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Laboratory-Clinic Interface

# Is the differentiation into molecular subtypes of breast cancer important for staging, local and systemic therapy, and follow up?

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ABSTRACT

Breast cancer complexity has long been known and investigated. After a first classification of the disease based on histology features, starting from the 1980s breast cancers have been distinguished on the basis of oestrogen receptor expression and later according to HER2. By 2000 the "microarray revolution" had shown that the phenotypic differences between breast cancers were a reflection of their mRNA expression profiles, while the more recent "genomic revolution" is revealing the genomic bases of breast cancer heterogeneity. However, how this huge amount of data and knowledge translate into clinically relevant practice is currently not clear. In the present review we discuss how the different breast cancer classification methods might translate into improved clinical guidelines with regard to staging, therapy, and follow up of patients with breast cancer.



## Background

It has long been known that breast cancers are actually a heterogeneous group of diseases with different phenotypes, prognoses and responses to treatment. Recognition of the importance of the oestrogen receptor (ER) [1] and the human epidermal growth factor receptor 2 [HER2] [2,3] in breast cancer, and the large scale use of immunohistochemistry (IHC), enabled almost every cancer centre in the world to differentiate breast cancer patients into three major groups: the hormone receptor positive group (which expresses ER and/or progesterone receptor [PgR]), the HER2-positive group (which expresses HER2 by IHC or amplification detected by fluoresce in-situ hybridization [FISH]) and the "triple negative" group (which is negative for ER, PgR, and HER2). Later, the ER-positive group was subdivided into two distinct prognostic groups, luminal A and luminal B, based on percentage of Ki-67 [4] (marker of proliferation) or the presence of PgR.

The next step in the evolution of tools able to differentiate breast cancers into molecular subtypes was the advent of gene expression arrays, which simultaneously measure thousands of genes to create a molecular portrait of the investigated tumours [5–8]. In 2000, Perou et al. [6] were the first to show that the phenotypic diversity of breast cancers is accompanied by a corresponding diversity in gene expression patterns that can be captured using cDNA arrays.

Based on the expression of an "intrinsic" gene subset, breast samples were segregated into two subtypes: the first was characterised by tumours that were clinically described as ER-positive and showed a relatively high expression of breast luminal genes. The second was characterised by tumours that were ER-negative and expressed genes typical of breast basal/myoepithelial cells. Luminal cases were then further distinguished into at least two subgroups on the basis of their proliferation levels [9], and additional subtypes have been defined among the basal tumours as well [10–12]; however, their clinical impact in the clinic is still limited.

*Abbreviations:* AI, aromatase inhibitors; BC, breast cancer; BCS, breast-conserving surgery; BCS, breast cancer survival; CAF, cyclophosphamide, doxorubicin and fluorouracil; CR, complete response; DFS, disease free survival; ER, oestrogen receptor; ET, endocrine therapy; FEC, fluorouracil epirubicin and cyclophosphamide; FISH, fluorescence in-situ hybridisation; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; HR, hazard ratio; IHC, immunohistochemistry; LRR, loco-regional relapse; PgR, progesterone receptor; pCR, pathological complete response; PMRT, post mastectomy radiotherapy; RFS, recurrence-free survival; RS, recurrence score; TIL, tumour infiltrating lymphocytic; TMA, tissue microarray; TNM, tumor-node metastasis; TS, tenon score; TNBC, triple negative breast cancer; SCM, subtype classification models; SDM, synchronous distant metastases.

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The latest level at which breast cancer complexity has been dissected is the genomic level. In the last few years, pivotal studies have been published in which breast cancer tumours were segregated on the basis of their mutation profiles or the presence of other genomic aberrations [13,14].

Altogether, the different strategies used to classify samples have led to a multidimensional understanding of the complex molecular make-up of breast cancers, but the major question is whether these efforts have an impact in clinical practice. In this review, we aim to discuss the relevance of molecular classification tools in the different areas of clinical practice, including basic staging, through local and systemic treatment decisions and to follow up practices (Fig. 1 and Table 1).

## Clinical definitions of subtypes and their role in staging and work-up

Sorlie et al. correlated tumour subtypes to clinical outcome [5]. Poor prognosis for the basal-like subtype and a significant difference in outcome for the identified ER-positive sub-groups were observed. The analysis described above used unsupervised or semi supervised hierarchical clustering, a strategy that reflects inherent biologic differences. However, it is hard to implement such an approach in clinical practice. For this reason a series of subtype sample predictors have been developed. Two different approaches have been used: single sample predictors (SSPs) [15–17] and subtype classification models (SCMs) [18–20]. While SSPs use hierarchical clustering followed by nearest centroid classification based on large sets of tumour-intrinsic genes to classify tumors, SCMs use a mixture of Gaussian distributions based on sets of genes (modules) with expression correlated with three relevant breast cancer genes: ER, HER2, and the proliferation gene aurora kinase A (AURKA). One of the SSPs, PAM50, obtained CE approval in 2012 and FDA 510(K) approval in 2013, and it is currently implemented in the clinic as a Nano-String based assay (PROSIGNA™) [16].

One of the major debates of recent years has been about whether IHC for different markers can serve as a surrogate of microarray-defined subtypes and, in particular, if either Ki67 or PgR levels could be used to differentiate between luminal A and luminal B cancers [21–23].

Moreover different studies mix their definition of molecular subtypes based on surrogate IHC markers. This is especially problematic for the luminal B subtype, which has been defined in many different ways in publications. The 13th St Gallen International Breast Cancer Conference (2013) Expert Consensus Panel recently refined its earlier approach to the clinico-pathological surrogate definitions of subtypes and in particular to the distinction between luminal A and luminal B tumours. It was suggested that either a level of <14% for Ki67% or a PgR cut-point of $\geqslant$20% best correlated with the gene-expression definition of luminal A [24,25]. However, according to St Gallen 2013 consensus, no definitive statement was released for Ki67 cut off for treatment decision. Although the majority of the Panel voted that a threshold of $\geqslant$20% was indicative of 'high' Ki67% status, others, concerned about the high degree of inter-laboratory variation in Ki67% measurement and the possibility for under treatment of patients with luminal disease who might benefit from chemotherapy, proposed the use of a lower cut-point to define 'high' Ki-67% [26]. One major drawback of IHC is its low reproducibility. This is true for ER, PgR, and HER2 [27,28], but especially for Ki67% [29,30]. That said, IHC currently still represents the preferred method of choice to define molecular subtypes in the clinical setting.

As for the question of whether staging should differ according to molecular subtype, currently no guidelines suggest different initial staging according to it. The updated ESMO Clinical Practice Guidelines [31] suggest that disease stage should be assessed according to the classic tumor-node metastasis (TNM) system with no influence of breast cancer subtypes. However, the current TNM staging system might not be enough to encompass the complexity of tumour biology and for predicting outcomes to make therapeutic decisions for all breast cancers, especially for patients with triple-negative breast cancer (TNBC). In a study performed by Park et al. [32], recurrence-free survival (RFS) curves of patients with TNBC showed overlap from stages 1 to 3A, and there was only wide separation of RFS curves between stages 1–3A and 3B–C.

Also, the presence of synchronous metastasis could differ according to molecular subtypes. In a study presented at the 2011 ASCO Annual Meeting [33], 2411 patients with breast cancer were included: 106 patients were found to have synchronous distant metastases (SDM) (4.4%). The median clinical size of lesions in patients with SDM was 35 mm (8–120), while it was 15 mm in patients without SDM ($p < 0.001$). Moreover, 7% of patients with

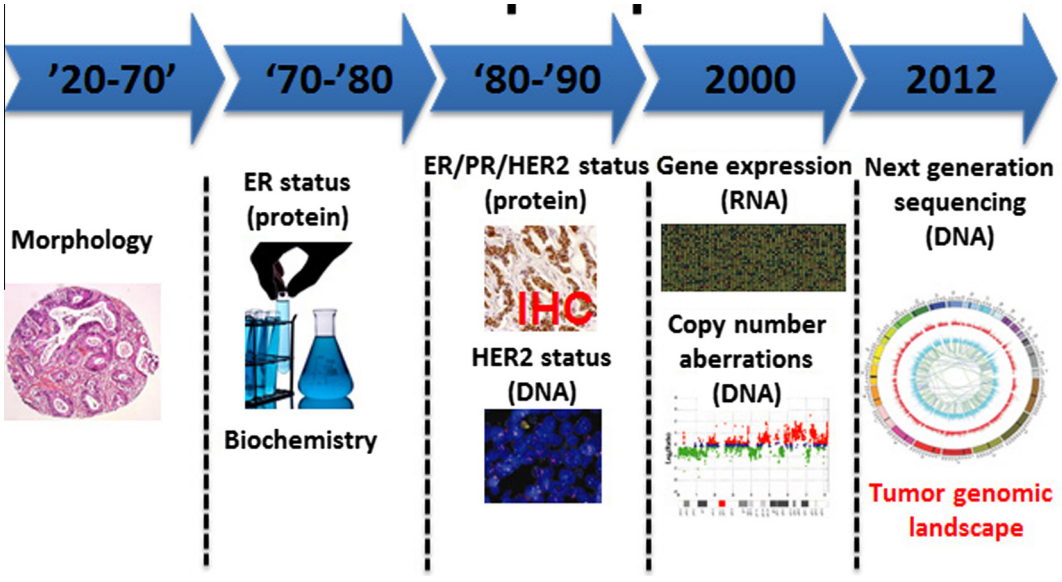


**Fig. 1.** Breast cancer "subtypes": historical perspective. IHC-immunohistochemistry, ER-oestrogen receptor, PgR-progesterone receptor.