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Review Article

Is "Basal-like" Carcinoma of the Breast a Distinct Clinicopathologic Entity? A Critical Review with Cautionary Notes

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ABSTRACT

This review deals with studies that have used cDNA microarrays and immunohistochemistry to identify a subtype of breast carcinoma recently known as "basal-like" carcinoma. The key breast carcinoma studies are critically discussed to highlight methodological problems in cohort selection, definitions, interpretation of results, and statistical analysis. It concludes that "basal-like" carcinomas do not reflect a single, biologically uniform group of breast cancers and show significant variations in their phenotypes, grades, immunoprofiles, and clinical behavior, just as a wide range of subtypes and behaviors is observed among epithelial/luminal-derived breast carcinomas. Well-designed studies with comparison of low grade non-basal versus low grade basal and high grade non-basal versus high grade basal carcinomas are necessary before one can be convinced that this subtype represents a distinct clinicopathologic entity.

Key Words: Breast cancer, Basal-like carcinoma, Microarray Analysis, Molecular, Classification Tumors, Immunohistochemistry

Introduction

The traditional clinicopathological parameters such as tumor size, involvement of axillary lymph nodes, histologic grade, nuclear grade, MIB-1 (Ki-67) index, expression of estrogen and progesterone receptors (ER, PR), over-expression (or amplification) of Her2/neu, and mutations in the TP53 gene all have been successfully correlated to prognosis of patients with breast carcinoma (1-9). With regard to the biology of breast cancer, however, the current prognostic factors provide limited information (9). Moreover, the well-established prognostic and/or predictive factors have significant limitations in distinguishing breast cancer patients who may benefit from aggressive chemotherapy from those who do not need any adjuvant treatment (10). Indeed, it has been shown that about 70% of patients with breast cancer receiving chemotherapy or anti-hormonal therapy would have survived without such treatments (10, 11).

The molecular-genetic heterogeneity and the large number of genes involved in controlling cell proliferation, apoptosis, and differentiation clearly underline the importance of investigating multiple genetic changes in a variety of phenotypically different breast carcinomas. The introduction of complementary DNA (cDNA) and oligonucleotide microarrays in the mid 1990s (12, 13), the increasing

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application of these high throughput techniques, and significant improvement in bioinformatic analyses have resulted into an era of genome-wide approaches to prognostification and outcome prediction in patients with breast cancer.

In 2000, Perou & Sorlie et al. showed in their seminal paper (14) that the phenotypic diversity of breast carcinomas is accompanied by a corresponding diversity in gene expression patterns that can be captured using cDNA microarrays. For the first time, Perou & Sorlie et al. demonstrated that breast cancers could be classified into distinctive subtypes distinguished by characteristic differences in their gene expression patterns (GEP) or their "molecular portraits" (14). In their first study, they were able to identify four groups of breast cancers related to different molecular features of mammary epithelial cells composing of ER+/luminal-like, basal-like, Her2/neu +, and normal breast. (14). This study and the subsequent study in 2001 (15) had a major impact on both molecular biologists and oncologists and also challenged the traditional histopathological classification system of breast carcinomas. An important implication of these two studies was that ER-negative breast carcinomas include at least two biologically distinct subtypes of tumors, namely basal-like and Her2/neu positive cancers, which may need to be treated as distinct entities (14, 15).

Essential information provided by two initial publications in 2000 and 2001

The first c-DNA study performed by Perou & Sorlie et al. analyzed variations of gene expression patterns in grossly dissected normal and malignant human breast tissues from 42 individuals consisting of 36 infiltrating ductal carcinomas, 2 lobular carcinomas, 1 ductal carcinoma in situ, 1 fibroadenoma, and 3 normal breast samples (14). Fluorescently labeled cDNA was prepared from mRNA from each experimental sample. The authors prepared a pool of mRNAs isolated from 11 different cultured cell lines that served as a common "reference" sample. The reference sample was labeled using a second distinguishable fluorescent nucleotide and provided an internal standard against which the gene expression of each experiment sample was compared. Using a hierarchical clustering method, the authors focused first on a set of 1.753 genes (about 20% of the 8.102 genes analyzed) in order to group genes on the basis of pattern similarity which their expression varied over all samples. Finally, the authors selected a subset of 496 genes ("intrinsic" gene subset) that consisted of genes with significantly greater variation in expression between different tumors than between paired samples from the same tumor. Using the "intrinsic" gene subset, the cluster analysis revealed 4 distinctive groups consisting of 1) luminal epithelial/ER+, 2) basal-like, 3) Her2+, and 4) normal-breast-like carcinomas. This study concluded that application of cDNA microarrays and hierarchical clustering provide a distinctive "molecular portrait" of each breast cancer and that the breast tumors could be classified into subtypes based on differences in their molecular patterns (14).

In the subsequent important study of Sorlie & Perou et al. which was published in 2001 (15), the authors refined their previous classification by analyzing a larger number of breast carcinomas and explored the clinical value of the subtypes by searching for correlations between cDNA gene expression pattern and clinically established prognostic factors. The authors analyzed a total of 85 cDNA microarray experiments representing 78 breast carcinomas (including 71 ductal, 5 lobular, and 2 DCIS), 3 fibroadenomas, and 4 normal breast tissues. Using hierarchical clustering of the variations in gene expression, the authors were able to classify breast cancers into basal-like, Her2-overexpressing, and normal-breast like groups. The previously identified luminal epithelial/ER+ group could be subdivided into at least two subgroups of luminal A and luminal B, each with a distinctive molecular genetic profile. In order to investigate whether the 5 distinctive groups represent clinically distinct subgroups of patients, univariate survival analyses comparing the subtypes with regard to disease-free survival (DFS) and overall survival (OS) were performed. This second study revealed a highly significant differences in survival between the subtypes, with the Her2+ and basal-like subtypes associated with the shortest DFS and OS. The results of the second study (15) indicated that basal-like subtype may represent a different clinical entity. Furthermore, this study suggested that ER+ cancers are highly heterogeneous with respect to their gene expression patterns and that luminal subtype can be divided into luminal A and luminal B (or even luminal C) associated with different clinical outcome. According to this study, the luminal subtype B (and C) carcinomas may represent a clinically distinct group with poorer prognosis as compared to luminal A breast cancers (15).

Issues with two major cDNA studies published in 2000 and 2001

Although, without any doubt, these two publications (14,15) had a major impact on our current understanding and classification of breast carcinoma, one needs to re-evaluate these more cautiously. Indeed, there are a number of shortcoming that hamper the enthusiasm about these two papers. The problems are briefly discussed as follow:

1)Use of different cancer cell lines as a common "reference" sample

As mentioned above, Perou & Sorlie et al. used a pool of mRNAs isolated from 11 different cultured cell lines that provided an internal standard or "reference" sample against which the gene expression of breast cancer was compared. According to the Supplementary Information of the first study (14), the "reference" sample was composed of equal mixtures of mRNA isolated from breast (MCF7, HS578T), ovarian cancer (OVCAR3), hepatoblastoma (HepG2), embryonal carcinoma (NTera2/D1), acute leukemia (NB4), T4 leukemia (MOLT4), multiple myoloma (RPMI8226), malignant melanoma (UACC62), liposarcoma (SW872), and colon carcinoma (COLO205) human cell lines. In contrast to the gene profiling study of van't Veer et al. (16) in which a pool of cRNA from each individual sporadic breast cancer patient as a "reference" was used, Perou & Sorlie et al. used a mixture of highly heterogeneous cell lines of epithelial, mesenchymal, and haematologic cancers of different organs as a control. The crucial question is whether the used "reference" sample, as used by Perou & Sorlie et al. is the appropriate one for genetic comparison, if one tries to better understand the biology of breast carcinoma. It is not clear why Perou & Sorlie et al. did not use a "reference" sample exclusively from frozen tissues of normal breast (tumor-free and normal breast tissue away from breast carcinoma) as an internal standard. Although human cell lines and primary cultures have been a popular choice due to their widespread availability, and the ease of obtaining large amounts of RNA for microarray analysis, a "reference" sample exclusively consisting of normal breast tissue or, at least breast cancer cell lines, is a much more appropriate standard for comparison, if one tries to demonstrate "up-regulation" and "down-regulation" of several thousands of genes in patients with breast carcinoma. As the field has evolved, investigators have realized the crucial issue of reference RNA samples used by different array platforms (17-19).

2)Issue of small sample size and statistical evaluation of enormous data bases

The first study examined 39 breast cancers consisting of 36 IDC, 2 ILC, and 1 DCIS. cDNA analysis and hierarchical clustering of the results of this study revealed 6 (15%) breast cancers with "basal-like" features (BLC) (14). In the subsequent study of Sorlie & Perou et al. which also included correlation to clinical outcome, 78 breast carcinomas comprising of 71 IDC, 5 ILC, and 2 DCIS were examined (15). This second study revealed 7 BLCs. It is important to point out that for survival (DFS, OS) analyses, only a subgroup of 49 patients with locally advanced tumors and no distant metastases were investigated (only by univariate analysis). In fact, the total number of cases and the number of BLC cases in both studies were too small to allow a meaningful and reliable statistical analyses. As correctly pointed out by Ioanndis (20), sample size plays a crucial role for analysis of results obtained by microarrays and molecular research. "Microarrays need evidence and this cannot be obtained from a couple of small studies, no matter how high tech". (20). Indeed, the same caveat applies not only in gene expression profiling, but also in proteomics, and all discovery-oriented molecular research where enormous data bases can be rapidly generated from just a small number of patients (20-22). To achieve reliable and reproducible results, Ein-Dor et al. (23) could recently prove that thousands of samples are needed to generate a robust gene list for predicting outcome in cancer.

3)Hierarchical clustering of luminal-like subtype using the "intrinsic" gene subset

A close look to the first study (see ref 14, Fig.3) reveals that genes identified in "luminal-like" subtypes included prolactin receptor, myosin VI, hepatocyte nuclear factor 3, angiotensin receptor 1, and most importantly estrogen receptor 1 as well as estrogen associated protein (LIV-1). It is however, completely unclear why luminal-type cytokeratins such as CK8/18 and/or CK19 are not shown in this subtype of breast cancer (see ref 14, Fig:3), although the terminology of "luminal-like" would imply this. Indeed, there is a well-known fact that "luminal-like" carcinomas which often express ER, show a strong immunoreaction for CK8/18 and or CK19 in the vast majority of cancer cells.

4) Hierarchical clustering of "basal-like" subtype using the "intrinsic" gene subset

The cluster analysis of the first study displayed a "basal-like" subtype of breast carcinoma. This subtype was characterized by two basal-type cytokeratins, namely CK5 and/or CK17 (but not CK14 as identified in many IHC-studies). Other genes of this subtype included laminin (gamma2), collagen type XVII, calponin1, caveolin 2, and heparin binding growth factor 8. The first study also described a second basal epithelial-cell-enriched gene cluster showing cytokeratins 7 and 13 (both are not regarded basal-type cytokeratins), epidermal growth factor receptor, fatty acid binding protein 7 (brain), P-cadherin placental, protein tyrosine phosphatase (receptor type, k), integrin (beta 4), and troponin. It is of note that this second gene cluster of "basal-like" subtype did not show basal-type cytokeratins such as CK5 and/or CK17.

The authors of these two studies also performed IHC on cases with gene profiling of CK5 and/or CK17 and reported positive staining for either CK5/6 or CK17 or both in all cases of "basal-like" subtype. The authors did not use a cut-off for positive immunoreaction in tumor cells of this subtype. Judging from one illustration (ref 14, Fig.2d), the immunostaining for CK5/6 was heterogeneous. On the other hand, several IHC-studies recognized a strong positivity for CK8/18 and/or CK19 (both being luminal-type cytokeratins) in BLCs. Indeed, it is extremely rare to observe immunostaining for CK5/6 or CK17 without positivity of breast cancer cells for CK8/18 or CK19. Moreover, the immunoreaction of tumor cells in BLCs for CK8/18 and/or CK19 is often much more intense and in much higher proportion than that of basal-type cytokeratins. This would imply that "basal-like" subtype should also show these luminal cytokeratins by means of cDNA microarrays, which is not the case. In other words, with respect to basal and luminal type cytokeratins, there is no clear correlation of GEP-results and IHC-results. This discrepancy requires further explanation.

Subsequent molecular-genetic findings

There have subsequently been a number of publications that have used variations of the "intrinsic" gene set to confirm the existence of the "molecular" portrait, with particular attention to the basal-like carcinoma and its prognostic significance (24- 29). It is important to note that GEP studies of primary breast carcinomas performed by different laboratories have resulted in the identification of a number of distinct prognosis profiles, or gene sets that share very little overlap in terms of gene identity (23, 30-32). A recent study (32) examined 295 breast cancer samples and applied five different gene-expression-based models. The results of GEP of the examined cases using "intrinsic subtype (14), 70-gene profile (16), wound response (33), recurrence score (34) and the two-gene ratio (35) were correlated with the probability of relapse-free survival and overall survival. An interesting finding of this current study (32) was that despite the absence of gene overlap, four of the five tested gene models (all but the two-gene ratio approach) showed significant agreement in the outcome predictions for individual patients. This study also revealed that a patient whose breast cancer is classified as basal-like based on the "intrinsic" gene set will most likely be classified as having a poor 70-gene profile , a poor activated wound response, and high recurrence score (32).

Using comparative genomic hybridization (CGH), a relatively recent publication (36) demonstrated that "basal-like" carcinomas show losses at 16p, 17q, 19q and Xp. The "basal-like" cancers displayed in this study chromosome gains at 20q, 1q, 4q, 6q, 7q, and 8q. This study have found a striking similarity in the pattern of CGH alterations in "basal-like" carcinomas and myoepithelial carcinomas (synonyms: malignant myoepithelioma, or carcinoma with myoepithelial differentiation) (36). Furthermore, the clinical outcome of patients with poorly differentiated non-basal-like carcinoma (infiltrating ductal carcinoma, NOS type) was compared with that of patients with "basal-like" carcinoma. A remarkable finding of this study was that the "basal-like" carcinomas on their own do not convey a poor prognosis and that these tumors represent a heterogeneous group with only a subset showing a shorter overall survival (36).

Immunohistochemical features

While BLC of the breast was initially defined by gene expression profiling, most of the recent published works have used immunohistochemistry to define BLC. Basal-like carcinomas commonly show a focal positive immunoreaction with antibodies against basal-type cytokeratins (or high molecular weight cytokeratin) such as CK5/6, CK14, and CK17. While most BLCs are negative for ER, PR, and Her2 (triple negative), many of them express

EGFR (Her1), immunohistochamically (37-42). The vast majority of basal-like carcinomas express both luminal type cytokeratins (such as CK8/18 and CK19) and vimentin (37, 39, 42). A positive reaction for c-kit (CD117) can also be identified in more than 50% of basal-like carcinomas (37, 39, 42).

It is important to keep in mind that some of the myoepithelial markers in the breast such as SM-actin, maspin, CD10, 14-3-3 sigma, and CD29 can also be positive in tumor cells of BLCs indicating that at least some of the basal-like carcinomas show a myoepithelial differentiation raising the possibility of myoepithelial origin of this subtype of breast carcinoma (41).

It is important to note that a wide variety of definitions have been used for immunohistochemical characterization of BLC. Using immunohistochemistry, most previous studies have noted a heterogeneous and focal expression of basal-type cytokeratins in BLCs. The vast majority of these studies have shown a much more intense/diffuser expression of luminal-type cytokeratins such as CK8/18 or CK19 in BLCs. Indeed, no minimal threshold for what constitutes a positive immunoreaction with antibodies against basal-type cytokeratin has been established or agreed upon. Consequently, there are several open questions that need to be addressed by an international consensus to define the precise immunohistochemical profile of BLCs. These are:

1) Considering the heterogeneous and often focal immunoreaction, what is the lowest threshold for accepting a lesion as positive? Is a positive reaction for K5/6 or K17 in a single cell sufficient to consider a given breast cancer basal-like? Or is 5%, 10%, or even more than 50% positivity a better requirement? Is there any difference between a lesion that is 10% positive and one that is diffusely (100%) positive?

2) What type(s) of basal-type or high molecular weight cytokeratins should be useded to confirm BLC? The original two studies of Perou & Sorlie identified K5/6 and K17 in basal-like subtype by using cDNA microarrays and immunohistochemical validation. CK14 was not mentioned in these two studies. A number of recent IHC- studies, however, have used cytokeratin14 as a marker for BLC. Is a positive immunoreaction for cytokeratin14 without positivity for keratins 5/6 and/or 17 be used as a marker of BLC? Should we require a panel of three basal-type cytokeratins such as CK5/6, CK14, and CK17 to characterize BLC? Should we also require expression of p63, CD10 and other myoepithelial markers?

4) How should one classify a poorly differentiated breast carcinoma that displays positive immunoreaction for a variety of basal-type cytokeratins and also exhibits some positive reaction for ER or PR, but is negative for Her2? One has to keep in mind that although all 6 cases of BLC in the first study of Perou & Sorlie et al. (14) were negative for ER, the second study performed by Sorlie & Perou et al. (15) included 2 ER positive breast cancers among the basal-like subtype (2 out of 7 cases). Should one classify a Her2 positive but ER and PR negative breast carcinoma which also express some positivity for basal-type cytokeratins BLC or do we need to reserve this subtype for triple negative cases that express basal-type cytokeratins?

Histopathologic features of BLC

The vast majority of publications use the designation of basal-like phenotype for this subtype of invasive breast carcinoma. This subtype of carcinoma is, However, defined either by genotyping (GEP, using "intrinsic" gene model) or immunohistochemistry (using antibodies against basal-type cytokeratins). Therefore, the designation of "phenotype" is not quite appropriate since it is actually the immunoprofile or immunotypic characteristics that define this subtype. In fact, the designation of basal-like phenotype would imply that carcinomatous cells histomorphologically look like basal cells. This is, definitely, not the case! In addition, it should be noted that epithelial and myoepithelial cells have long been recognized in the breast (1,2). It has also been known for quite some time that the normal epithelial cells variably express CK8, CK18, ER and PR, while the myoepithelial cells generally express CK5/6, CK14, CK17, p63, CD10, actin, and calponin, but never express either ER or PR (1,2). Does replacing epithelial with "luminal" and myoepithelial with "basal" add anything? Nevertheless, there are some phenotypic features of carcinomas that are more likely to be associated with this recently recognized subtype of breast cancer. Basal-like carcinomas usually, but not always, show high nuclear atypicality, high mitotic activity including several atypical mitotic figures, high MIB-1 (Ki-67) index, solid aggregates of tumor cells often with pushing border of invasion, and not infrequently a marked lymphocytic stromal reaction (40, 42). Areas of central "comedo" type or acellular ("geographic") necrosis within the invasive, solid

aggregates can be present (40, 42- 44). The above mentioned morphologic features are commonly observed in poorly differentiated invasive ductal carcinoma (grade 3 IDC, NOS type).

The vast majority of metaplastic (sarcomatoid) carcinomas of the breast show the characteristic immunohistochemical features of BLC (2, 41, 45-47). Indeed, there is no sharp dividing line between metaplastic carcinoma and BLC. The metaplastic carcinomas with basal-like immunophenotype include adenosquamous carcinoma, spindle cell carcinoma (including carcinoma with myoepithelial differentiation), carcinoma with heterologous chondroid and/or osseous differentiation (including so-called matrix-producing carcinoma and carcinosarcoma) (1,2,41). In addition, most of medullary carcinomas and so-called atypical medullary carcinomas (IDC with medullary features) are triple negative and express basal-type cytkeratins (48).

It is important to note that although most examples of basal-like subtype are of high grade and show a very high mitotic activity, there are certain low grade primary breast carcinomas which definitely show the immunoprofile of BLCs. These rare BLCs which clinically have a very low aggressive behavior include adenoid-cystic carcinoma, low grade adenosquamous carcinoma, low grade squamous carcinoma, low grade spindle cell ("fibromatosis-like") carcinoma, and secretory ("juvenile") carcinoma (1, 2). All these primary low grade breast carcinomas express basal-type cytokeratins and often are negative for ER, PR, and Her2 (triple-negative) (2). In sharp contrast to the high grade BLCs, the low grade variant of BLC lacks significant nuclear atypia and shows a very low MIB-1 (Ki-67) index or mitotic activity (2).

A distinct clinicopathologic entity?

Since the first two publications of Perou & Sorlie et al. in 2001 and 2001 (14,15), there have been a marked increase of publications concerning BLC. Although, several publications have confirmed the "distinctive" nature of this cancer subtype, both genetically and clinicopathologically, there are some recent publications raising serious questions, particularly with regard to the poor prognosis of this subtype. (49,50).

The main reasons for advocating this newly recognized subtype as a distinct clinicopathologic entity are discussed as follow:

1) Prognosis

Gene expression profiling (GEP) studies have repeatedly shown that the basal-like and Her2-overexpressing subtypes of breast carcinomas have significantly poorer disease-free survival (DFS) and overall survival (OS) than their luminal A/ER+ counterparts (15, 24, 27, 51). However, many of these studies did not specifically describe histopathologic features of the cohort of BLCs whether they were mostly of high grade nuclear atypia, contain high number of mitotic figures, show a high MIB-1 (Ki67) index, or were strongly positive for p53 immunostaining. In other words, if one takes high grade BLCs in her/his study and compare the clinical outcome of this subtype with that of the luminal A/ER+ which often represents low (grade1) or intermediate (grade2) breast cancer, the prognosis (OS and DFS) will be, of course, much poorer in BLCs. On the other hand, none of the GEP-studies seems to include special subtypes of breast carcinomas that are of low grade nuclear atypia but characteristically are triple negative and express basal-type cytokeratins such as CK5/6, CK14, or CK17. A typical example of this low grade variant of BLC is adenoid cystic carcinoma of the breast which is well-known to have an excellent prognosis (1, 2, 52). Other examples of low grade breast carcinomas with basal-like immunotype that do not seem to be examined by previous GEP-studies are low grade metaplastic carcinomas (low grade adenosquamous carcinoma, low grade squamous carcinoma, low grade "fibromatosis"-like or spindle cell carcinoma, and low grade "matrix-producing carcinoma"). All these special types of breast carcinomas express several basal-type cytokeratins, are mostly triple negative and are associated with a very low proliferative activity (low MIB-1 index, low mitotic activity) (1, 2). It is well-known that all these low grade carcinomas have a good (or excellent) prognosis, yet not included in the category of BLCs in GEP-studies. Therefore, the selection of BLCs in the previous GEP-studies with clinicopathologic correlation is not representative of all variants.

The situation is more complex and confusing if one focuses on a number of studies that solely used immunohistochemistry (IHC) for their definition and characterization of BLCs. Due to the lack of uniform IHC-definition and probably some biased selection of the cases, these IHC-studies revealed contradictory results with regard to the prognosis of BLCs as being an independent poor prognostic factor.

In this section, 8 recent IHC-studies are analyzed, in order to discuss relevant issues concerning

contradictory results in BLCs. First, the focus is on three large studies from the Nottingham group (53-55) in which the basal-like subtype was defined as IHC positivity for CK 5/6 and/or CK14 and have shown BLCs to be associated with poorer OS and/or DFS than non-basal breast carcinomas (ref). In the following discussion, these three publications are referred to as Nottingham study1, 2 and 3.

Nottingham study1: Using tissue microarray (TMA) technology, the first study from this group which was performed by Abd El-Rehim et al (53), examined a high number of 1944 cases of invasive carcinoma with antibodies against basal (CK5/6, CK14) and luminal cytokeratins (CKs 8, 18, 19) to determine the frequency of expression of each cytokeratin subtype and compare their relationships with well-established clinicopathologic prognostic factors. Any positive immunoreaction (even single tumor cells) for CK 5/6 and/or CK 14 was regarded positive. This study has found that basal marker expression was significantly related to poor prognosis, ER negativity, and younger patient age (53). Multivariate analysis revealed that CK 5/6 (but not CK14) was an independent indicator for DFS. Overall survival of BLCs, however, was not significantly different from that of non basal-like carcinomas. The multivariate analysis of the results of this study included grade, nodal status, tumor size, Nottingham prognostic index (NPI), status of ER, vascular invasion, and patient age. It is of note that 2 well-established and important pathologic parameters, namely MIB-1 (Ki67) index and immunoreactivity for p53 were not included in the multivariate analysis.

Nottingham Study 2: The second TMA-study performed by Rakha EA et al. (54) also examined 1944 cases and evaluated morphological and immunophenotypic characteristics of breast carcinomas with basal and myoepithelial immunotype (differentiation). The results of this study were correlated with outcome data. The authors were able to indentify two groups of breast cancers: a) tumors with basal immunotype that expressed one or both CK5/6 and/or CK14 and b) carcinomas with myoepithelial immunotype expressing smooth muscle actin (SMA) and/or p63. Positivity was defined as the detection of 10% or more of tumor cells positive for CK5/6, CK14, SMA, and p63 staining. Multivariate analysis in this study showed that tumors with basal, but not myoepithelial, immunotype has an independent value in predicting outcome, associating with reduced DFS and OS (54). On the other hand, carcinomas with the combined basal and myoepithelial immunotype showed the shortest DFS and OS (54).

What is missing in the second Nottingham study is a comparison of outcome (DFS and OS) between grade3 invasive ductal carcinomas (NOS-type) of non basal-type with grade3 BLCs. In other words, this study is unclear whether non basal, but triple negative, grade 3 carcinomas of NOS-type have a different clinical behavior than that of grade 3 BLCs. It is also of note that the second study used the cut-off of 10% for all basal and myoepithelial markers, whereas in the first Nottingham study any immunoreaction for CK5/6 and/or CK14 was considered positive.

Another issue with the second study (54) is the separation of tumors with basal versus myoepithelial (immuno) phenotype (differentiation) just based on very few immunohistochemical markers. It is well-known that normal and neoplastic myoepithelial cells can show positive immunoreaction for some myoepithelial markers but can also be completely negative for several other established myoepithelial markers (1,2). Like salivary glands, depending on functional activity and state of differentiation of myoepithelial cells, the immunoreaction may vary significantly among several established myoepithelial markers such as SM-actin, SM-myosin, calponin, p63, CD10, maspin, 14-3-3sigma, CD29, etc (2). In the second study, Rakha et al. (54) used IHC for only 2 myoepithelial markers (SMA, P63). Clearly, the negative immunoreaction for these 2 markers, by no means, excludes the possibility of myoepithelial differentiation in a given breast cancer. Furthermore, p63 is a marker for both myoepithelial and basal cells (basal cells of skin, prostate, etc.) and therefore is not an appropriate marker, if one tries to distinguish between carcinomas with myoepithelial cell differentiation and carcinomas with basal-like differentiation.

Nottingham study 3 : The third paper which was published in 2006 (55), focused on a large number of 1872 invasive breast carcinomas with a long term follow-up to investigate the clinical significance of BLCs as defined by IHC for CK5/6 and/or CK14 (The cut-off 10% was used). This study confirmed the previous findings that BLCs as a whole were associated with shorter OS and DFS in both lymph node (LN) negative and LN positive subgroups.

An important additional finding of the third study (55) was that when tumors were stratified by histological grade, basal phenotype was not of a significant prognostic value in grades 1 or 2, indicating that expression of basal type cytokeratins by its own has no prognostic value. It is of note, that the third study,

like the two previous studies, is unclear whether grade 3 and triple negative, non basal-like cancers behaved differently from grade 3 BLCs or not.

An IHC-study performed by van de Rijn et al (56) has found expression of cytokeratins 17 and 5 as an independent factor (poor clinical outcome) in breast carcinomas. Using TMA-technology, the authors examined 600 breast cancers immunohistochemically. Tumors with even focal and weak immunoreaction for CK17 and/or CK5 were considered basal-like. The authors of this study found that in the group of breast cancer patients with known lymph node metastases (229 cases), the expression of CK17 and/or CK5/6 had no predictive value. However, in the group of patients without lymph node metastases (245 patients), CK17 and/or CK5/6 expression was associated with significantly shorter survival. Multivariate analysis on all patients (with and without LN-metastases) revealed that the prognostic association of basal cytokeratin expression with poor outcome was not independent from tumor size, LN status, and histologic grade (56). Only in patients without lymph node metastases, the expression of basal cytokeratins was identified as an independent prognostic factor (56).

It is of note that van de Rijn et al. (56) did not state in their study what they exactly meant with poor clinical outcome. It is unclear whether they meant reduced OS, reduced DFS, or both. Furthermore, it is not clear whether patients with low grade carcinomas with some positivity for CK17 and/or CK5/6 showed different clinical outcome than those with grade 1 cancers without expressing basal cytokeratins.

In contrast to the Nottingham studies (53, 54, 55) that found worse prognosis in BLCs in both LN positive and LN negative patients, van de Rijn et al. (56) have found a poor prognosis in BLCs, only in LN negative patients. Moreover, in contrast to the van de Rijn et al. study, Malzahn et al. (57) reported a statistically significant association of basal/myoepithelial keratin expression with poor prognosis only in patients with LN positive patients but not in LN negative breast cancer patients.

With regard to the prognosis of BLCs, there are four more publications that need to be addressed. In contrast to the above mentioned studies, all of these studies have found that BLC on its own does not convey a poor prognosis.

A study performed by Johns et al. (36) have investigated 43 grade 3 invasive ductal carcinoma positive for basal cytokeratins 14 as well as 43 grade- and age-matched CK14 negative controls by means of immunohistochemistry and comparative genomic hybridization (CGH). CK14 was the only basal cell marker examined in this study. In the cohort of grade 3 carcinomas, CK14 expression was not associated significantly with prognosis. Only a subset of grade 3 IDC with basal immunotype showing certain CGH pattern revealed significantly shorter OS than other grade 3 tumors, indicating that even high grade BLCs represent a heterogeneous group of breast cancer (36). In addition, the authors found striking similarities of genetic alterations between BLCs and previously reported myoepithelial carcinomas (carcinoma with myoepithelial differentiation) (58). In a following study performed by Fulford et al. from the same group (Breakthrough Breast Cancer Research, London, see ref 59), 443 grade 3 invasive ductal carcinomas were examined by immunohistochemistry for CK14 and the results were correlated with the established clinicopathological parameters (59). An important finding of this study which drastically contrasts to many other studies was that in patients without metastatic disease, DFS in CK14-positive cases was significantly better than in CK14-negative carcinomas. The overall survival in CK14-positive and-negative patients was similar at 5 years, but long-term survival was much better in CK14-positive patients (59). Moreover, Fulford et al. (59) reported that both OS and DFS were significantly better for diffuse than for focal CK14 immunostaining. While focally positive tumors had OS and DFS similar to the non-basal carcinomas, the prognosis for diffuse simmunostaining was markedly better (59). It is of note that CK14 was the only basal cell marker used in the study of Fulford et al. and patients with special type carcinomas such as metaplastic carcinoma and medullary carcinoma were not included in this study.

A recent IHC study performed by Kim et al. (49) analyzed 776 patients with invasive breast carcinoma. Positivity for CK5, CK14, and CK8/18 was defined as detection of at least 1% of malignant tumor cells showing strong cytoplasmic and membranous staining. EGFR (Her1), C-kit (CD117), ER, Her2 and p53 were also evaluated immunohistochemically. Clinicapathologic characteristics of breast cancers included age, histologic grade, nuclear grade, tumor size, status of nodal metastasis, tumor type, locoregional recurrence, and distant metastasis. Histologically, most basal-like carcinomas were invasive ductal carcinoma, NOS type (98 cases, 86%), with high nuclear and/or histologic grades, and most metaplastic carcinomas (6 out of 8 cases) were

of the basal-like subtype. All BLCs indentified in this study were histologic grades 2 (22 cases, 19%) or 3 (92 cases, 81%). Both histologic and nuclear grades of BLCs were significantly higher than those of other subtypes. On multivariate analysis adjusting other prognostic factors, Her2-overexpressing subtype was the worst subgroup of breast cancers with poorer prognosis than other subtypes and showed poorer prognosis than BLCs. In contrast to earlier findings, no statistically significant survival differences were evident between BLCs and other subgroups except for Her2-overexpressing subtype (49). It is of note that the cohort of 776 breast cancer patients in this study were exclusively from South Korea which should be taken into consideration for the analysis of contradictory results obtained in western countries.

Finally, another recent IHC-study, done by Potemski et al. (50) is of particular interest. The authors examined 195 breast carcinomas and defined BLC as tumors positive for CK5/6 and/or CK17 but negative for ER, PR, and Her2. Twenty five percent of tumors were classified BLC. Positive immunostaining for CK5/6 and/or CK17 (no cut-off) was associated with worse cancer-specific survival in all examined breast cancer cases, and in the node negative group but not in the node positive group. To determine the real prognostic value of Basal-like cytokeratins, cancer-specific survival in a group of ER negative patients was investigated depending on CK5/6 and/or CK17 expression. Importantly, no influence of basal-type cytokeratins on survival was identified (50). In multivariate analysis, independent prognostic factors affecting survival in the whole group included nodal involvement, Her2 status and cyclin E expression. Petemski et al. concluded that the poor prognosis associated with the BLC is not related to positive immunostaining for CK5/6 and/or CK17 but is determined by ER absence and cyclin E expression (50).

In summary, with regard to the poor prognosis of BLCs and expression of basal type cytokeratins in breast cancers as an independent prognostic factor, it is fair to state that the results of current studies are contradictory. The observed discrepancies on the prognostic significance could be due to the lack of uniform IHC definition of BLCs, analytic methods, patient populations, and treatment modalities. For future studies it is crucial to investigate and compare age and grade-matched low grade basal versus non basal as well as high grade basal versus non basal carcinomas. Important independent prognostic factors such as MIB1 index and immunoreactivity for p53 need to be included in multivariate analysis of both GEP and IHC studies. Indeed, these 2 prognostic parameters are missing in the vast majority of current studies of BLCs.

It is highly likely that breast carcinomas with high nuclear atypia, high MIB-1 index (or high mitotic activity) and positivity for p53 have a very poor prognosis regardless of expression of basal cytokeratins such CK5/6, CK14 or CK17. The fact that triple negative, special type carcinomas of the breast such as adenoid cystic carcinoma, secretory ("juvenile") carcinoma, and low grade metaplastic carcinoma with low grade nuclear atypia and low mitotic activity express basal cell markers (CK5/6, CK14, or CK17, p63, etc.) and yet are associated with excellent prognosis clearly indicates that expression of basal (myoepithelial) cell markers by its own does not affect the prognosis in patients with breast cancer.

2) The Basal-like subtype and BRCA-1 and BRCA-2 mutations

Several recent studies have characterized the morphological and immunohistochemical features of breast carcinomas arising in patients harboring germline mutations in the BRCA1 and BRCA2 genes (60- 62). Breast carcinomas in patients with BRCA1 germline mutations are mostly of higher grade, have higher mitotic index, usually are triple-negative, and show mutations in Tp53 gene than aged-matched sporadic breast cancers (60). A number of studies have demonstrated a strong association between the BLCs and BRCA-1 gemline mutations (60,61), as this subtype is present in 44 to 88% of BRCA-1 associated breast cancers. However, there is little information on association of sporadic breast carcinomas with basal-like features (which represent the vast majority of BLCs) and BRCA-1 mutations. A recent study, performed by Lakhani et al (60), investigated 183 breast cancers in BRCA1 mutation carriers, 63 BRCA2 mutation carriers, and 109 controls (breast cancers unselected for mutation status). The authors used IHC for five basal markers (CK5/6, CK14, CK17, EGFR and osteoponin) and ER in order to develop predictive tests for identification of high-risk patients. In multivariate analysis, CK14, CK5/6, and ER were significant predictors of BRCA carrier status (60). In contrast, the frequency of all basal markers in BRCA2 cancers was not significantly different from controls. Based on the results of this study, the

authors suggested that such information can be used to predict more accurately the probability of carrying a BRCA1 mutation. Accordingly, a screening test based on selecting women who are ER negative and CK5/6 positive, would have a sensitivity in BRCA1 carriers of 56% and a specificity of 97%, with a positive predictive value of 28% and a negative predictive value of 99% (60).

Concerning BRCA2, there is no significant association between the BLCs and BRCA-2 gene mutations at present time. Interestingly, a recent study (62) demonstrated that BRCA-2 associated breast carcinomas are predominantly high grade NOS-type ductal carcinoma of non-basal subtype often showing positivity for ER.

3) Patient age and race

The average age of patients with BLCs ranged from 47 to 55 years in 3 large IHC-studies (28, 49, 63) and was 54 years in one GEP-study. (27). On the contrary, two large populations based studies (27, 28) have found significant differences between the "intrinsic" subtypes regarding patient's age, with the basal-like subtype having the lowest average age among the classifiable cases in both studies. As apposed to this finding, there were no significant differences between the "intrinsic" molecular subtypes regarding patient age among the 804 patients that were enrolled in the Polish Breast Cancer Study (63).

One study showed that almost 40% of breast carcinomas in premenopausal African Americans (AA) were basal-like, as compared to 14% in postmenopausal AA and 16% in non-African Americans (pre- or postmenopausal). (28).

4) Pattern of distant metastasis

Three recent studies have found an increased rate of brain metastasis for BLCs and BRCA-1 related carcinomas (43, 44, 59). Furthermore, one study (59) have found BLC to be less likely associated with liver and bone metastases as compared with poorly differentiated, non-basal ductal carcinomas (IDC, NOS, type). One study reported that the likelihood of lung and pleural metastases in basal and non-basal breast carcinomas was not different (59).

5) Therapeutic response

A few recent studies examined whether the different molecular subtypes of breast carcinoma responded differently to (neo)adjuvant chemotherapy. One study performed by Rouzier et al. (65) examined fine needle aspiration of 82 breast carcinomas obtained before starting preoperative (neoadjuvant) paclitaxel followed by 5-fluorouracil, doxorubicin, and cyclophosphamide chemotherapy. Gene expression profiling was done with Affymetrix microarrays and the previously reported "intrinsic" gene set was used for hierarchical clustering and molecular classification. The authors of this study have found that basal-like and Her2+ subtypes were associated with the highest rates of pathologic complete response (CR), 45% and 45%, respectively, whereas the luminal subtype cancers had a pathologic CR rate of 6%. However, it is important to point out that the molecular class in this study was not independent of conventional clinicopathologic predictors of response such as estrogen receptor status and nuclear grade (65).

In another study performed by Sorlie et al. (66), the authors analyzed cDNA expression data from 81 breast carcinomas from two patients series, one treated with doxorubicin alone and the other treated with 5-fluorouracil and mitomycin. Sorlie et al. observed a low frequency of progressive disease within the luminal A subtype from both series and a high frequency of progressive disease among patients with luminal B subtype treated with doxorubicin (66). However, aside from these two observations, no other consistent association between response to chemotherapy and tumor subtype were observed in this study. Using supervised analysis, Sorlie et al could not uncover a gene profile that could reliably (more than 70% accuracy and specificity) predict response to either treatment regimen (66).

With regard to the clinical outcome and response to chemotherapy of patients with BLCs, one recent retrospective study performed by Banerjee et al. (67) analyzed 49 patients with BLC (as defined by CK5, CK14 and CK17) and 49 controls matched for age, nodal status, and histologic grade. Histological features, status of ER and PR as well as Her2 and clinical outcome (DFS, OS) after adjuvant chemotherapy (anthracycline) were compared between the two groups. This study showed that patients with BLC had a significantly higher recurrence rate and were associated with significantly shorter DFS and OS. Furthermore, in the group of patients who received anthracycline-based chemotherapy, both DFS and OS were found to be significantly shorter in the patients with BLC (67). The authors of this study concluded that BLC is a distinct clinical and pathologic entity, with a more aggressive clinical course. The authors

also concluded that standard adjuvant chemotherapy seems to be less effective in BLC and new therapeutic modalities are indicated (67). A critical review of the above mentioned publication of Banerjee et al. reveals that it suffers from some methodological problems with significant impact on its conclusions. According to the material and methods of this study, all tumors were of grade 3 using the modified Bloom-Richardson-Scarff grading system (Nottingham or Elston-Ellis grading system). Medullary carcinomas and high grade metaplastic carcinomas were excluded from this study. Tumors were considered to be positive for ER and PR when nuclear reactivity was observed in more than 10% of tumor cells at any intensity (Caveat: This cut-off is no longer used in most breast cancer centers). For CK5, CK14 and CK17, any cytoplasmic expression in neoplastic cells or tissue was considered to be positive. Most importantly, carcinomas with expression of at least one basal cytokeratins were considered to be the basal-like, regardless of the expression of ER, PR, or Her2. In other words, even triple positive tumors with very focal imunoreaction for basal cytokeratins were considered BLC. One needs to precisely define characteristic immunohistochemical features of BLC, if one claims that poorly differentiated BLC as a distinct pathologic and clinical entity has a much more aggressive clinical course compared to high grade non-basal and triple-negative carcinoma. It is well-known that the vast majority of BLCs are triple-negative and this property in conjunction with expression of basal type cytokeratins distinguish this subtype from luminal and Her2-overexpressing subtypes. It is likely that Banerjee et al. included some cases of breast carcinomas with ER and/or Her2 positivity as BLC in their study. Therefore, the comparison of the two groups of high grade carcinomas in this study is probably inaccurate and even misleading. According to the definition of BLC, as described in material and methods of this study, a case with G3, IDC that shows positivity for CK5/6 in only very few tumor cells while displaying strong positivity for ER and Her2 would be classified as BLC. On the other hand a high grade carcinoma which is completely negative for ER, PR and Her2 but reveals immunoreaction for CK5/6 or CK17 in more than 50% of tumor cells would also be considered BLC. Obviously, these two tumors represent biologically completely different groups and, therefore, cannot be included in the same category of BLC as indicated by the above mentioned study. Interestingly, a publication of the same group two years earlier has found that IDC with basal-like immunotype on its own does not convey a poor prognosis (36).

In contrast to the study of Banerjee et al (67). another recent study (65) showed that basal-like and Her2+ subtypes were more sensitive to anthracycline-based neoadjuvant chemotherapy than luminal A breast carcinomas (see also ref:68).

With regard to chemotherapy, there is some evidence that patients with lymph node positive high grade BLC may benefit significantly more from high dose adjuvant chemotherapy (high dose chemotherapy accompanied by autologous peripheral blood progenitor cell transplantation) than conventional chemotherapy (69,70). In the West German Study Group AM-01 Trial, in which 236 node positive breast cancer patients were randomized into conventional dose-dense and high dose adjuvant chemotherapeutic arms, patients with basal-like and Her2+ carcinomas that received the high dose therapy had an event-free (ES) and overall survival (OS) comparable with luminal A/ER+ group (69). Patients with BLC that were treated with the conventional chemotherapy had an ES and OS that was significantly worse than those whose breast carcinomas were luminal A/ER+ (69). Currently, there is no information available with respect to high-dose chemotherapy (accompanied by authologous peripheral blood progenitor cell transplantation) of node negative patients with basal-like and Her2+ breast carcinomas.

Finally, it has been suggested, and it is certainly of hope, that BLC may represent a group of breast carcinoma that could benefit from EGFR-targeted therapeutic strategies using either monoclonal antibodies against extracellular domain of the receptor or small inhibitory molecules binding to its intracellular domain (tyrosine kinase inhibitors) (71, 72). Indeed, epidermal growth factor receptor (EGFR, Her1) is over-expressed by immunohistochemistry in more than 50% of cases with BLC (including a variety of metaplastic carcinomas and sarcomatoid carcinomas with myoepithelial differentiation). (42, 71-73). Future studies are needed to show whether determination of EGFR (Her1) by IHC versus in situ hybridization (FISH or CISH) could be used as a reliable method for selection of patients that may benefit from EGFR-targeted therapies.

Conclusions with cautionary notes

The aim of this review is to critically analyze several major publications to find out whether the "basal-like" subtype represents a distinct clinicopathologic entity. A critical survey of the current publications reveals that it is too early to consider this subtype as a distinct entity and the scientific and medical communities need to interpret these studies more cautiously. There are several major issues and problems that need to be resolved before one can reliably consider this subtype a distinct entity . The major issues/problems are summerized as follow:

BLC was originally defined by GEP-analyses showing basal-type cytokeratins (CK5 and/or CK17) in a subgroup of breast cancers which were negative for ER and Her2. The designation of "basal-like" was chosen mainly because the basal-type cytokeratins (CK5,CK17) are typically expressed in basally located myoepithelial cells (14,74-77). The main issues with the first GEP-study (14) include small sample size while analyzing a huge data set (see also ref: 23,78), lack of any clinical correlation, and use of a common "reference" sample which was a mixture of 11 different human cell lines consisting of breast cancer, ovarian cancer, hepatopblastoma, teratocarcinoma (embryonal carcinoma), malignant melanoma, liposarcoma, acute leukemia, and colon cancer cell lines. It is important to note that the used "reference" sample in the first GEP-study constitutes a mixture of highly heterogeneous cancer cell lines that by no means allows any conclusion concerning the biology of breast carcinomas.

The subsequent GEP-study (15) was the one with correlation to the clinical outcome. In order to investigate whether the five different groups identified by hierarchical clustering may represent clinically distinct subgroups of patients, univariate survival analyses comparing the subtypes with respect to DFS and OS were performed. One major limitation of this GEP-study is the lack of a multivariate analyses including well-established clinicopathologic factors such as nuclear grade, MIB-1 index (or mitotic index), tumor size, age, etc. Needless to say that without performing a multivariate analysis that includes all known clinicopathologic parameters, as unfortunately occured in the second GEP-study, the "distinctive" nature of BLC cannot be proved. The second GEP-study also suffers from small sample size and the recently recognized major mathematical problem of statistical analyses of thousands of genes, particularly in a substantially small group of patients (for more detail see ref:23, 78).

As shown in the second GEP-study, the vast majority of Her2+ and BLC (71% and 82%, respectively) show TP53 gene mutations (15). One could reasonably argue that the poor OS and DFS as shown in this study is merely a reflection of poorly differentiation, particularly in association with TP53 mutations. In other words, the poor prognosis of BLC could mainly be the results of TP53 mutations, already known to be an independent prognostic factor in breast carcinoma.

The results of several recent studies that used IHC for their definitions and analyses of BLC cannot be directly compared to those of the GEP-studies. While several IHC-studies used CK14 as the only basal cell marker, others used CK5/6 and/or CK17 (with or without CK14) for their definition of BLC. While the first GEP-study (14) showed that all 6 BLCs were negative for ER, the second GEP-study (15) included 2 ER-positive cases in the category of BLC. Several IHC-studies regard BLC as a triple negative tumor (negativity for ER, PR and Her2) in conjunction with positivity for at least one basal-type cytokeratins, whereas others included ER+ and Her2+ cases in the BLC. Many investigators used a cut-off of 1% for their definition, while others required at least 10% positivity in cancerous cells. Clearly, due to the lack of a uniform approach and standardized definition, the results of IHC-studies cannot be compared with each other and, therefore, are not conclusive. One should also keep in mind that the basal type cytokeratins identified in GEP-studies of BLC were CK5 and CK17 but not CK14.

Even by using a uniform approach by the same group of investigators, there are a number of IHC-studies on BLC that show contradictory results (36,59). While the investigators of one group have reported BLC to be associated with poor clinical outcome (after performing multivariate analyses), the same group has published completely divergent and even contradictory results showing that basal phenotype on its own does not convey a poor prognosis or even has shown that the prognosis of high grade BLC is much better than non-basal, high grade invasive ductal carcinoma of NOS-type (36, 59).

One can hardly find a GEP or IHC study on BLC which could demonstrate that poorly differentiated BLC has a significantly poorer prognosis than that of a high grade, non-basal, triple negative breast carcinoma. Similarly, there is no study of clinical outcome of patients suffering from low grade basal-

like carcinoma in comparison to low grade, non-basal carcinoma. This kind of information is absolutely necessary before one claims that BLC by its own is of important prognostic relevance.

While the vast majority of BLCs are of high grade and show a high mitotic activity (including atypical ones), there are some special type breast carcinomas which are triple negative but express basal-type cytokeratins (CK5/6, CK14, CK17). A typical example of these special type breast cancers is adenoid cystic carcinoma. Although all basal-like features (basal-type cytokeratins, EGFR, c-kit, p63, triple negativity, etc.) are present in adenoid cystic carcinoma of the breast, this special type has an excellent prognosis. Despite showing basal-like features, adenoid cystic carcinoma characteristically lacks significant nuclear atypia and increased mitotic activity. Another example of a low grade carcinoma with basal-like immunotype is secretory ("juvenile") carcinoma, which also has an excellent prognosis, particularly in patients younger than 20! Other examples include low grade spindle cell ("fibromatosis-like") carcinoma and examples of low grade metaplastic carcinoma (low grade "matrix-producing" carcinoma, etc) all expressing basal-type cytokeratins and yet are not associated with a poor prognosis.

So-called basal-like carcinomas of the breast often express one or several myoepithelial marker(s), if one uses a panel of conventional (SM-actin, SM-myosin, p63, calponin, CD10, S100-protein) and novel (14-3-3 sigma, CD29, NGFR/p75, etc.) myoepithelial markers. While some of the myoepithelial markers are completely negative in "basal-like" carcinomas, others are, at least focally, positive in tumor cells that also express basal-type cytokeratins. One should also be aware of the fact that there are no known basal cells but basally located myoepithelial cells in the breast. It is possible that the cells of origin in so-called basal-like carcinomas are, at least in some cases, related to the myoepithelial cells (2, 41). It is also likely that neoplastic cells in "basal-like" carcinoma are related to mammary "progenitor" cells (79, 80).

As mentioned above, the prognostic value of expression of basal-type cytokeratins in BLC is controversial. However, what is much more important is the possible predictive value of this subtype with respect to the chemotherapeutic response or targeted therapies. Similar to its prognostic value, the current data on chemotherapeutic response of BLC are not conclusive. It is of note that even Sorlie et al, who introduced BLC, were not able to show a consistent association between response to chemotherapy and BLC (66). In fact, using supervised analysis, they could not uncover a gene profile that could reliably predict chemotherapy response in their data set (66). It is important to note that another study (65) showed that subtypes of BLC and Her2+ carcinomas were not independent of conventional clinicopathologic predictors of response such as estrogen receptor status and nuclear grade.

In summary, the "basal-like" subtype of breast carcinoma is enthusiastically regarded by many investigators as a distinct clinicopathologic entity. A critical review of the literature, however, reveals several limitations and methodological problems of the current studies on this subject (14, 15, 20- 23, 78, 81- 83). Due to the major problems/limitations and because of divergent or even contradictory results of the current studies, as discussed in this review, one has to seriously question the common claim of "distinctive" nature of this breast cancer subtype. It is important to emphasize that "basal-like" carcinomas do not reflect a single, biologically uniform group of breast carcinomas. Indeed, there is a range of myoepithelial or "basal"-derived carcinomas with variation in their phenotype, immunoprofile, grades, and clinical behavior, just as a wide range of subtypes and behaviors is observed among epithelial/luminal-derived breast carcinomas. As a subtype of breast carcinoma, however, it is of hope that at least some patients with "basal-like" carcinoma could benefit from EGFR-targeted therapies and/or certain type of chemotherapy.

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