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Understanding and Treating Triple-Negative Breast Cancer Across the Age Spectrum

Molecular Stratification of Triple-Negative Breast Cancers

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ABSTRACT

Research focused on the analysis and classification of breast tumors, primarily using DNA microarrays and patterns of gene expression, has resulted in distinct tumor subtypes. Although no knowledge of patient survival or outcomes was used to derive these gene descriptions, these different classes based upon patterns of gene expression have important prognostic implications. Predictive markers in estrogen receptor–negative and triple-negative disease will be particularly important because in the absence of therapy, these tumor subtypes tend to have a poor prognosis. In addition, the claudin-low subgroup has been found to be common within the triple-negative cancers and may have further prognostic and therapeutic implications. Patients with triple-negative breast cancer do benefit from chemotherapy, but better treatment options are needed that are less toxic, reduce the risk of disease progression, and are more targeted to this patient population. Potential treatments include poly (ADP-ribose) polymerase inhibitors, and therapies that target cancer stem cells could also have an important impact in these patients. This article will focus on the molecular stratification of triple-negative breast cancers and the therapeutic implications of these classifications. ***The Oncologist*** 2010;15(suppl 5):39–48

INTRODUCTION

Research focused on the analysis and classification of breast tumors, primarily using DNA microarrays and patterns of gene expression, have resulted in five different tumor subtypes and a normal breast-like group. Although no knowledge of patient survival or outcomes was used to derive these gene descriptions, these different classes based upon patterns of gene expression have important prognostic implications. Predictive markers in estrogen receptor $(ER)^-$ and triple-negative disease have been particularly important because, in the absence of therapy, these tumor subtypes tend to have a poor prognosis. Triple-negative disease does respond to chemotherapy, but there is a high risk for recurrence and disease progression with these tumors. Therefore, the need still exists to develop more targeted, less toxic therapies for these specific subtypes of tumors. This article focuses on the molecular stratification of triple-negative breast cancers and the therapeutic implications of these classifications.

Over the years, researchers at the University of North

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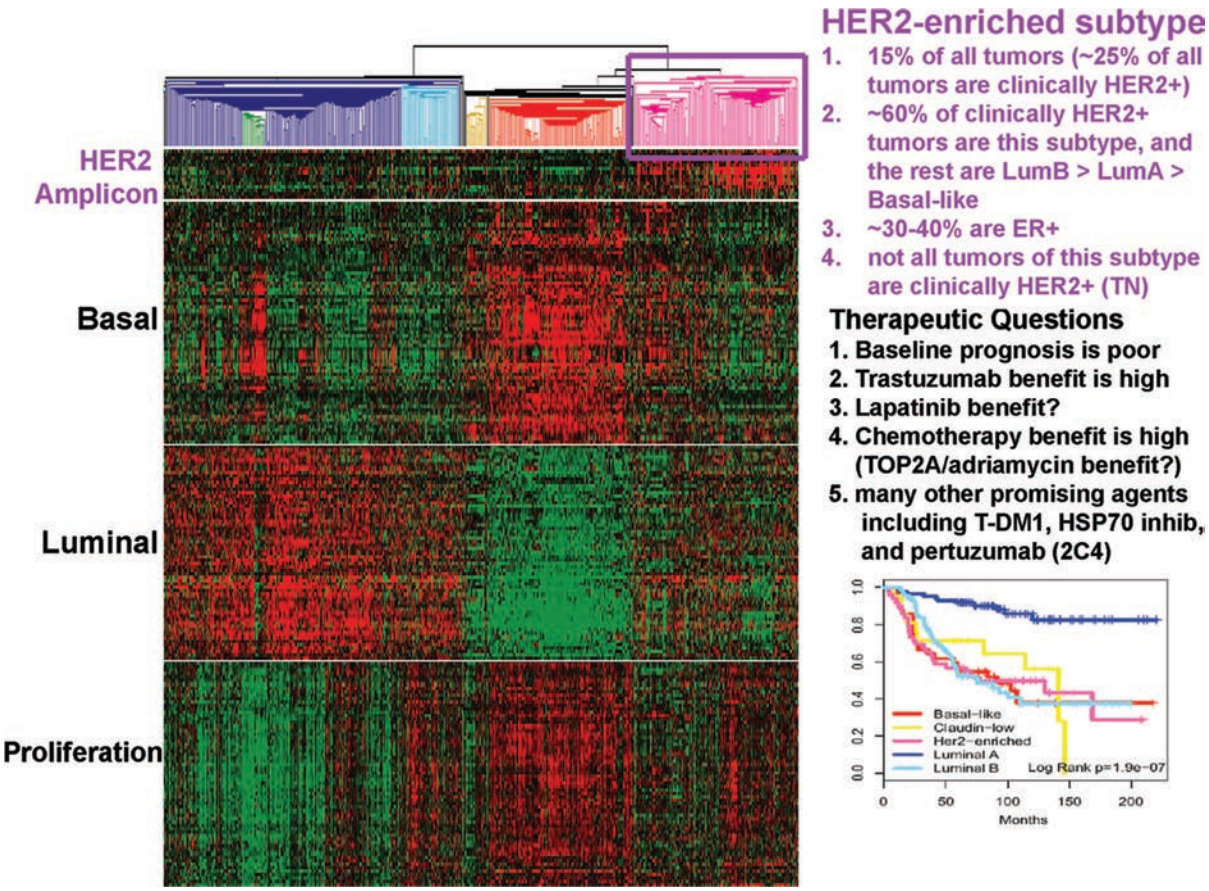

Figure 1. Cluster diagram of breast cancer subtypes highlighting the HER-2-enriched subtype.

Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor; Lum, luminal; TN, triple negative; TOP2A, topoisomerase 2A.

Carolina at Chapel Hill have collected a data set of approximately 470 different breast tumors, including clinical ER, progesterone receptor (PR), and human epidermal growth factor receptor (HER)-2 data, as well as microarray data [1–9]. Researchers applied molecular classification to these tumors and then broke out the group of triple-negative tumors. The vast majority of triple-negative breast cancers were found to be of the basal-like phenotype. However, all the other molecular subtypes were present; these include the HER-2–enriched group, luminal A, luminal B, claudin-low, and a few normal-like tumors [9]. There is still significant biological heterogeneity within triple-negative disease, and it is likely that therapies will need to specifically target this heterogeneity to be effective against these tumors.

HER-2–Enriched Triple-Negative Tumors

One minor constituent of the triple-negative breast cancer group is the HER-2–enriched gene expression subtype/subset. Some of these tumors are clinically triple negative, despite having the gene expression signatures of the HER-2–enriched subtype. Clinically HER-2^+ tumors represent about 15%–20% of all tumors, and the majority of clinically HER-2^+ tumors fall into this HER-2–enriched subtype. However, about 30%–40% of these are ER^+ and the majority are ER^-. Looking at gene expression patterns of HER-2, not every tumor within the HER-2–enriched subtype is HER-2^+ or HER-2 amplified. Hence, researchers have adopted the term "HER-2 enriched," as opposed to simply HER-2^+, to signify the fact that not all these tumors are HER-2^+ (Fig. 1).

This distinction is important for this discussion, because tumors that are in the HER-2–enriched subtype and are not HER-2^+ are triple negative. Consequently, there is a biology possibly being dictated by HER-2 that may have some bearing upon a subset of the triple-negative group. It may be that these tumors have a mutation of HER-2 in the kinase domain, for instance, but this is speculation. Researchers have yet to identify this activating event, but nonetheless, a few HER-2–enriched subtype tumors are indeed constituents of the triple-negative group.

One of the pressing questions regarding patients with the HER-2–enriched subtype who are triple-negative con-

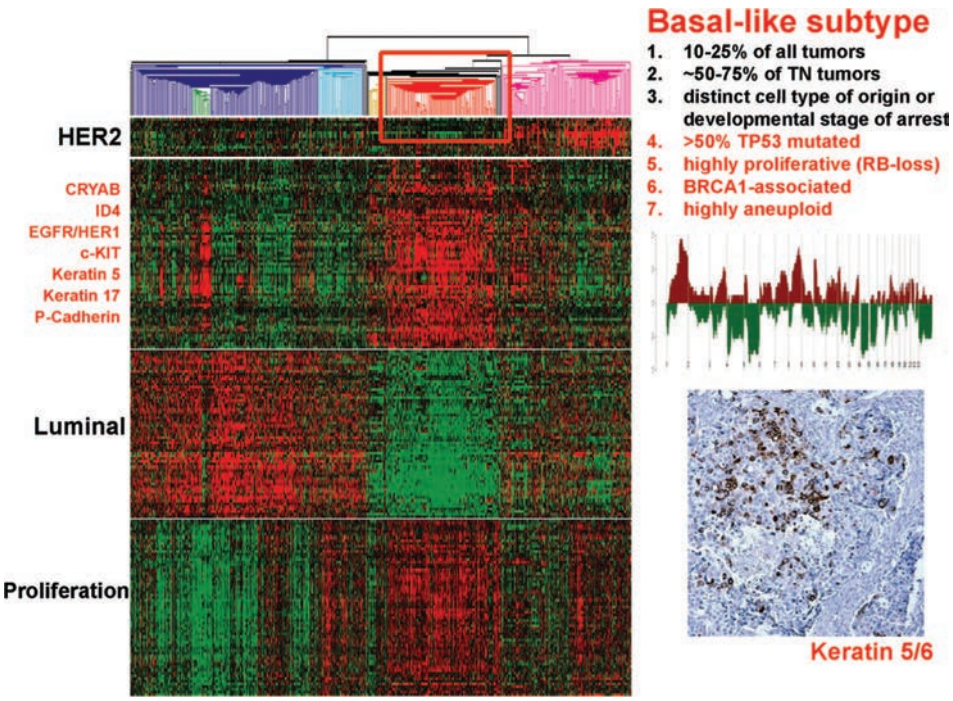

Figure 2. Cluster diagram of breast cancer subtypes highlighting the basal-like subtype.
Abbreviations: EGFR, epidermal growth factor receptor; HER-2, human epidermal growth factor receptor; TN, triple negative.

cerns whether HER-2–targeted therapies have any benefit. Clinical studies, such as the Cancer and Leukemia Group B (CALGB) neoadjuvant trial 40601, are focused on HER-2^+ patients receiving trastuzumab, lapatinib, or the combination. It is somewhat unfortunate that triple-negative, HER-2–enriched subtype patients were not eligible for enrollment in that study. As such, researchers will not actually know from this study, or many like it, whether patients with the HER-2–enriched subtype and who are clinically HER-2^- gain a benefit from HER-2–targeted agents. However, there is a hint that some HER-2^- patients gain a benefit from HER-2–targeted therapies, and it might be those HER-2– patients who are triple negative.

Basal-Like Breast Cancer Classification

Tumors referred to most commonly as triple-negative cancers can often be classified as basal-like breast cancer. Basal-like breast cancer represents 10%–25% of all tumors, depending on the demographics of the population, and make up about 50%–75% of the triple-negative subtype. It is important to note that, whereas triple-negative cancers are frequently found to be basal-like tumors, triple-negative cancers can less commonly fall into any of the other intrinsic subtypes. From a biologic perspective, it is now thought that basal-like tumors are derived from a distinct cell type or a distinct developmental stage of mammary–epithelial cell development.

These tumors were referred to as basal-like many years ago because of the unique expression of cytokeratins 5, 6, or 17, which are typically expressed in the basal epithelial layer of the skin and airways. Over the years, researchers have come to discover many important features of the basal-like subtype, including the fact that the vast majority of these tumors are *p53* mutated. These tumors are highly proliferative, on average, as illustrated by the proliferation index, regardless of whether proliferation is measured by Ki-67, proliferating cell nuclear antigen, immunohistochemistry, or gene expression proliferation.

This group is homogeneously highly proliferative, and this is in large part a result of the lack of RB1 protein function, which is a critical regulator of the cell cycle. These cells are RB deficient and p53 deficient, and thus they are likely to grow quite quickly. These cells have also been found to be associated with *BRCA1* mutation status, whereby the majority of *BRCA1* mutation carriers, when and if they develop breast cancer, develop basal-like breast cancer. Researchers believe that this p53 loss, RB loss, and BRCA1 pathway association is responsible for the high aneuploidy seen in these tumors, including a huge number of chromosomal changes, translocations, and losses (Fig. 2).

The BRCA1 pathway association now has important therapeutic implications with the introduction of the poly-(ADP-ribose) polymerase (PARP) inhibitors. The PARP inhibitors target an alternate DNA repair pathway in

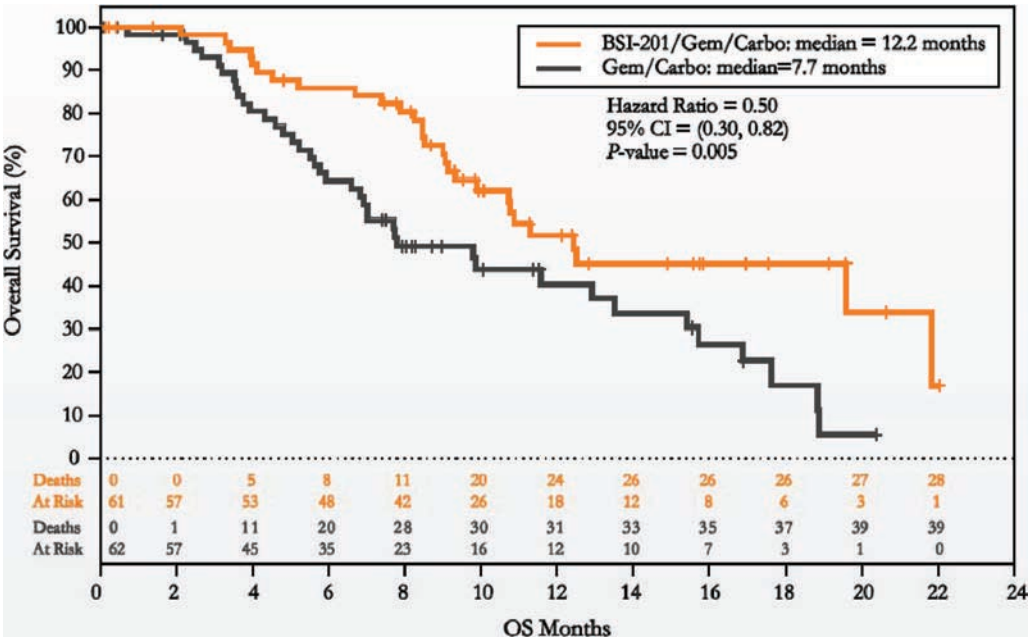

Figure 3. Kaplan-Meier survival analysis of patients from ClinicalTrials.gov Identifier NCT00540358.
Abbreviations: Carb, carboplatin; CI, confidence interval; Gem, gemcitabine; OS, overall survival.
From O'Shaughnessy J, Osborne C, Pippen J et al. Updated results of a randomized phase II study demonstrating efficacy and safety of BSI-201, a poly (ADP-ribose) polymerase (PARP) inhibitor, in combination with gemcitabine/carboplatin (G/C) in metastatic triple negative breast cancer (TNBC) [abstract 3122]. Presented at the 2009 San Antonio Breast Cancer Symposium, San Antonio, Texas, December 9–13, 2009.

BRCA-deficient cells, producing a synthetic lethal effect. A phase II trial of one PARP inhibitor was recently published, showing promising survival data in patients with triple-negative metastatic disease (Fig. 3) [10].

Another important question in triple-negative or basal-like breast cancers is with respect to the chemotherapy benefit in these patients, particularly the benefit of carboplatin. This question is being addressed in the CALGB 40603 neoadjuvant trial, in which patients are randomized to receive neoadjuvant paclitaxel, with or without carboplatin, followed by doxorubicin–cyclophosphamide (AC) chemotherapy. The important question here is whether carboplatin is more effective than other agents in this group. Patients in that study are also being treated with bevacizumab, an antiangiogenesis agent that targets vascular endothelial growth factor (VEGF) (Fig. 4). The effect of antiangiogenesis therapy is a particularly attractive and important question in triple-negative tumors. In one unpublished analysis, average VEGF expression was highest in the basal-like and HER-2–enriched subtypes.

A 13-gene hypoxia signature has been developed to further characterize triple-negative tumors. This signature is at its highest level in the basal-like and claudin-low groups, again suggesting that angiogenesis inhibitors should be tested within basal-like tumors, and possibly also within the HER-2–enriched and claudin-low subtypes (Fig. 5) [11].

Therapeutic Targets for Basal-Like Breast Cancer

The baseline prognosis for patients with basal-like breast cancer is poor in the absence of therapy, so it is critical to identify the best therapeutic targets for these patients. The chemotherapy benefit is high, but there are many promising new targets and approaches for this group, including PARP inhibitors, a potential carboplatin benefit, angiogenesis inhibitors, and HER-1–RAS–mitogen-activated protein kinase/extracellular signal–related kinase kinase pathway inhibitors. Some preclinical and actual clinical data are available to suggest that the HER-1–RAS pathway is active and important in the basal-like group. The key issue is how to effectively target the pathway.

One analysis was conducted that evaluated the pathologic complete response (pCR) rate according to intrinsic subtype, combining data from three different neoadjuvant studies that had similar anthracycline- and taxane-containing regimens. These included the doxorubicin–cyclophosphamide plus docetaxel (AC-T) arm from the National Surgical Adjuvant Breast and Bowel Project B-27 trial, the taxane plus fluorouracil– doxorubicin–cyclophosphamide neoadjuvant chemotherapy study, and the AC-T arm of the I-SPY study. Results from combining these data nicely illustrate what has been shown in many different publications, which is that, in these neoadjuvant studies, luminal A

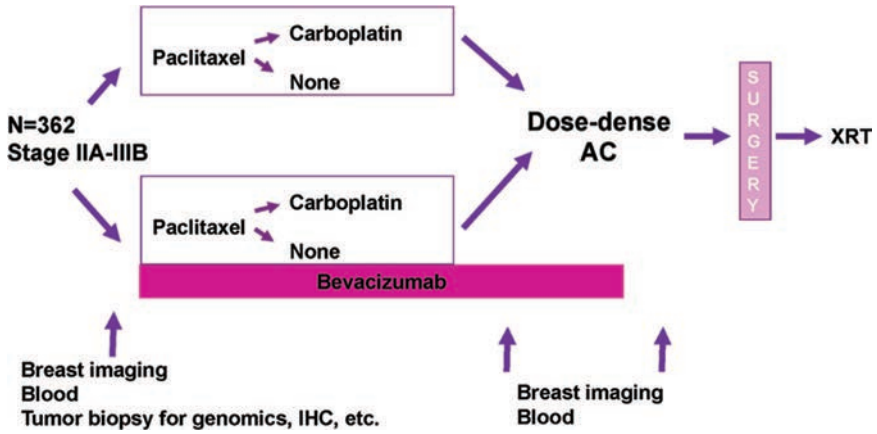

Figure 4. Cancer and Leukemia Group B Neoadjuvant Trial 40603: Randomized phase II 2 × 2 factorial trial of the addition of carboplatin with or without bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC in hormone receptor-poor/HER-2⁻ resectable breast cancer (ClinicalTrials.gov identifier, NCT00861705; triple-negative patients, ~75% basal-like).

Abbreviations: AC, doxorubicin and cyclophosphamide; IHC, immunohistochemistry; XRT, radiotherapy.

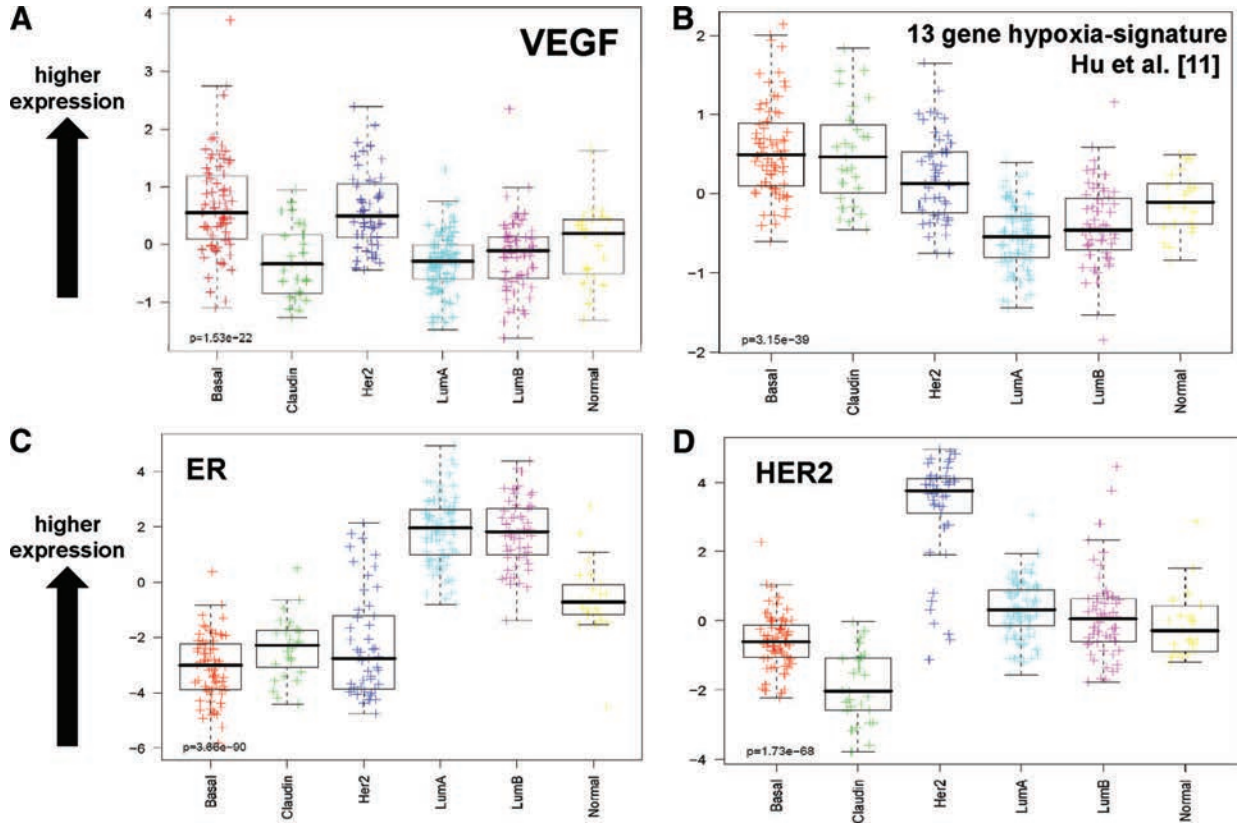

Figure 5. Hypoxia-related features are common in basal-like tumors. Analysis of variance plots of relative gene expression according to intrinsic subtypes: **(A)** VEGF. **(B)** 13-gene hypoxia signature. Based on signature from Hu Z, Fan C, Livasy C et al. A compact VEGF signature associated with distant metastases and poor outcomes. BMC Med 2009;7:9. Originally published by BioMed Central. **(C)** ER. **(D)** HER-2.

Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor; VEGF, vascular endothelial growth factor.

subtype patients tend to be the most common; however, they have a very low pCR rate, in this case only 7%. The basal-like group had the highest pCR rate at 43%. The HER-2–enriched subtype had a pCR rate of approximately 38%, in the absence of any trastuzumab. Overall, this analysis showed that this group is chemotherapy sensitive, and it is likely that the addition of trastuzumab improves the pCR rate even more. Meanwhile, the luminal B group had

Table 1. Neoadjuvant response rates according to intrinsic subtypes and other clinical parameters.

Variable		Prevalence	RD	pCR	% pCR	UNI OR	UNI p-value	MVA OR	MVA p-value
ER	+	62%	189	27	13%	0.1	<0.0001	0.8	0.725
	−	38%	75	55	42%				
PgR	+	44%	96	12	11%	0.2	0.0002	0.6	0.401
	−	56%	87	48	36%				
HER2	+	24%	34	23	40%	4.3	0.0109	2.0	0.715
	−	76%	146	32	18%				
Grade	1	4%	7	1	13%	1.0	0.0054	1.0	0.503
	2	43%	83	12	13%	1.1		0.3	
	3	54%	77	42	35%	3.6		0.4	
T	1	6%	11	5	31%	1.0	0.72	—	—
	2	48%	92	29	24%	0.7			
	3	31%	59	19	24%	0.9			
	4	15%	30	7	19%	0.5			
N	0	32%	66	15	19%	1.0	0.30	—	—
	1	51%	91	39	30%	2.1			
	2	9%	18	6	25%	1.8			
	3	7%	18	0	0%	0.0			
Subtype	Luminal A	30%	100	7	7%	1.0	<0.0001	1.0	
	Luminal B	20%	59	12	17%	6.0		4.8	
	Her2-enriched	16%	36	20	36%	20.7		8.4	0.033
	Basal-like	27%	55	42	43%	28.6		18.3	
	Normal-like	7%	21	5	19%	NA		NA	

Related multi-agent neoadjuvant regimens (no trastuzumab), overall pCR rate = 24%.
- NSABP B-27: AC/T arm only (collaboration with Soon Paik and the NSABP).
- Hess et al. 133 T/FAC from MDACC (analyzed in Parker et al., JCO 2009).
- ISPY: AC/T (collaboration with Laura Esserman and ISPY investigators).
- Multivariable analysis performed on T/FAC and AC/T (ISPY) studies only (N=186 patients).

From Parker JS, Prat A, Cheang MCU et al. Breast cancer molecular subtypes predict response to anthracycline/taxane-based chemotherapy [abstract 2019]. Presented at the San Antonio Breast Cancer Symposium, San Antonio, Texas, December 9–13, 2009.

an appreciable pCR rate, but it was still rather low (Table 1) [12].

A multivariate analysis of these data suggests that the most important piece of information is a patient's intrinsic subtype, and that knowing the ER, PR, or HER-2 status does not provide additional clarification on top of knowing the intrinsic subtype in terms of predicting pCR.

Immunohistochemistry Subtypes and Basal-Like Breast Cancer

Over the years, researchers have attempted to develop immunohistochemical surrogates for the intrinsic subtypes so that they can look at rich pathology archives to expand the breadth of information from available studies [13–21]. Recently, an immunohistochemical surrogate was applied to a unique population-based study called the Carolina Breast Cancer Study, which purposely oversampled for African-Americans and younger women with breast cancer (Fig. 6).

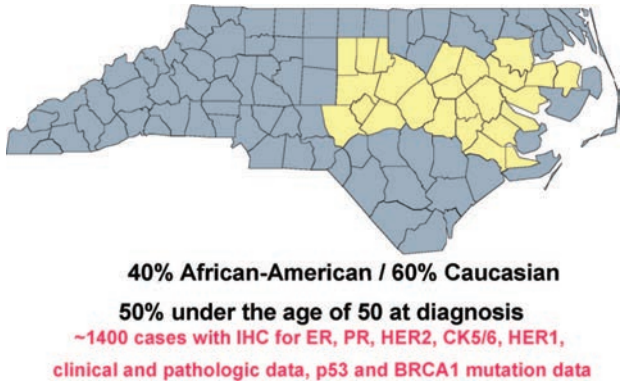

Figure 6. Carolina Breast Cancer Study population-based case-control study.

Abbreviations: ER, estrogen receptor; HER-2, human epidermal growth factor receptor; IHC, immunohistochemistry; PR, progesterone receptor.

This was done because African-Americans and young women have a lower incidence of breast cancer, but a

Table 2. Frequency of intrinsic subtypes across breast cancer cases of the CBCS.

Breast cancer	African-American premenopausal	African-American postmenopausal	Caucasian premenopausal	Caucasian postmenopausal
Subtype	N (%)	N (%)	N (%)	N (%)
Luminal A	108 (41.4%)	179 (56.3%)	216 (57.4%)	293 (66.5%)
N = 796				
Basal-like	70 (27.2%)	52 (16.0%)	54 (14.5 %)	49 (9.3%)
N = 225				
HER2+/ER-	22 (8.4%)	26 (7.7%)	24 (5.6%)	44 (6.0%)
N = 116				
Luminal B	19 (7.3%)	26 (8.7%)	46 (12.4%)	46 (10.7%)
N = 137				
Unclassified	41 (15.7%)	38 (11.3%)	38 (10.1%)	33 (7.5%)
N = 150				
Total: 1424	260 (100%)	321 (100%)	378 (100%)	465 (100%)
$p < 0.0001$				

From Millikan RC, Newman B, Tse CK et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008;109: 123–139, with kind permission from Springer Science+Business Media B.V. Data first presented in Carey LA, Perou CM, Livasy CA et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006;295:2492–2502.

higher mortality risk with the disease. The frequency of this poor outcome, basal-like subtype differed according to age and race, whereby its frequency was lowest in postmenopausal whites, higher in premenopausal whites, and higher still in postmenopausal African-Americans. It was at its highest level, approximately 27%, in premenopausal African-Americans. This finding alone can explain some of the racial outcome disparity differences, because African-Americans tend to be getting more of the aggressive, poor outcome breast cancer. Interestingly, the reverse finding was shown with the luminal A subtype, which is a good-prognosis tumor subtype: its frequency was lowest in young African-Americans and highest in postmenopausal whites (Table 2) [16, 18].

These findings were taken a step further in a study by Olopade and colleagues, in which they studied breast cancer in indigenous African women. The frequency of triple-negative cancers was higher in this population, suggesting that there must be environmental and/or genetic factors that have an influence on the frequency of the subtypes in different populations [22].

One method of getting at what might be some of the etiologic causes of these frequency differences was to look at some of the risk factors from the Carolina Breast Cancer Study. In fact, there were a number of risk factors that actually had opposite effects according to tumor subtype. Importantly, features like having multiple children and a younger age at first birth were protective for luminal A disease, but predisposing to basal-like cancer. Other features, like a high waist-to-hip ratio, which is a measure of obesity, were predisposing for both types of ER^+ and ER^- cancers, but it was quite stunning to see that some of these well-known risk factors, like having multiple children, might actually be predisposing to one subtype and protective for another (Table 3) [18]. Clearly, additional studies are needed, but this again suggests that basal-like breast cancer is a unique and distinct disease from ER^+ disease and needs to be treated as such.

Table 3. Luminal A and basal-like patients in the CBCS.

	Luminal A	Basal-like
	N = 796	N = 225
Menarche < 13	1.1 (0.9–1.3)	1.4 (1.1–1.9)
> 3 children	0.7 (0.5–0.9)	1.9 (1.1–3.3)
First birth < 26	0.7 (0.5–0.9)	1.9 (1.2–3.2)
Breastfeeding > 4m	0.9 (0.7–1.1)	0.7 (0.4–0.9)
Parity > 3 and No breastfeeding	0.7 (0.5–0.9)	1.9 (1.1–3.3)
Waist: Hip > 0.84	1.5 (1.1–1.9)	2.3 (1.4–3.6)

Adjusted ORs (95% CI).
N = 1424 cases and N = 2022 controls from the CBCS.
From Millikan RC, Newman B, Tse CK et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008; 109:123–139, with kind permission from Springer Science+Business Media B.V.

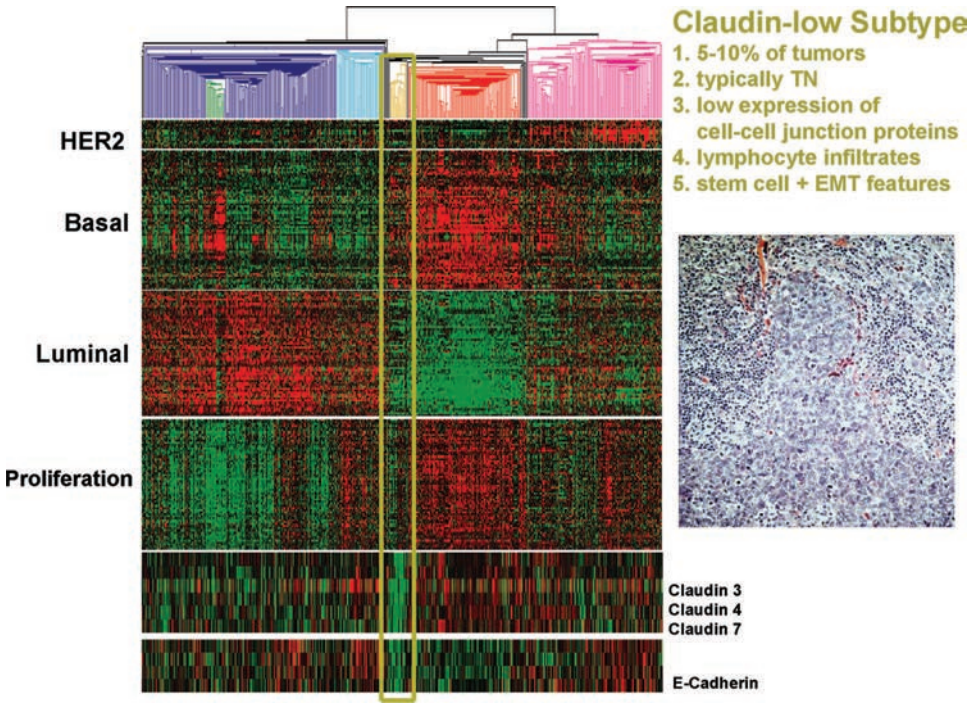

Figure 7. Cluster diagram of intrinsic subtypes highlighting the claudin-low subtype.
Abbreviations: EMT, epithelial–mesenchymal transition; HER-2, human epidermal growth factor receptor; TN, triple negative.

Claudin-Low Subtype Tumors

The most recently identified tumor group is the claudin-low subtype. The subtype name is based on the defining feature of this group, specifically that these tumors exhibit low expression of many of the claudin genes, including 3, 4, and 7. The claudins are involved in epithelial cell tight–tight junctions. Another epithelial cell interaction protein is E-cadherin. These claudin-low tumors lack cell–cell junction proteins, including E-cadherin. The claudin-low tumors are also triple negative, and so can be considered another subtype of triple-negative disease, in addition to the basal-like group. As shown in the clustering analysis in Figure 7, claudin-low tumors are somewhat similar to basal-like cancers, but distinct (Fig. 7). Other important features of claudin-low tumors is that they almost always have an intense immune cell infiltrate, and they also have stem cell features and features of epithelial–mesenchymal transition (EMT).

Histology findings from four claudin-low tumors were of interest because the low expression of E-cadherin hinted that they might be lobular cancers. Although most of these tumors do not have lobular features, they are generally characterized by a high tumor grade, particularly little differentiation, and again, they very often have an intense immune cell infiltrate [6].

One of the reasons that researchers believe that claudin-low tumors have features of tumor-initiating cells or stem cells was based on a study in which fluorescence-activated cell sorting on primary human breast tumors was used to isolate the stem cell fraction of the tumor-initiating cell fraction, by isolating the $CD44^{+}CD24^{-}$ fraction. Researchers then compared this tumor-initiating cell fraction with the other fractions and identified what they called a tumor-initiating cell signature, which was highly enriched for genes that have mesenchymal features including *ZEB1*, *twist*, and *snail*, and other EMT features. Comparing the signature of tumor-initiating cells with each of the intrinsic subtypes, only claudin-low tumors were statistically enriched for this tumor-initiating cell signature. Virtually every claudin-low tumor was enriched. These data suggest that claudin-low tumors are somewhat naturally enriched for tumor-initiating cells or stem cells, and so this group may offer us a unique opportunity to be able to study tumor initiating cells and stem cells through the examination of the unique properties of this subtype (Fig. 8) [23].

From a biological perspective, intrinsic breast tumor subtypes may be reflective of arrest at different stages of epithelial cell development. The claudin-low group represents the most primitive tumors that are the most similar to the mammary stem cell, and the next step on the pathway is what is sometimes referred to as the luminal progenitor, which is the basal-like phenotype. A *BRCA1* mutation is linked to this luminal progenitor/basal-like phenotype, and thus, somehow loss of BRCA1 may block further differentiation and keep a cell in this step of development.

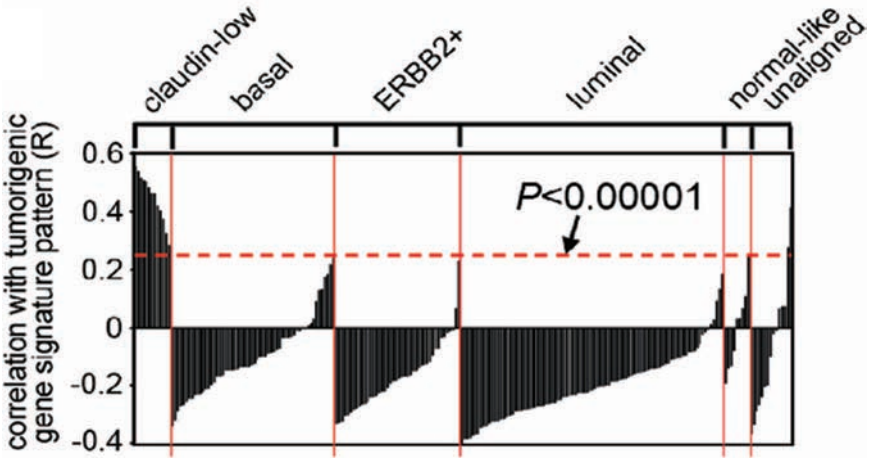

Figure 8. Tumor-initiating cell signature is enriched in claudin-low subtype.

From Creighton CJ, Li X, Landis M et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci U S A 2009;106:13820–13825. Reprinted with permission.

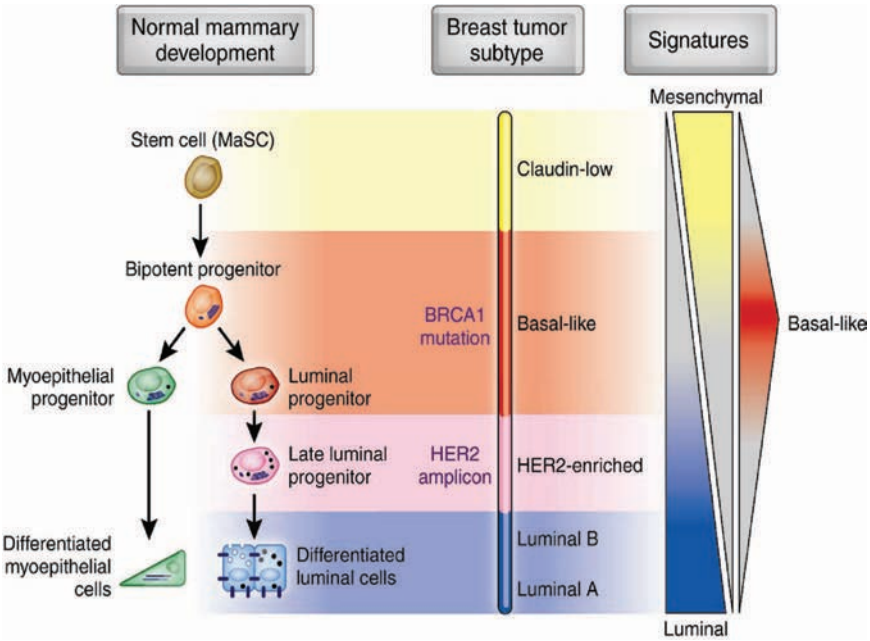

Figure 9. Mammary epithelial development and its possible relationship to the intrinsic subtypes of breast cancer.

Abbreviation: HER-2, human epidermal growth factor receptor.

From Prat A, Perou CM. Mammary development meets cancer genomics. Nat Med 2009;15:842–844. Reprinted by permission from Macmillan Publishers Ltd: Nature Medicine, copyright 2009.

The next step in development may take a luminal progenitor/basal-like cell to the HER-2–enriched subtype state, which is basically a loss of basal characteristics and a gain of luminal characteristics. The most differentiated groups are the luminal A and luminal B tumors (Fig. 9) [24].

Conclusions

In the absence of therapy, patients with claudin-low tumors have a poor prognosis, similar to the prognosis of patients with basal-like tumors and HER-2–enriched subtypes. It is therefore critical to treat these patients. However, these patients are likely to have triple-negative tumors, and treatment options are limited. This group does benefit from chemotherapy, to some degree, but better treatment options are needed. An important outstanding question is whether PARP inhibitors are effective in this group, because they too may be linked to BRCA1 pathway alterations, and another intense area of study is to target cancer stem cells in these patients.

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