=0.022)* by a likelihood ratio test. The subtypes better predicted pCR status than did the PAM50 intrinsic subtypes (basal-like vs non basal-like).

Conclusions—Classifying TNBC by 7 subtypes predicts high vs. low pCR rate. We confirm the clinical relevancy of the 7 subtypes of TNBC. We need to prospectively validate whether the pCR rate differences translate into long-term outcome differences. The 7-subtype classification may spur innovative personalized medicine strategies for TNBC patients.

Keywords

Triple negative breast cancer; Molecular subtypes; Predictive factor; Pathological complete response; mRNA array analysis

Introduction

Triple-negative breast cancer (TNBC) is defined by lack of expression of estrogen receptor (ER) and of progesterone receptor (PR) and lack of amplification or overexpression of human epidermal growth factor receptor 2 (HER2). TNBC represents approximately 15–20% of all patients with breast cancer and, compared with other breast cancer types, is associated with a high recurrence rate and short survival duration (1, 2). Reasons for this unfavorable prognosis include the heterogeneity and aggressive nature of the disease and the absence of well-defined molecular targets that could form the basis for targeted therapy (3).

In previous studies, 20–30% of patients with TNBC achieved pathological complete response (pCR) neoadjuvant chemotherapy, and pCR was strongly associated with prolonged overall survival (OS) and event-free survival (4–7). In fact, patients with TNBC who achieved pCR had the same prognosis as did patients with non-TNBC (4). In contrast, among patients who did not achieve pCR, patients with TNBC had a significantly poorer outcome (a shorter survival duration driven by higher relapse rates) than did patients with non-TNBC (4). TNBC's heterogeneous response to chemotherapy in the neoadjuvant setting suggests that different subtypes of primary TNBC may be associated with high or low pCR rates. Subtype-specific individualization by molecular profiling would help us predict benefit from standard chemotherapy and develop personalized targeted drugs for TNBC.

Gene expression analyses have identified molecular subtypes of TNBC that are refining our understanding of breast cancer biology and enabling development of targeted therapy. Recently, Lehmann and Bauer et al (3) reported that TNBC can be classified into 7 subtypes (6 defined subtypes and an unstable group) by gene expression microarray. The 7 TNBC subtypes were characterized on the basis of gene ontologies and differential gene expression and labeled as basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), luminal androgen receptor (LAR), and unstable (UNS).

In this study, we confirmed Lehmann and Bauer's findings and applied their methodology to a population of TNBC patients. For each molecular subtype, we then evaluated pCR rates after standard neoadjuvant chemotherapy regimens, as well as clinical outcomes. Finally, we compared the predictive performance of the 7 subtypes with that of previously defined "intrinsic subtypes" whose gene expression profiles are established through the 50-gene Prediction Analysis of Microarray (PAM50) assay (8), and also evaluated the residual breast cancer burden (RCB) index (9), a post-chemotherapy pathologic measure that considers the size and invasive proportion of the primary tumor and the number and size of nodal metastases, and has potential to predict the clinical outcome after neoadjuvant chemotherapy more accurately than the current pCR vs. non-pCR category.

Materials and Methods

Validation of Lehmann and Bauer's Gene Expression Analysis

Datasets and normalization of data—Reproducing the method of Lehmann and Bauer et al (3), we obtained 12 public datasets (323 patients' DNA microarrays) from their TNBC training set and all 7 public datasets (201 patients' DNA microarrays) from their TNBC validation set. The raw data for 63 of the 386 samples from their training set were not included in our analysis. Datasets GSE5364 and GSE22513 (56 of the 63 samples) were not available to collect. The remaining 7 of the 63 samples were not used because we could not confirm them as TNBC based on Lehmann and Bauer et al's data. In their study, triple-negative status was identified by using mRNA expression of ER, PR, and HER2; they reported these data in supplemental tables. We identified TNBC status from these tables according to their criteria.

All of the arrays used were a type of Affymetrix U133 array: U133A, U133 Plus 2.0, or U133AAofAv2. Lehmann and Bauer et al used the robust multiarray average (RMA) algorithm to normalize and quantify the data (10). The data pre-processing and quantification were performed using R statistical software. We used this approach with two modifications. First, we used the frozen robust multiarray analysis (fRMA) algorithm, which allows arrays to be analyzed individually or in small batches and then combines the data for analysis, to normalize and quantify all of the datasets. Second, considering the potential effect of platforms on the gene expression profiles, we converted all of the other platforms to HGU133A by using the "fRMA Tools" R package. We later used linear mixed models to correct for any remaining platform-specific differences.

k-means clustering—We followed the methods used by Lehmann and Bauer et al. to identify TNBC subtypes and relative gene signatures. A total of 14,644 probes were selected to represent unique genes. We performed principal component analysis to check for the existence of batch effects. If batch effects were present, feature-by-feature linear models were applied to remove them. Principal component analysis results revealed an obvious batch effect, which was reduced after the application of linear models. We applied consensus clustering using 1000 iterations of k-means clustering to assess the classification robustness and to determine the optimal number of clusters. We performed k-means clustering on the genes (n = 1192) with standard deviation>0.8 and used silhouette width ($s[i]$) to measure the relative closeness of individual samples to their cluster centers. K-means clustering resulted in 6 stable clusters ($s[i]>0$) with a total of 261 samples and 1 unstable cluster ($s[i]<0$) containing 62 samples. Our consensus clustering results confirmed that it is reasonable to set k to 7. We labeled these 7 clusters as subtypes 1 to 7. We computed centroids for each cluster from the consensus clustering and then determined the Pearson correlation of each centroid with each sample in the validation set from Lehmann and Bauer et al. The highest correlation (and lowest *P* value) was used as the criterion with which to determine the subtype that a specific sample belonged to.(detail in supplementary)

Comparison of Lehmann and Bauer's and our subtype classification—We compared our clustering of the training and validation samples with the clustering by Lehmann and Bauer et al by using 7×7 contingency tables and X^2 tests. We also used Cohen's kappa to assess the association between our classifications and those reported by Lehmann and Bauer et al.

Application of the Method to Our Population

Patients and samples—We next applied the Lehmann and Bauer et al method to a separate population of patients with TNBC. This group consisted of 146 patients treated for

TNBC at MD Anderson. This dataset derived from a prospective study by Hatzis et al (11), which was conducted from June 2000 to March 2010 to develop genomic predictors for neoadjuvant chemotherapy; 97 patients overlapped the group reported by Hatzis et al. We also added new samples that were included in the cohort since the 2011 data were released (n=49). Among the 146 patients, 132 patients had received neoadjuvant chemotherapy containing sequential taxane and anthracycline-based regimens and had evaluable neoadjuvant pathological response. We excluded 2 of these patients because they had received neoadjuvant radiotherapy. Thus, 130 patients were included in our study. Patients had provided written informed consent to participate in an institutional review board–approved research protocol (LAB99-402, USO-02-103, 2003-0321, I-SPY-1) that allowed obtaining a tumor biopsy sample by fine-needle aspiration or core biopsy prior to any systemic therapy, for genomic studies to develop and test predictors of treatment outcome (9).

We identified TNBC by immunohistochemical (IHC)or fluorescence in situ hybridization (FISH) testing. ER status and PR status were considered negative if fewer than 10% of cells stained positively on IHC. HER2 status was considered negative if (a) IHC results were 0 to +1, (b) IHC results were +2 and FISH results were negative. To exclude samples falsely identified as negative by IHC, we implemented a secondary filter that removed samples in which ER expression (205225_at) was greater than the 75th percentile at the transcriptome level, as it was previously demonstrated that >96% of TNBC samples from the original 386-patient TNBC cohort had ER expression below the 75th percentile of all genes (12).

We collected the patient age, clinical stage, treatment regimen, and nuclear grade. We defined pCR as no evidence of invasive carcinoma in the breast and the axillary lymph nodes at the time of surgery. The median follow-up from diagnosis to death was 68.1 months (range 5.1–147.5).

All gene expression microarrays were profiled in the Department of Pathology at MD Anderson. Gene expression levels were derived by exposing the microarray to fluorescent probe sets (multiple oligonucleotide probes that hybridize to specific sequences of gene transcripts) (13, 14).

Gene expression analysis—We classified 146 TNBC samples as defined by Lehmann and Bauer's gene signatures. As for the public datasets, we performed data pre-processing and quantification in R and applied the fRMA algorithm. Before classification, we applied gene-by-gene mean-centering to our data.

Association of subtype with pCR status and clinical outcome—We constructed a contingency table and performed the Fisher exact test to assess the association between TNBC subtype and pCR status. To assess the independent utility of TNBC subtype for predicting pCR status, we fit a logistic regression model to our data and used age, clinical stage, treatment regimens, and nuclear grade as potential explanatory factors. In this context, using a likelihood ratio test, we examined whether adding TNBC subtype provided a significant improvement in predictive value over a model already containing the other 4 explanatory factors.

We used Cox proportional hazards models to estimate the association between TNBC subtype and OS and distant metastasis-free survival (DMFS). We identified OS and DMFS from the diagnosis date to death or to diagnosis of distant metastasis (15).

Relationship between the 7 subtypes and PAM50 subtypes—Hatzis et al (11) identified intrinsic subtypes in their study; thus, for 92 of our patients, we had data on

PAM50 subtypes (luminal A, luminal B, HER2-enriched, basal-like, and normal-like) (8). We investigated the relationship between the PAM50 intrinsic subtypes (grouped as basal-like vs. non-basal-like) and the 7 subtypes identified by Lehmann and Bauer et al.

Relationship between the 7 subtypes and RCB index—We also assessed the association between TNBC subtype and the RCB index reported by Symmans et al (9). Primary tumor and nodal metastasis samples were evaluated for residual disease as previously described, and tumors were classified as pCR, RCB-I (minimal residual disease), RCB-II (moderate residual disease), or RCB-III (extensive residual disease).

Results

Validation of Lehmann and Bauer's TNBC classification

In our analysis, 7×7 contingency tables (Table 1) indicated high correlation ($P < 2.2 \times 10^{-16}$) between our clustering and the original clustering. We also used Cohen's kappa coefficient to assess the association between our classifications and those reported by Lehmann and Bauer et al. For the training set, the value was 0.662 (n = 277); for the validation set, 0.462 (n = 200). Using large-sample normal approximations, the kappa2 function in the R package gave approximate z-values of 26.1 and 16.2, respectively; the *P* values were essentially 0.

The contingency table for the training set (Table 1) indicated that the BL1, M, IM, MSL, and LAR subtypes are more stable than the BL2 and UNS subtypes. The contingency table for the validation set (Table 1) indicated that subtypes IM, MSL, and LAR are more stable than the other subtypes.

In summary, there was high correlation between the results of the Lehmann and Bauer et al study and the results we obtained by using approximately the same methods.

TNBC Subtypes Predict pCR Status of Patients Treated with Neoadjuvant Chemotherapy

Of the 130 patients with evaluable pathological response after neoadjuvant chemotherapy, 16 patients received an anthracycline regimen alone, 3 patients received a taxane regimen alone, and 111 patients received both anthracycline and taxane regimens. We classified patients into subtypes as follows: BL1, 21 patients; BL2, 8 patients; M, 26 patients; IM, 27 patients; MSL, 13 patients; LAR, 20 patients; and UNS, 15 patients. There was no statistically significant difference in treatment regimens between subtypes *(P=0.651).*

The pCR rate for all patients was 28% (37/130). BL1 had the highest pCR rate (52%), and BL2 and LAR had the lowest pCR rates (0 and 10%, respectively). The Fisher exact test indicated a significant association between TNBC subtype and pCR status *(P=0.04379)* (Table 3). TNBC subtype was an independent predictor of pCR status *(P=0.022)* by a likelihood ratio test (based on logistic regression models with and without TNBC subtype as the variant).

Clinical Outcomes by Subtype

We excluded 8 patients from the survival analysis because of their history of other types of cancer. The Cox models showed no significant association between TNBC subtype and OS *(P= 0.287)* or DMFS *(P= 0.371).* The Kaplan-Meier plots for each subtype with respect to OS and DMFS are shown in Figures 1A and 1B, respectively. Owing to the limited sample size, the median survival rates were not available for all TNBC subtypes. However, despite its lower pCR rate, LAR had the best OS rate; M had the worst.

Relationship between the 7 Subtypes and PAM50 Subtype

Since PAM50 gene expression analysis has been incorporated into the clinic and shown to be strongly related to the clinical outcome of breast cancer, we determined the relationship between these classification systems. Figure 2 shows the distribution of the 7 Lehmann and Bauer TNBC subtypes between the PAM50 basal-like subtype and non-basal-like subtypes (grouped). All tumors in the BL1 and BL2 subtypes belonged to the basal-like PAM50 subtype, and most tumors in the LAR subtype belonged to the non-basal-like PAM50 group. In the non-basal-like group, there were only 3 TNBC subtypes, LAR, MSL, and M; the majority (59%) of these tumors were the LAR subtype.

Relationship between the 7 Subtypes and RCB Index

Figure 3 shows the relationship between the 7 subtypes and RCB index. Consistent with Symmans et al's previous study, RCB index clearly predicted the clinical outcomes in the TNBC population. There was no statistical difference in OS rate between patients with pCR and those with RCB-I status, and patients with RCB-III showed the lowest OS rate. As seen in Figure 3, combining the distribution of pCR and RCB-I revealed that current neoadjuvant chemotherapy has low efficacy for the BL2 subtype in particular.

Discussion

This is the first report that the TNBC subtype can serve as an independent predictor of pCR status in patients who receive current standard chemotherapy regimens. We confirmed that TNBC is heterogeneous and can be classified with high correlation into 7 subtypes, including 1 unstable subtype, by Lehmann and Bauer's algorithm. In our population, the subtypes better predicted pCR status than did the PAM50 intrinsic subtypes. RCB index predicted prognosis more accurately.

Although Lehmann and Bauer's classification is not the only strategy for defining molecular subtypes of TNBC, it has had a strong impact because it classifies the entire TNBC population into homogeneous subtypes and establishes 7 subtypes, and because subtype classification had a preclinical predictive effect on the outcome of therapy selected to incorporate specific targeted treatments, such as an androgen receptor antagonist and aPI3K/mTOR inhibitor. Our study has extended these findings by showing that the 7 subtypes predicted the rate of pCR to current standard chemotherapy regimens. Gene expression analyses have identified molecular subtypes of TNBC, such as the claud in-low subtype (16, 17) and basal-like subtype. However, these subsets are still not well defined enough to enable development of targeted therapy or prediction of pathological response.

It was interesting that while the BL1 subtype had the highest chemosensitivity (pCR rate), as we hypothesized, the BL2 subtype had the lowest pCR rate, contrary to our hypothesis. These subtypes have similar biology, such as high Ki-67 mRNA expression and enrichment of proliferation genes; however, BL2 has unique gene ontologies involving growth factor signaling, such as the EGF, MET, and IGF1R pathways. The difference in gene ontologies might explain the difference in pCR rates and could provide a basis for individualized therapy. For example, BL2 tumors could be targeted with EGFR or IGF1R inhibitors.

Tumors of the LAR subtype are heavily enriched in hormonally regulated pathways. Consistent with LAR's low pCR rate, which was in accord with our hypothesis, the luminal A and Bintrinsic subtypes, which are hormonally regulated tumors, showed less response to chemotherapy (6, 8). However, when we evaluated the RCB index, 33% of patients belonged to the pCR and RCB-I categories. This result might have affected the clinical outcomes. The LAR group had delayed recurrences compared with the other groups and did not have the lowest OS rate despite having a low pCR rate. Also, 75% of distant metastasis

in the LAR subtype occurred more than 3 years after diagnosis. The LAR group showed a clearly different clinical process from that of the other subtypes. These results suggest that within TNBC, we need to distinguish the LAR subtype and design a different treatment strategy for this group.

For other groups, consistent with the current knowledge about TNBC, most recurrences and deaths happened within 3 years of diagnosis. For these groups, to achieve pCR is the most desirable result for improving prognosis; thus, we need ways of predicting chemotherapy sensitivity, resistance, or both to guide selection of a treatment regimen, and these predictors also should be associated with DMFS and OS. The 7 subtypes have the potential to solve these problems. Further studies are needed to better characterize them.

In a previous study of the PAM50 intrinsic subtypes (2), the basal-like intrinsic subtype showed high chemosensitivity and the difference between the basal-like and non-basal-like subtypes was reported to be the main biological difference seen among patients with TNBC. (18)In our study, all patients in the group with the highest pCR rate, BL1, had tumors characterized as basal-like, and all but 1 of the patients in the LAR population, which had a low pCR rate, had non-basal-like tumors. Interestingly, although all those with the BL1 and BL2 subtypes belonged to the basal-like intrinsic subtype, BL1 had the highest pCR rate and BL2 the lowest. The 7 subtypes more accurately predicted pCR status.

RCB index was significantly associated with OS rate. This index has potential to predict clinical outcomes more accurately than pCR status after neoadjuvant chemotherapy. Combining the distribution of pCR and RCB-I revealed that current neoadjuvant chemotherapy has low efficacy for the BL2 subtype in particular. We conclude that identifying the BL2 population and developing specific treatments for this group would improve the clinical outcome in TNBC patients as a whole.

Although we reproduced Lehmann and Bauer's 7 subtypes and could apply their algorithm to new data, the results didn't match perfectly. Microarray-based molecular classification always involves this risk (19, 20). Even for the well-known intrinsic subtypes, several types of gene signatures have been derived in each study, and none of the classification systems tested have produced perfect agreement (8, 21–23). Although we had 130 patients, and there were a number of additional new samples that were included in the cohort since the 2011 data were released, once the patients were classified into 7 groups, we didn't have enough power to show clinical relevance. Further, one of our most interesting groups, BL2, had the smallest sample size. It is almost impossible to collect samples from consistently treated patients for whom uniform, clear clinical information is also available. Thus, prospective future studies are needed to establish the clinical relevance of using gene profiling to divide patients by subtype. Further, prospective validation by methods such as reverse-phase protein array or IHC staining is needed to measure whether the targets noted in the 7 subtypes are truly overexpressed or are functionally activated at the protein expression level. The clinical utility of these TNBC subtypes will not reach its full potential until markers for each subtype can be validated on reliable platforms, which can define the protein expression level or functionality of determine the specific targets which drived the tumorigenicity and metastasis of each subtype (IHC or NanoString assay).

In summary, our results suggest that we especially need to distinguish the BL2 and LAR subtypes in order to apply specific treatment strategies for them. These 2 subtypes showed the lowest pCR rates; however, appropriate treatment strategies might be quite different for these 2 subtypes. For the BL2 subtype, we need to develop a novel targeted therapy in the neoadjuvant setting to achieve a higher pCR rate. In addition, we need to distinguish the LAR subtype from other TNBCs and apply a new treatment strategy similar to that for the

luminal intrinsic subtype, such as targeting the androgen receptor pathway as a long-term adjuvant treatment. Prospective validation of our findings is needed. We conclude that the 7 subtypes may lead to innovative personalized medicine clinical trials for patients with TNBC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.
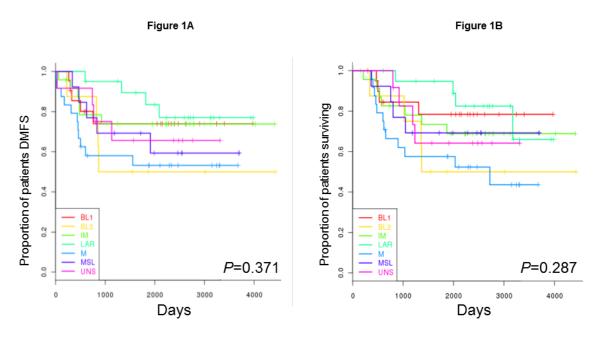
Acknowledgments

References

1. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer. 2007; 109:1721–8. [PubMed: 17387718]

2. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res. 2007; 13:2329–34. [PubMed: 17438091]

3. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011:121.

4. Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol. 2008; 26:1275–81. [PubMed: 18250347]

5. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol. 2012; 30:1796–804. [PubMed: 22508812]

6. Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res. 2005; 11:5678–85. [PubMed: 16115903]

7. Dawood S, Broglio K, Kau SW, Green MC, Giordano SH, Meric-Bernstam F, et al. Triple receptor-negative breast cancer: the effect of race on response to primary systemic treatment and survival outcomes. J Clin Oncol. 2009; 27:220–6. [PubMed: 19047281]

8. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol. 2009; 27:1160–7. [PubMed: 19204204]

9. Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol. 2007; 25:4414–22. [PubMed: 17785706]

10. Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res. 2003; 31:e15. [PubMed: 12582260]

11. Hatzis C, Pusztai L, Valero V, Booser DJ, Esserman L, Lluch A, et al. A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. JAMA. 2011; 305:1873–81. [PubMed: 21558518]

12. Chen X, Li J, Gray WH, Lehmann BD, Bauer JA, Shyr Y, et al. TNBCtype: A Subtyping Tool for Triple-Negative Breast Cancer. Cancer Inform. 2012; 11:147–56. [PubMed: 22872785]
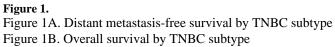
13. Hess KR, Anderson K, Symmans WF, Valero V, Ibrahim N, Mejia JA, et al. Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. J Clin Oncol. 2006; 24:4236–44. [PubMed: 16896004]

14. Symmans WF, Hatzis C, Sotiriou C, Andre F, Peintinger F, Regitnig P, et al. Genomic index of sensitivity to endocrine therapy for breast cancer. J Clin Oncol. 2010; 28:4111–9. [PubMed: 20697068]

15. Hudis CA, Barlow WE, Costantino JP, Gray RJ, Pritchard KI, Chapman JA, et al. Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. J Clin Oncol. 2007; 25:2127–32. [PubMed: 17513820]

16. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res. 2010; 12:R68. [PubMed: 20813035]

17. Herschkowitz JI, Simin K, Weigman VJ, Mikaelian I, Usary J, Hu Z, et al. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol. 2007; 8:R76. [PubMed: 17493263]

18. Prat A, Adamo B, Cheang MC, Anders CK, Carey LA, Perou CM. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist. 2013; 18:123–33. [PubMed: 23404817]

19. Mackay A, Weigelt B, Grigoriadis A, Kreike B, Natrajan R, A'Hern R, et al. Microarray-based class discovery for molecular classification of breast cancer: analysis of interobserver agreement. J Natl Cancer Inst. 2011; 103:662–73. [PubMed: 21421860]

20. Pusztai L, Mazouni C, Anderson K, Wu Y, Symmans WF. Molecular classification of breast cancer: limitations and potential. Oncologist. 2006; 11:868–77. [PubMed: 16951390]

21. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001; 98:10869–74. [PubMed: 11553815]

22. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A. 2003; 100:8418–23. [PubMed: 12829800]

23. Hu Z, Fan C, Oh DS, Marron JS, He X, Qaqish BF, et al. The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics. 2006; 7:96. [PubMed: 16643655]

Statement of Translational Relevance

Triple-negative breast cancer (TNBC) could be classified into 7 subtypes (1). These TNBC subtypes were identified in 21 public mRNA gene expression (GE) profiles of breast cancer with use of cluster analysis. However, the clinical relevancy of the heterogeneity of TNBC is unknown. To address the clinical relevancy, we hypothesized that the subtypes of TNBC have different rates of pathological complete response (pCR) to standard neoadjuvant chemotherapy regimens. In this study, we revalidated that TNBC is heterogeneous and the pCR differs by TNBC subtype using Lehmann and Bauer et al's algorithm. This is the first report that the TNBC subtype can serve as an independent predictor of pCR status in patients who received standard chemotherapy regimens. This confirms the possible clinical relevance of the 7 molecular subtypes and these subtypes may lead to innovative personalized medicine clinical trials for patients with TNBC.

Figure 1.
Figure 1A. Distant metastasis-free survival by TNBC subtype
Figure 1B. Overall survival by TNBC subtype

Figure 2A
Basal (N=75)

Figure 2B
Non-basal (N=17)

Basal (n=75)	
BL1	16
BL2	6
M	15
IM	17
MSL	7
LAR	1
UNS	13

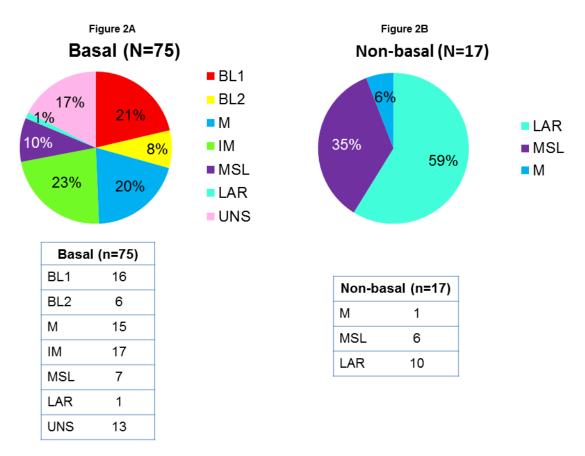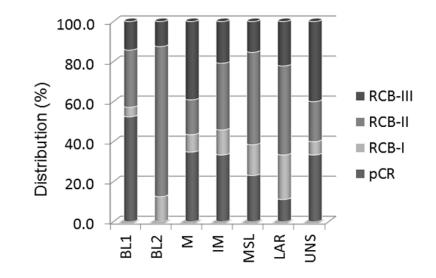| Non-basal (n=17)	
M	1
MSL	6
LAR	10

Figure 2.
Figure 2A. The relationship between PAM50 subtypes and the 7 subtypes (basal-like subtype)
Figure 2B. The relationship between PAM50 subtypes and the 7 subtypes (Non-basal like subtypes)

Figure 3.
The relationship between RCB index and the 7 subtypes

Table 1

The 7 ×7 contingency tables showing the clustering of samples in the training set and validation set using 2 sets of gene signatures as determined by Lehmann and Bauer and by us.

	Training set	Lehmann, Bauer et al							
		BL1	BL2	M	IM	MSL	LAR	UNS	P value
MD Anderson	BL1	40	10	1	8	0	0	9	
	BL2	0	3	0	9	0	4	0	
	M	0	3	49	0	0	0	3	
	IM	4	0	0	36	0	0	0	$P{<}2.2{\times}10^{-16}$
	MSL	0	3	3	0	20	0	0	
	LAR	0	4	0	0	0	26	0	
	UNS	5	9	4	0	0	4	24	

	Validation set	Lehmann, Bauer et al							
		BL1	BL2	M	IM	MSL	LAR	UNS	P value
MD Anderson	BL1	40	10	1	8	0	0	9	
	BL2	0	3	0	9	0	4	0	
	M	0	3	49	0	0	0	3	
	IM	4	0	0	36	0	0	0	$P{<}2.2{\times}10^{-16}$
	MSL	0	3	3	0	20	0	0	
	LAR	0	4	0	0	0	26	0	
	UNS	5	9	4	0	0	4	24	

Table 2

Patient characteristics by TNBC subtype (n = 130)

Characteristic	Category	BL1 n=21	BL2 n=8	M n=26	IM n=27	MSL n=13	LAR n=20	UNS n=15	P-value
Age (yr)	>50	14	2	13	12	7	6	7	
	50	7	6	13	15	6	14	8	0.283
Clinical stage	I	0	0	0	2	1	0	0	
	IIA	3	2	6	9	4	7	2	
	IIB	8	4	13	5	1	6	7	
	IIIA	4	1	2	3	1	2	1	
	IIIB	1	0	3	6	1	3	1	
	IIIC	5	1	2	2	5	2	4	0.276
Nuclear grade	2	2	1	4	0	2	7	0	
	3	19	7	22	27	11	13	15	0.008
Treatment regimen	A	1	1	4	4	0	4	1	
	T	0	0	2	1	0	0	0	
	A+T	20	7	20	22	13	16	14	0.651
pCR	Yes	11	0	8	8	3	2	5	
	No	10	8	18	19	10	18	10	0.043
Distant recurrence	Yes	5	4	12	6	4	4	4	
	No	16	4	14	21	9	16	11	0.348
Local recurrence	Yes	3	2	1	3	0	1	3	
	No	18	6	25	24	13	19	12	0.269
Survival status	Dead	4	4	12	9	4	4	5	
	Alive	17	4	14	18	9	16	10	0.369

pCR: pathological complete response A: anthracycline T: taxane

Table 3

Distribution of pCR/non-pCR status by TNBC subtype

	pCR	Non-pCR	pCR rate	95%CI	*P* value
BL1	11	10	0.52	0.31–0.73	
BL2	0	8	0.00	0.00–0.00	
M	8	18	0.31	0.13–0.48	
IM	8	19	0.30	0.12–0.46	*P*=0.043
MSL	3	10	0.23	0.0001–0.45	
LAR	2	18	0.10	0.03–0.23	
UNS	5	10	0.33	0.09–0.57	

Likelihood ratio test:

Adjusting clinical features: age, clinical stage, nuclear grade, and treatment type.

TNBC subtype was an independent predictor of pCR status *(P=0.022)*