The Breast 24 (2015) S26–S35

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Clinical implications of the intrinsic molecular subtypes of breast cancer

Aleix Prat [a, b, c, *], Estela Pineda [a, b], Barbara Adamo [a, b], Patricia Galván [a, c], Aranzazu Fernández [a, b], Lydia Gaba [a, b], Marc Díez [a, b], Margarita Viladot [a, b], Ana Arance [a, b], Montserrat Muñoz [a, b]

[a] Translational Genomics and Targeted Therapeutics in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain
[b] Medical Oncology Department, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain
[c] Translational Genomics Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

ARTICLE INFO

Article history:
Available online 5 August 2015

Keywords:
Breast cancer
Subtype
PAM50
Gene expression

ABSTRACT

Gene-expression profiling has had a considerable impact on our understanding of breast cancer biology. During the last 15 years, 5 intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like and Claudin-low) have been identified and intensively studied. In this review, we will focus on the current and future clinical implications of the intrinsic molecular subtypes beyond the current pathological-based classification endorsed by the 2013 St. Gallen Consensus Recommendations. Within hormone receptor-positive and HER2-negative early breast cancer, the Luminal A and B subtypes predict 10-year outcome regardless of systemic treatment administered as well as residual risk of distant recurrence after 5 years of endocrine therapy. Within clinically HER2-positive disease, the 4 main intrinsic subtypes can be identified and dominate the biological and clinical phenotype. From a clinical perspective, patients with HER2+/HER2-enriched disease seem to benefit the most from neoadjuvant trastuzumab, or dual HER2 blockade with trastuzumab/lapatinib, in combination with chemotherapy, and patients with HER2+/Luminal A disease seem to have a relative better outcome compared to the other subtypes. Finally, within triple-negative breast cancer (TNBC), the Basal-like disease predominates (70–80%) and, from a biological perspective, should be considered a cancer-type by itself. Importantly, the distinction between Basal-like versus non-Basal-like within TNBC might predict survival following (neo)adjvuvant multi-agent chemotherapy, bevacizumab benefit in the neoadjuvant setting (CALGB40603), and docetaxel vs. carboplatin benefit in first-line metastatic disease (TNT study). Overall, this data suggests that intrinsic molecular profiling provides clinically relevant information beyond current pathology-based classifications.

Introduction

Despite that breast cancer mortality has been moderately reduced by current treatments, more than 450,000 estimated deaths due to breast cancer are expected annually worldwide [1]. The most plausible explanation for this scenario is that we lack a complete picture of the biologic heterogeneity of breast cancers. Importantly, this complexity is not fully reflected by the main clinical parameters and pathological markers (oestrogen receptor [ER], progesterone receptor [PR] and human epidermal growth factor 2 [HER2]), all of which are routinely used in the clinic to stratify patients for prognostic predictions, to select treatments and to include patients in clinical trials.

Gene expression profiling has had a considerable impact on our understanding of breast cancer biology. During the last 15 years, we and others have extensively characterized 5 intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER-2 enriched, Basal-like and Claudin-low) and a normal breast-like group [2–6]. These entities have shown significant differences in terms of their incidence, risk factors, prognosis and treatment sensitivity. Regarding prognosis, the Luminal A subtype has shown repeatedly

* Corresponding author. Translational Genomics and Targeted Therapeutics in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Rosselló, 149, 08036, Barcelona, Spain.
E-mail address: alprat@clinic.ub.es (A. Prat).

http://dx.doi.org/10.1016/j.breast.2015.07.008

to have a better outcome than the rest of subtypes across many datasets of patients with early breast cancer, including 6 phase III clinical trials (TransATAC, GEICAM9906, CALGB9741, ABCSG08, NCIC-CTG MA.5 and NCIC-CTG MA.12), where patients received various adjuvant systemic treatments.

A particular piece of data that highlights the importance of intrinsic subtyping in breast cancer comes from one of the most complete molecular characterization studies that have ever been performed in breast cancer. In this study, led by The Cancer Genome Atlas Project (TCGA), more than 500 primary breast cancers were extensively profiled at the DNA (i.e. methylation, chromosomal copy-number changes and somatic and germline mutations), RNA (i.e. miRNA and mRNA expression) and protein (i.e. protein and phosphor-protein expression) levels using the most recent technologies [6]. In a particular analysis of over 300 primary tumours (i.e. shown in Figure 2 of that publication [6]), 5 different data-types (i.e. all except DNA mutations) were combined together in a cluster of clusters in order to identify how many biological homogenous groups of tumours one can identify in breast cancer. The consensus clustering results showed the presence of 4 main entities of breast cancer but, more importantly, these 4 entities were found to be very well recapitulated by the 4 main intrinsic subtypes (Luminal A, Luminal B, HER2-enriched and Basal-like) as defined by mRNA expression only [7]. Overall, these results suggest that intrinsic subtyping captures the vast majority of the biological diversity occurring in breast cancer.

Since 2011, the St. Gallen international expert consensus panel adopted an intrinsic subtype-based approach for recommending adjuvant systemic therapies (i.e. endocrine, chemotherapy and anti-HER2 therapy) in early breast cancer [8]. Although the panel acknowledged the superior accuracy and reproducibility of multigene expression molecular assays, these assays are not readily available for all our patients. Thus, over the years, we and others have proposed pathology-based surrogate definitions especially for distinguishing Luminal A from B tumours [9–11]. However, despite important efforts to improve the various pathology-based surrogate definitions of the intrinsic subtypes, these continue to be suboptimal.

Here, we review the current and the potential future clinical implications of the intrinsic molecular subtypes of breast cancer beyond the pathological-based surrogate classification endorsed by the 2013 St Gallen Consensus Recommendations [8].

Intrinsic subtyping based on gene expression versus histopathology

To date, numerous studies have evaluated and compared the classification of tumours based on the PAM50 gene expression predictor with the pathology-based surrogate definitions [6,10,12–26]. To better understand the concordance between the 2 classification methods, we have combined the data from all of these studies for a total of 5994 independent samples (Table 1). Of note, the vast majority of these studies performed central determination of pathology-based biomarkers, so this needs to be taken into account since this is not what is currently being done in the clinical setting where each hospital determines these biomarkers. Of note, large discrepancies (~20%) between local and central determination of ER, PR, Ki67 and HER2 are expected [27–31].

In this combined analysis, the discordance rate between both classifications was found to be present in almost 1 out of 3 patients (rate = 30.72% across all patients; kappa statistic = 0.564, "moderate agreement"; rate = 44.0% within non-triple-negative disease; kappa statistic = 0.314, "fair agreement"). Across the IHC-based subtypes, the discordance rate was 37.8%, 48.9%, 53.8%, 33.9% and 13.9% for the IHC-Luminal A, IHC-Luminal B, IHC-Luminal B/HER2+ (to identify PAM50 Luminal B), HR−/HER2+ (to identify PAM50 HER2-enriched) and triple-negative (to identify PAM50 Basal-like) subtypes, respectively. These results clearly suggest that the 2 methods to identify intrinsic biology should not be considered the same. The most likely explanation is that 3 or 4 biomarkers do not fully recapitulate the intrinsic subtypes of breast cancer. For example, we compared the prognostic and predictive ability of a 3-gene subtype classifier based on ESR1, ERBB2 and AURKA compared with the 50-gene PAM50 intrinsic classifier, and the 50-gene assay was significantly better [4]. In fact, during the development of the clinically applicable PAM50 intrinsic subtype predictor, 50 genes was found to be the minim number of genes needed to robustly identify the 4 main intrinsic subtypes without compromising its accuracy [4].

Main molecular features of the intrinsic subtypes

Four main intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched and Basal-like) have been characterized over the last 15 years. At the RNA and protein level, Luminal A and B subtypes are largely distinguished by the expression of two main biological processes: proliferation/cell cycle-related and luminal/hormone-regulated pathways (Fig. 1). Compared to Luminal A tumours, Luminal B tumours have higher expression of proliferation/cell cycle-related genes or proteins (e.g. MKI67 and AURKA) and lower expression of several luminal-related genes or proteins such as the progesterone receptor (PR) [32] and FOXA1, but not the oestrogen receptor [10], which is found similarly expressed between the two luminal subtypes and can only help distinguish luminal from non-luminal disease. At the DNA level, Luminal A tumours show a lower number of mutations across the genome, lower number of chromosomal copy-number changes (e.g. lower rates of CCND1 amplification), less TP53

Table 1
Distribution of the PAM50 intrinsic subtypes within the pathology-based groups.[a]

IHC-based group	References	N	PAM50 intrinsic subtype distribution			
			Luminal A	Luminal B	HER2-enriched	Basal-like
HR+/HER2−	[10,14,16–22]	4295	60.3%	31.9%	6.6%	1.2%
Luminal A	[10,14,17,21]	637	62.2%	27.0%	10.2%	0.6%
Luminal B	[10,14,17,21]	317	34.1%	51.1%	11.0%	3.8%
HER2+	[6,23–26]	831	17.6%	26.8%	44.6%	11.0%
HER2+/HR+	[25,26]	182	33.0%	46.2%	18.7%	2.2%
HER2+/HR−	[25,26]	168	19.0%	4.2%	66.1%	10.7%
TNBC	[12–15]	868	1.6%	3.2%	9.1%	86.1%

[a] The data has been obtained from the different publications. Several studies have performed a standardized version of the PAM50 assay (RT-qPCR-based or nCounter-based) from formalin-fixed paraffin-embedded tumour tissues [10,14,17,19–22], while others have performed the microarray-based version of the PAM50 assay [6,16,18,23–26].

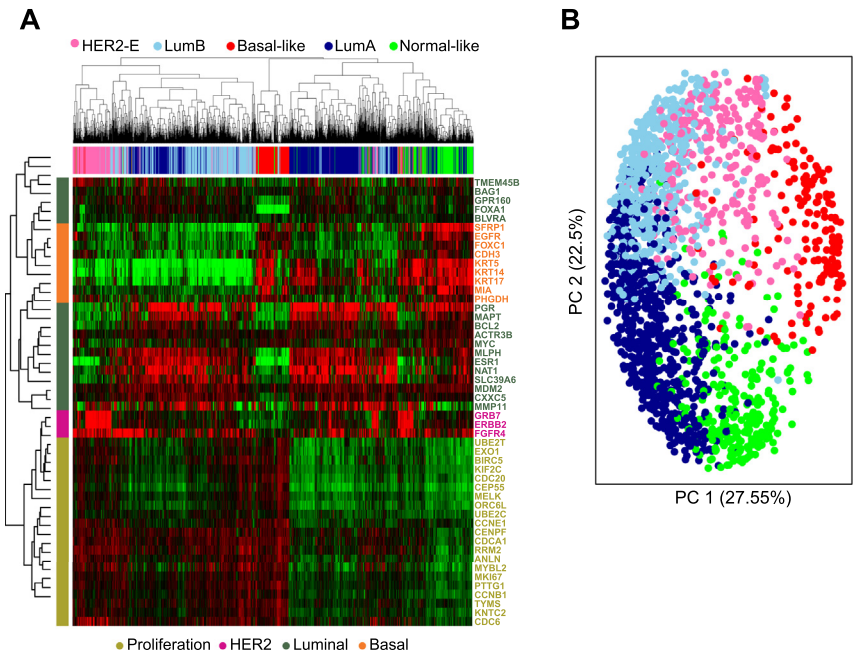

Fig. 1. Intrinsic subtype identification using the PAM50 subtype predictor. (A) PAM50 unsupervised gene expression heatmap of 1,834 breast cancer samples profiled at the Translational Genomics Group at VHIO. The subtype calls of each sample are shown below the array tree. Each square represents the relative transcript abundance. (B) Principal component 1 and 2 loading plots of the same dataset shown in (A) using the PAM50 genes-only.

mutations (12% vs. 29%), similar GATA3 mutations (14% vs. 15%) and more PIK3CA (45% vs. 29%) and MAP3K1 mutations (13% versus 5%) compared to Luminal B tumours [6]. Interestingly, a subgroup of Luminal B tumours is found hypermethylated, and a subgroup of Luminal A (6.3–7.8%) and Luminal B (16.4–20.8%) tumours show HER2-amplification/overexpression (*see below*).

The HER2-enriched subtype is characterized at the RNA and protein level by the high expression of HER2-related and proliferation-related genes and proteins (e.g. ERBB2/HER2 and GRB7), intermediate expression of luminal-related genes and proteins (e.g. ESR1 and PGR) and low expression of basal-related genes and proteins (e.g. keratin 5 and FOXC1). At the DNA level, these tumours show the highest number of mutations across the genome, and 72% and 39% of HER2-enriched tumours are TP53 and PIK3CA mutated, respectively. Although the majority (68%) of HER2-enriched tumours have ERBB2/HER2 overexpression/amplification, we should expect to identify the HER2-enriched subtype within HER2-negative disease (*see below*). Interestingly, the HER2-enriched subtype has been found uniquely enriched for tumours with high frequency of APOBEC3B-associated mutations [33]. APOBEC3B is subclass of APOBEC cytidine deaminases, which convert cytosine to uracil and has been implicated as a source of mutations in many cancer types [34].

The Basal-like subtype is characterized at the RNA and protein level by the high expression of proliferation-related genes (e.g. MKI67) and keratins typically expressed by the basal layer of the skin (e.g. keratins 5, 14 and 17), intermediate expression of HER2-related genes, and very low expression of luminal-related genes. At the DNA level, these tumours show the second highest number of mutations across the genome, mostly are hypomethylated, and 80% and 9% of Basal-like tumours are TP53 and PIK3CA mutated, respectively. BRCA1-mutated breast cancer is associated with Basal-like disease [35,36]. Finally, ERBB2/HER2 overexpression/amplification is found in 2.1–17.4% of tumours with a Basal-like profile.

Basal-like versus not classification: biological and epidemiological implications

The TCGA comprehensive molecular characterization of breast cancer confirmed that among all the intrinsic subtypes, the Basal-like is the most distinct [6]. This observation fits with previous molecular studies and with clinical data that shows that triple-negative breast cancer tends to affect young women, is associated with BRCA1 mutations and is a highly aggressive disease [37]. However, how different is Basal-like disease from the rest of breast cancer subtypes?

Two recent studies have addressed this question from a biological perspective [38,39]. In the first one, we evaluated global microarray-based gene expression profiles of a combined dataset composed of 6 different cancer-types obtained from the TCGA project and that included 542 primary breast cancers [38]. The unsupervised results revealed that a subgroup of breast cancers, virtually all Basal-like by PAM50, should be considered a molecular entity by itself just like ovarian or colorectal cancer, and that >70% of Basal-like breast cancers were more similar to squamous cell lung cancer than to Luminal A or B disease [38]. In the second study, the panCancer TCGA study group combined all the available molecular data (except mutations) across 12 cancer types, including 845 primary breast cancers [39]. Unsupervised classification using all data-types revealed a similar finding as the previous study, namely that Basal-like breast cancer is a unique entity and much different from the rest of breast tumours. Interestingly, the other cancer-type that showed such a large biological heterogeneity was bladder cancer which could be re-classified into 3 distinct molecular entities, one being similar to the Basal-like breast cancer subtype [39].

Despite *in vivo* preclinical data suggesting that breast cancer disease arises from the transformation of a common luminal progenitor [40–42], this biological result with human tumours strongly suggest that 2 very different cell-types of origin exist in the mammary gland; one whose transformation gives rise to Basal-like

disease and another one whose transformation gives rise to non-Basal-like disease. This hypothesis is further supported by epidemiological data [43]. For example, a bimodal age distribution at diagnosis with peak frequencies near ages 50 and 70 years is a known fundamental characteristic of breast cancer [43]. The bimodal peak frequencies at diagnosis suggest that a "mixture" of two main populations exist in breast cancer. Not surprisingly, when intrinsic subtyping was evaluated, the two main populations were largely explained by the Basal-like versus not nature of the tumour [43], with Basal-like tumours explaining the early-onset age peak.

Another example is work by Millikan and colleagues [44] looking at risk factors of breast cancer in a population-based, case–control study of African-American and white women. The results revealed that Luminal A disease exhibits risk factors typically reported as protective for the development of breast cancer, including increased parity and younger age at first full-term pregnancy; on the other hand, Basal-like cases exhibits several associations that were opposite to those observed for Luminal A, including increased risk for parity and younger age at first term full-term pregnancy [44]. Moreover, longer duration breastfeeding, increasing number of children breastfeed, and increasing number of months breastfeeding per child were each associated with reduced risk of Basal-like breast cancer, but not luminal A [44]. Overall, this data suggests that we should clearly separate these two entities when we talk about breast cancer.

Clinical implications within HR+/HER2-negative disease

Prognostic implications

Within HR+/HER2-negative breast cancer, 90–95% of tumours fall into the Luminal A and B subtypes. In early breast cancer, Luminal B disease has worse baseline distant recurrence-free survival at 5- and 10-yrs regardless of adjuvant systemic therapy compared to Luminal A disease. This has now been observed in multiple retrospective datasets, including 6 phase III clinical trials (i.e. CALGB9741 [45], GEICAM9906 [46], TransATAC [19], ABCSG-08 [20], MA.5 [47] and MA.12 [17] trials) coming from different countries and populations and with different adjuvant systemic therapies (i.e. endocrine-only, chemotherapy-only and both). In the vast majority of studies, the three main variables that predict outcome in early breast cancer are nodal status, tumour size and intrinsic subtyping.

Of note, the vast majority of these studies with long-term follow-up show that the survival curves of Luminal B tumours cross the survival curves of Basal-like disease at around ~10-years of follow-up. Thus, although at 5-years of follow-up, Basal-like disease has a worse outcome than Luminal B tumours, this is not the case at 10 years. This result suggest that if we are to make an impact in decreasing the mortality of early breast cancer, we should focus on finding additional therapies for Luminal B disease since this tumour subtype is very frequent (i.e. represent ~30–40% of all breast cancer diagnoses) and chemotherapy and endocrine therapies are not enough for the majority of these patients.

A part from predicting baseline prognosis, the Luminal A vs B classification, together with tumour size and nodal status, predicts the residual risk of recurring at a distant site within the 5–10-years of follow-up (the so called late recurrence). In a retrospective analysis of the ABCSG-08 study, late distant relapse-free survival was found significantly different between Luminal A and B subtypes in all patients and in the node-negative subgroup [48]. Similar results have been observed in the TransATAC study and in a pooled analysis of both studies looking at the PAM50 Risk of Recurrence (ROR) score [49,50]. These results suggest that intrinsic subtype has the ability to inform decisions concerning the length of endocrine therapy (i.e. 5 vs 10 years), being the low-risk Luminal A tumours with low tumour burden (e.g. tumour size 1 cm and node-negative) the group were 5 years of endocrine therapy might be sufficient.

Cytotoxic therapy benefit

Most of the direct evidence of general chemo-sensitivity of the Luminal A and B subtypes comes from the neoadjuvant setting. For example, in a cohort of 208 patients with luminal disease treated with anthracycline/taxane-based chemotherapy and with pathologic complete response (pCR) data, the pCR rates in patients with the Luminal A and B subtypes were 3% and 16% (odds ratio = 6.01, p-value = 0.003), respectively [4,51]. Similar results have been obtained with the pathology-based surrogate definitions of both luminal subtypes [52–54]. Overall, this data suggest that among the 2 luminal subtypes, the Luminal A tumours are less chemosensitive than Luminal B tumours. This hypothesis is further sustained by the fact that pCR is not predictive of survival outcome in IHC-Luminal A tumours [53] and in patients with HR+/HER2−/low-grade [55], but it is predictive of outcome in IHC-Luminal B/HER2-negative [53] and in HR+/HER2−/high-grade [55]. Furthermore, indirect evidence comes from the retrospective results of the OncotypeDX Recurrence Score, a prognostic gene expression-based test, where low-risk patients, which are basically a subset of Luminal A tumours, did not show a survival benefit from multi-agent adjuvant chemotherapy in the NSABP-B20 (CMF or CF regimens) [56] and SWOG8814 (FAC regimen) [57].

Even if one assumes that all patients benefit to the same extend from multi-agent chemotherapy [58], intrinsic subtyping together with prognostic factors such as tumour size and nodal status can be used to help decide when adjuvant chemotherapy should not be administered because the risk of relapsing without it is very low. For example, if the risk of relapsing at distant sites of a patient is estimated to be $\leq$10% without chemotherapy, the absolute benefit from chemotherapy would be $\leq$3% (assuming a 30% reduction of the risk of 10-year overall mortality for all patients as suggested by the Oxford overview [58]). This absolute benefit barely exceeds the approximately 1% life-threatening toxicities of chemotherapy [59,60].

Although Luminal A tumours seem to benefit less from multi-agent chemotherapy than Luminal B tumours, this does not preclude that this group of tumours cannot benefit from particular cytotoxic agents or regimens. For example, a retrospective analyses of the GEICAM9906 study (FECx6 vs. FECx4 and weekly paclitaxel) and CALGB9342/9840 (3-weekly paclitaxel vs. weekly paclitaxel) showed that low-proliferative tumours, mostly a subset of Luminal As, benefit substantially from the weekly paclitaxel regimen whereas high proliferative tumours did not [46]. Although this was a hypothesis-generating result, one should wonder if lower-dose but more continuously administered chemotherapy might be more beneficial in these low-proliferative Luminal A tumours instead of 3-weekly and high-dose regimens. In fact, in a retrospective analysis of a 10-year follow-up of the phase 3 randomized BCIRG-001 trial (FAC vs. TAC), the only subtype that did not benefit from 3-weekly docetaxel instead of 3-weekly 5-FU in terms of disease-free survival was the IHC-Luminal A (IHC-Luminal A vs. IHC-nonLuminal A interaction p-value = 0.031) [62]. Further studies are needed to determine if Luminal A tumours benefit from chemotherapy or specific chemotherapeutic agents/regimens or even CDK4/6 inhibitors. This answer would be especially relevant in the clinic for those patients with Luminal A tumours with high tumour burden (intermediate or high-risk).

Endocrine therapy benefit

Regarding the benefit from endocrine therapy, both tumour subtypes have shown to derive a similar relative benefit by looking

at the proportional fall in the proliferation marker Ki67 upon treatment with an aromatase inhibitor in the neoadjuvant setting [16]. However, since Luminal A tumours have a lower baseline proliferation status than Luminal B tumours, a larger proportion can achieve low post-treatment values. In fact, in a retrospective analysis of the ACOSOG Z1031 phase II trial, where patients with stage 2 or 3 ER-high/HER2-negative disease were treated for 4–4.5 months with a neoadjuvant aromatase inhibitor [18], a larger proportion of Luminal A tumours achieve a pre-operative prognostic index (PEPI) score of 0 (i.e. good outcome without chemotherapy) compared to Luminal B tumours (27.1 vs. 10.7%, P-value = 0.004). PEPI compares 4 clinical-pathological variables before and after treatment (i.e. tumour size, nodal status, Ki67 and Allred ER score) and has been prognostically validated in an independent study of 203 postmenopausal women of the IMPACT trial [63].

At the adjuvant setting, the only data that we are aware of is the result coming from the BCIRG-001 phase III adjuvant clinical trial (TAC vs FAC), where IHC-Luminal A tumours showed a higher benefit from adjuvant tamoxifen than IHC-Luminal B tumours (tamoxifen vs. no tamoxifen hazard ratio = 0.15 vs. 0.44) [64]. However, allocation to tamoxifen therapy was not based on randomization and thus this result should be interpreted with caution.

Implications of the nonluminal subtypes

Within HR+/HER2-negative early disease, it is expected to identify a subpopulation of non-luminal subtypes (i.e. HER2-enriched and Basal-like) by gene expression (Table 1). HER2-enriched tumours seem to represent 5.5–11.0% of all HR+/HER2-negative cases depending on the dataset evaluated, whereas Basal-like tumours represent around ~1–5%. Based on the molecular features of these two non-luminal subtypes, one would expect to identify these tumours in patients with tumours that express low-ER. In fact, a study performed intrinsic subtyping in 25 tumour samples with 1–9% ER-positive tumour cells, and found that 80% were nonluminal (48% Basal-like and 32% HER2-enriched) [65]. On the other hand, a combined analysis of 48 borderline cases (1–10% ER+ tumour cells) from the MA.5, MA.12 and GEICAM9906 revealed that 46.0% were nonluminal (29% HER2-enriched and 17% Basal-like) [66]. Moreover, HER2-enriched and Basal-like tumours can still be identified in tumours that have very high expression of ER as exemplified by the 6 non-luminal tumours (representing 2.9% of the entire cohort) identified in the Z1031 trial where patients' tumours were all Allred ER score of 6–8.

In terms of survival outcome, we evaluated the prognostic value of the intrinsic subtypes in a cohort of 1380 patients with ER+/HER2-unknown early breast cancer treated with 5 years of adjuvant tamoxifen-only across several retrospective studies [67]. Non-luminal subtypes represented 9% (7% HER2-enriched and 2% Basal-like) of the samples, and each nonluminal subtype showed a significant worse outcome compared to Luminal A subtype in both node-negative and node-positive disease. This data suggest that HER2-enriched and Basal-like diseases might not benefit much from endocrine therapy despite being ER+. In fact, in the Z1031 trial [18], the single Basal-like patient demonstrated high pre- and post-treatment Ki67 values consistent with endocrine therapy resistance (38% and 26.8%, respectively). All five HER2-enriched patients had persistently high surgical Ki67 levels (20%), consistent with high-level oestrogen-independent growth.

In another retrospective study [16] of 112 postmenopausal women with stages I–IIIB ER+ early breast cancer before and after 2-weeks' anastrozole treatment in a neoadjuvant trial, Basal-like (n = 3 [2.7%]; mean Ki-67 change of +15.3%) and HER2-enriched (n = 9 [8.0%]; mean Ki-67 change of −50.7%) subtypes generally showed poorer response compared to Luminal A or B subtypes (mean Ki-67 change of −75%) [16]. Interestingly, this study profiled post-treatment samples. As expected, the vast majority of Luminal A samples (31/32, 97%) continued being Luminal A or became normal breast-like (likely suggesting tumour response and contamination by normal tissue) [16]. On the other hand, although the majority of Luminal B tumours became Luminal A (9/17, 53%) or normal breast-like (3/17, 18%), a substantial proportion of Luminal B tumours (5/17, 30%) remained Luminal B or became HER2-enriched [16]. Although encouraging, further data is needed in order to use intrinsic subtyping during treatment as a biomarker of treatment sensitivity.

Clinical implications within HER2-positive disease

Distribution and biology of the intrinsic subtypes

Similarly as the other pathology-based groups, all the intrinsic molecular subtypes can be identified within clinically HER2-positive disease albeit with different proportions. In our combined analysis of 831 HER2+ tumours (Table 1), 44.6%, 26.8%, 17.6% and 11.0% were identified as HER2-enriched, Luminal B, Luminal A and Basal-like. Interestingly, HR-status by IHC does not fully recapitulate the intrinsic subtypes since 20.9% (1 out of 5 patients) of HER2+/HR+ tumours will be identified as non-luminal (18.7% HER2-enriched and 2.2% Basal-like), and 23.2% of HER2+/HR− tumours will be identified as luminal (19.0% Luminal A and 4.2% Luminal B) and 10.7% as Basal-like.

From a biological perspective, a particular unanswered question was how different is an intrinsic subtype based on HER2 status. For example, how different is HER2+/Luminal A disease from a classical HER2-negative/Luminal A disease? We recently approached this question by interrogating The Cancer Genome Atlas (n = 495) and Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) datasets (n = 1730) of primary breast cancers for molecular data derived from DNA, RNA and protein, and determined intrinsic subtype. Within each subtype, only 0.3%–3.9% of genes were found differentially expressed between HER2+ and HER2-negative tumours. As expected, the vast majority of differentially expressed genes originated in the 17q12 DNA amplicon where the ERBB2 gene is located. Within HER2+ tumours, HER2 gene and protein expression was statistically significantly higher in the HER2-enriched subtype than either luminal subtype. Thus, this result suggests that intrinsic subtype dominates the biological phenotype within HER2+ and HER2-negative disease.

Prognostic implications

Two large studies have evaluated the prognostic value of HR status (i.e. a surrogate manner of looking at luminal vs non-luminal disease) within HER2+ breast cancer [68,69]. In the 4-year follow-up of the N9831 and National Surgical Adjuvant Breast and Bowel Project B-31 adjuvant trials of trastuzumab in HER2+ disease (n = 4045), HR-positive disease was found statistically significantly associated with approximately 40% increased disease-free survival and overall survival, compared to hormone receptor-negative disease [68]. This association of hormone receptor status with survival was found to be independent of the main clinical-pathological variables, including trastuzumab administration. Similar results were observed in a prospective cohort study of 3394 patients with stage I to III HER2+ breast cancer from National Comprehensive Cancer Network centres [69]. In both studies, HR-negative disease experienced more cancer relapse in the first 5 years than HR-positive [69]. Interestingly, patients with HR-negative tumours

were less likely to experience first recurrence in bone and more likely to recur in brain, compared to patients with hormone receptor-positive tumours [69]. Better outcomes independently of treatment in the HR-positive group compared to the HR-negative have also been observed in the NeoALTTO [70] and ALTTO [71] clinical trials.

Regarding intrinsic subtyping, we have recently evaluated the prognostic value of these entities in a large retrospective cohort of 1730 patients from the UK and Canada with and without HER2+ disease treated in the adjuvant setting with different treatments except trastuzumab [24]. The results revealed that intrinsic subtypes are an independent prognostic variable beyond tumour size and nodal status, and HER2+/Luminal A tumours showed a similar outcome compared to HER2-negative/Luminal A tumours [23]. Overall, this data suggests that Luminal A disease could be used, in the future, together with tumour size and nodal status, to help better identify those patients with a low risk of relapsing and thus safely treated with less intense chemotherapy such as the adjuvant regimen paclitaxel and trastuzumab recently proposed for "small" (i.e. <3.0 cm) and node-negative HER2+ breast cancer [72].

Anti-HER2 treatment benefit

In early breast cancer, two randomised neoadjuvant clinical trials tested trastuzumab versus not in combination with chemotherapy in HER2+ breast cancer [73,74]. In the NOAH study, HR status was found predictive of pathological complete response (48% for HR-negative vs. 18% for HR+; p = 0.002) [75]. Overall, pCR was found predictive of event-free survival (EFS) and overall survival; concordant with this, patients with HR-negative tumours benefited more from trastuzumab than HR+ patients (hazard ratio for EFS = 0.58 in HR-negative vs. = 0.74 in HR+ disease) [76]. When intrinsic subtyping was evaluated in a subset of patients (46.7%) of the NOAH study, patients with HER2-enriched disease showed a higher benefit of adding trastuzumab in terms of pCR and EFS compared to non-HER2-enriched disease [25].

Although the NOAH results make a lot of sense from a biological perspective, the larger benefit from trastuzumab in HR-negative disease compared to HR-positive disease has not been observed in the 3 large adjuvant clinical trials evaluating 1-year of trastuzumab vs. placebo and both HR groups seem to benefit similarly [77,78]. Concordant with this, in one of these studies, the NSABP-B31 trial, all the intrinsic subtypes benefit similarly from trastuzumab [79]. Somewhat surprising, intrinsic subtyping in this study was not found to be prognostic. One main difference between the NOAH and the adjuvant trials is that the former was a poorer outcome population (e.g. overall survival at 5 years with trastuzumab: ~75% in the NOAH trial vs. ~90% in the combined N9831 and NSABP-B31 dataset). Although risk by itself should not be a predictor of therapeutic benefit, it might reflect differences in underlying biology which should be the ultimate responsible for the differentially response.

Indirect data from the neoadjuvant setting suggests that even patients with Luminal A/HER2+ or B/HER2+ tumours benefit from trastuzumab. In the CALGB40601 trial, where patients with HER2+ disease were randomized to neoadjuvant paclitaxel with anti-HER2 therapy (i.e. lapatinib, trastuzumab or the combination), the pCR rates of Luminal A/HER2+ tumours or Luminal B/HER2+ tumours were 30–40% [26]. These pCR rates are clearly higher than the ones expected with a single taxane without trastuzumab. For example, in the same study, the pCR rates in Luminal A/HER2+ and Luminal B/HER2+ tumours treated with paclitaxel and lapatinib were 9% and 22%, respectively.

Another important clinical question is which patients with HER2+ disease benefit the most from adding a second anti-HER2 agent to trastuzumab and chemotherapy. In a retrospective analysis of the CALGB40601 neoadjuvant trial, the HER2-enriched subtypes seemed to be the only intrinsic subtype to increase pCR rates (although not statistically significantly) with dual HER2 blockade versus trastuzumab-only (80% vs. 71%) [26]. This fits with recent survival data from the NeoALTTO and ALTTO trial in HER2+ early breast cancer showing that HR-negative tumours obtain a larger benefit (although not statistically significant) than HR+ tumours following (neo)adjuvant chemotherapy and dual HER2 blockade (i.e. trastuzumab and lapatinib) compared with chemotherapy and trastuzumab-only [70,71]. Overall, the evidence so far suggests that all the intrinsic subtypes benefit from trastuzumab (although HER2-enriched might benefit the most), and the benefit of adding a second anti-HER2, especially in the context of chemotherapy, might reside in the HER2-enriched subtype. Further studies are needed to shed light on the prognostic value and anti-HER2-sensitivity of the intrinsic subtypes within HER2+ breast cancer.

Finally, the intrinsic subtypes might be to help identify those patients with HER2+ early breast cancer that might be successfully treated with dual HER2 blockade (+/− endocrine therapy) but without chemotherapy since theirs tumours are exquisitely sensitive to anti-HER2 therapy. Indeed, there is clinical evidence suggesting that these patients exist. For example, the pCR rates in the chemotherapy-free arm of the NeoSphere (n = 107; 4 cycles of pertuzumab + trastuzumab) and TBCRC006 (n = 64; 12 weeks of lapatinib + trastuzumab and endocrine therapy if HR+) neoadjuvant studies were 16.8% and 27.0%, respectively [80–82]. In both studies, HR-negative tumours achieved higher pCR rates than HR+ tumours. Interestingly, in a recently reported neoadjuvant study, the TBCRC023, comparing 12-week versus 24-week of lapatinib + trastuzumab treatment (and endocrine therapy if HR+), the pCR rate in the HR+ tumours was 33.2%, suggesting that longer treatment in HR+ tumours might reach similar pCR rates as chemotherapy plus two anti-HER2 agents [83]. However, no data on intrinsic subtype is available to date from these studies. Based on the prior knowledge, one can speculate that, regardless of HR status, the HER2-enriched subtype enriches for the identification of patients that are more likely to achieve a pCR with dual HER2 blockade without chemotherapy. We are currently testing this hypothesis in a prospective neoadjuvant clinical trial called PAMELA (NCT01973660), which is similar to TBCRC006 and TBCRC023 trials, but treatment lasts for 18-weeks.

Clinical implications within triple-negative (TN) disease

Distribution and biology of the intrinsic subtypes

In the past, we have used the word TN and Basal-like interchangeably. However, within TN disease, all the intrinsic molecular subtypes can be identified, although the vast majority fall into the Basal-like subtype (86%; range 56%–95%, depending from the study). In our combined analysis of 868 TN tumours (Table 1), 86.1%, 9.1%, 3.2% and 1.6% were identified as Basal-like, HER2-enriched, Luminal B and Luminal A, respectively. Although the correlation between pathological and gene expression profiling is moderate, this pathology-based subset is the one with the greatest consistency between both classifications. Of note, we did not evaluate the presence of the Claudin-low subtype [5].

At the same time, other gene expression-based classifications of TN disease have emerged over the years. For example, Lehmann and colleagues described 6 molecular subtypes of TN breast cancer: two Basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL) and a luminal androgen receptor subtype (LAR) [84,85]. As expected, Lehmann's

classification identified most TN tumours as Basal-like (80.6%) [85] and, with the exception of LAR group, all other subtypes were mostly identified as Basal-like by PAM50 (BL1 99%, BL2 95%, IM 84%, M 97%, MSL 50%). Interestingly, the LAR subtype was predominantly identified as either HER2-enriched (74%) or Luminal B (14%). In another recent study, Burstein and colleagues [86] classified TN disease into 4 main groups: LAR, mesenchymal (MES), Basal-like immune-suppressed (BLIS) and Basal-like immune-activated (BLIA). Again, most PAM50 non-Basal-like tumours were identified as LAR by this classification, and most PAM50 Basal-like were BLIS and BLIA. Thus, we can conclude that TN disease is biologically heterogeneous and that although Basal-like disease predominates (+/− immune activation and/or infiltration), there is a small group of non-Basal-like tumours (mostly LARs, or HER2-enriched) [15,87].

Prognostic implications

No data is available regarding the prognostic impact of the intrinsic molecular subtypes defined by PAM50 within TN disease. Regarding the Lehmann's classification, the 7 subtypes have been evaluated retrospectively in several publicly available cohorts of TN disease treated with different adjuvant therapies [84,85,87]. Although no clear results were obtained, several tendencies were observed in both studies. For example, the M group showed the worse outcome and the IM group showed a relatively better outcome. Regarding the LAR group, one study showed a worse outcome and another one a tendency for the best outcome. In Burstein and colleagues [86], the only group that showed a different outcome from the rest was the BLIA, which is consistent with the known prognostic impact of immune infiltration in TN disease [88,89]. However, the BLIA group, or the Basal-like with immune infiltration, has a high risk of relapsing (~20%). Thus, this data suggests that subtyping within TN will not have a clinical impact based on prognosis-only since no group has such an outstanding outcome that would allow avoiding chemotherapy.

Polychemotherapy benefit

Polychemotherapy is highly effective in high proportion of patients with TN early breast cancer [55,90,91]. In the neoadjuvant setting, pCR rates following anthracycle/taxane-based chemotherapy is 25–35% and patients that achieve a pCR have a better outcome than those that do not (hazard ratio for EFS = 0.24, 0.09–0.27) [55,90]. Thus, two relevant clinical questions are the following: 1) can we identify at diagnosis or after primary surgery those patients that are likely to be cured with local therapy and polychemotherapy and 2) can we identify biological processes and/or targets associated with chemotherapy sensitivity or resistance?

To try to address the first question, we analysed intrinsic subtypes and various gene expression-based signatures in 5 independent cohorts (2 neoadjuvant and 3 adjuvant), including 1 phase II and 2 phase III clinical trials (GEICAM2006/03, GEICAM9906 and CALGB9741), of patients with TN disease and/or Basal-like disease treated with polychemotherapy [12]. In this study, we identified significant associations between genomic signatures and response and survival after polychemotherapy within Basal-like disease-only and not within TN disease as a whole. In other words, non-Basal-like tumours within TN disease were contaminating the population and adding noise to the association being evaluated. This observation makes sense based on the huge biological and epidemiological differences among the two groups (*see above*). Overall, this result argues that clinical trials evaluating TN disease should consider using gene expression data to stratify patients by the Basal-like versus not classification given the impact that this heterogeneity might have on the primary end points of these trials.

To try to address the second question, we observed that, within TN disease falling into the Basal-like subtype, high expression of a previously identified proliferation signature, or low expression of a luminal signature, was independently associated with pCR and improved survival following polychemotherapy across different cohorts [12]. Thus, gene expression-based signatures within an already defined homogenous group of tumours (TN and Basal-like) can further stratify patients and provide clinically relevant information. For example, high-risk tumours of the Basal-like subtype with high proliferation scores are very sensitive to chemotherapy and it may be possible that the typical standard of care treatment for this group (i.e. anthracycline/taxane neo/adjuvant regimens) is effective and sufficient. Conversely, those Basal-like tumours predicted to be less responsive, or to have a worse prognosis despite the standard polychemotherapy, may be appropriate for studies of novel agents or approaches. Some patients might be particularly sensitive to specific additional drugs like platinum agents, which increase pCR rates in TN disease [91,92], or other cytotoxics, as will be tested in the upcoming CIBOMA/2004-01/GEICAM/2003-11 phase III clinical trial that focuses on adjuvant capecitabine maintenance therapy after conventional induction chemotherapy in 876 patients with TN disease. Novel therapies that target lower-proliferating cells (i.e. mesenchymal/claudin-low-like and/or luminal-like cells) might be warranted in patients with TN tumours of the Basal-like subtype that show low expression of proliferation features.

Carboplatin treatment benefit

The standard polychemotherapy regimen in early breast cancer is based on anthracycline/taxane-based combinations. However, preclinical data suggest that Basal-like breast cancers are more sensitive to interstrand crosslinking agents that damage the DNA such as platinum, because of deficiencies in the BRCA-associated DNA repair mechanism. To date, two clinical trials (i.e. CALGB40603 and GeparSixto) have tested the impact of adding carboplatin to an anthracycline/taxane-based regimen in TN early breast cancer in the neoadjuvant setting [91,92]. The two studies showed that carboplatin increases the pCR rate by an absolute 15% in the entire population. In the CALGB40603 trial, a retrospective gene expression-based analysis identified a 13.0% of nonBasal-like disease at baseline. In patients with Basal-like tumours (83.0%), pCRs rose from 47% to 61% with the addition of carboplatin ($p = 0.014$), an increment which did not differ significantly from the overall study population (interaction $p = 0.93$). Thus, further studies (e.g. GeparSixto) are needed to evaluate if the Basal-like vs. not classification is useful for identifying those patients that might benefit or not from adding carboplatin to standard polychemotherapy in early TN breast cancer.

In the first-line metastatic setting, the TNT phase III trial randomized 376 patients with TN or BRCA1/2 breast cancer disease to carboplatin versus docetaxel [93]. Overall response rate and median progression-free survival did not differ between the two arms [93]. However, intrinsic subtyping in 210 patients with TN disease identified 17.1% tumours as nonBasal-like. More importantly, the Basal-like vs. not classification predicted ORR to carboplatin vs. docetaxel (interaction $p = 0.01$). Patients with nonBasal-like disease were more sensitive to docetaxel than carboplatin (73% vs. 16%), whereas patients with Basal-like disease showed similar sensitivity (32.6% vs. 35.2%). Of note, in this study, BRCA1/2 mutated tumours ($n = 43$) benefited more from carboplatin than docetaxel compared to nonBRCA1/2 mutated tumours (ORR 68% vs. 33%; interaction $p = 0.01$), thus this is likely another biomarker of platinum sensitivity, most likely within Basal-like disease.

Anti-VEGF therapy benefit

Bevacizumab, an anti-VEGF monoclonal antibody, is approved in Europe for HER2-negative metastatic breast cancer in combination with paclitaxel or capecitabine. However, in the USA, safety and toxicity risks do not seem to outweigh the ability of bevacizumab to significantly prolong progression-free survival. Thus, finding biomarkers that can help us identify which patients benefit the most from this treatment strategy seems logical. In this regard, the CALGB40603 trial recently tested the impact of adding bevacizumab to standard neoadjuvant anthracycline/taxane-based chemotherapy in TN early breast cancer. The results showed that in the general TN population ($n = 443$), bevacizumab increased the pCR rates in the breast (59% vs. 48%; $p = 0.0089$). In a subsequent retrospective analysis of 367 samples, a greater benefit from bevacizumab was observed in Basal-like disease (i.e. pCR rose from 45% to 64%, $p = 0.0009$), but not in non-basal-like disease (i.e. pCR decreased from 60% to 43%; interaction $p = 0.024$). Thus, this is another example of how the Basal-like versus not classification can predict sensitivity to a particular treatment strategy. Further studies are needed to determine if Basal-like disease is a biomarker of response or benefit from bevacizumab, especially in the metastatic setting where it is still approved in some countries.

Antiandrogen therapy benefit

Based on the biology of the different intrinsic subtypes within TN disease, one can speculate that the non-Basal-like (i.e. Luminal A, Luminal B and HER2-enriched) or LAR group might benefit from antiandrogens. In fact, a preclinical study revealed that TN cell lines that overexpress the androgen receptor, and which fall into the LAR or Luminal B subtypes (i.e. MDA-MB453 and SUM185PE), are highly sensitive to bicalutamide [84,85]. In the clinical setting, we have indirect evidence suggesting that patients with TN disease that fall into these subtypes benefit from anti-androgens. Gucalp and colleagues [94] completed a phase II trial of bicalutamide 150 mg daily in patients with metastatic ER-/PR-negative but AR-positive by IHC. Among 424 ER-/PR-negative patients, 12% tested AR+. The clinical benefit rate was 19% (7–39%). In any case, further studies are needed to evaluate if the LAR or non-Basal-like subtypes benefit from antiandrogen. A phase II study with enzalutamide in 95 patients with metastatic TN but AR+ disease has completed recruitment (NCT01889238).

Conclusions

Breast cancer is a clinically and biologically heterogeneous disease. However, the vast majority of the biological diversity coming from the DNA, miRNAs and proteins is captured by the 4 main intrinsic subtypes defined by gene expression-only. At the same time, and contrary to popular belief, intrinsic biology is not sufficiently captured by standard clinical-pathological variables. In this review, we have argued how intrinsic biology identified by gene expression analyses provides, today and specially in the future, clinically relevant information beyond the current pathology-based classification endorsed by the St. Gallen Consensus Panel. In the upcoming years, we should expect more wealth of data regarding the clinical utility of intrinsic subtyping in a variety of clinical scenarios, and in combination with other biomarkers such as somatic mutations.

Conflict of interest statement

Aleix Prat has served in an uncompensated advisory role to Nanostring Technologies. There are no other potential conflicts of interest.

References

[1] La Vecchia C, Bosetti C, Lucchini F, Bertuccio P, Negri E, Boyle P, et al. Cancer mortality in Europe, 2000–2004, and an overview of trends since 1975. Ann Oncol 2010;21:1323–60.

[2] Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747–52.

[3] Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5:5–23.

[4] Prat A, Parker JS, Fan C, Perou CM. PAM50 assay and the three-gene model for identifying the major and clinically relevant molecular subtypes of breast cancer. Breast Cancer Res Treat 2012;135:301–6.

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

[6] Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.

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

[8] Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thurlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2013. Ann Oncol 2013;24:2206–23.

[9] Maisonneuve P, Disalvatore D, Rotmensz N, Curigliano G, Colleoni M, Dellapasqua S, et al. Proposed new clinicopathological surrogate definitions of luminal A and luminal B (HER2-negative) intrinsic breast cancer subtypes. Breast Cancer Res 2014;16:R65.

[10] Prat A, Cheang MC, Martin M, Parker JS, Carrasco E, Caballero R, et al. Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol 2013;31:203–9.

[11] Cheang MCU, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 Index, HER2 status, and prognosis of patients with luminal B breast Cancer. J Natl Cancer Inst 2009;101:736–50.

[12] Prat A, Lluch A, Albanell J, Barry WT, Fan C, Chacon JI, et al. Predicting response and survival in chemotherapy-treated triple-negative breast cancer. Br J Cancer 2014;111:1532–41.

[13] Sikov W, Barry W, Hoadley K, Pitcher B, Singh B, Tolaney S, et al. Impact of intrinsic subtype by PAM50 and other gene signatures on pathologic complete response (pCR) rates in triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy (NACT)+/− carboplatin (Cb) or bevacizumab (Bev): CALGB 40603/150709 (Alliance). San Ant Breast Cancer Symp 2014;2012:S4–05.

[14] Bastien RR, Rodriguez-Lescure A, Ebbert MT, Prat A, Munarriz B, Rowe L, et al. PAM50 breast cancer subtyping by RT-qPCR and concordance with standard clinical molecular markers. BMC Med Genomics 2012;5:44.

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

[16] Dunbier AK, Anderson H, Ghazoui Z, Salter J, Parker JS, Perou CM, et al. Association between breast cancer subtypes and response to neoadjuvant anastrozole. Steroids 2011;76:736–40.

[17] Chia SK, Bramwell VH, Tu D, Shepherd LE, Jiang S, Vickery T, et al. A 50-Gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. Clin Cancer Res 2012;18:4465–72.

[18] Ellis MJ, Suman VJ, Hoog J, Lin L, Snider J, Prat A, et al. Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor–rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype—ACOSOG Z1031. J Clin Oncol 2011;29:2342–9.

[19] Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. J Clin Oncol 2013;31:2783–90.

[20] Gnant M, Filipits M, Greil R, Stoeger H, Rudas M, Bago-Horvath Z, et al. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 risk of recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. Ann Oncol 2014;25:339–45.

[21] Martín M, González-Rivera M, Morales S, de la Haba J, González-Cortijo L, Manso L, et al. Prospective study of the impact of the Prosigna™ assay on adjuvant clinical decision-making in women with estrogen receptor-positive, HER2-negative, node-negative breast cancer: a GEICAM study. San Ant Breast Cancer Symp 2014;2012. P6-08-10.

[22] Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res 2010;16:5222–32.

[23] Prat A, Carey LA, Adamo B, Vidal M, Tabernero J, Cortés J, et al. Molecular features and survival outcomes of the intrinsic subtypes within HER2-positive breast cancer. J Natl Cancer Inst 2014;106.

[24] Curtis C, Shah SP, Chin S-F, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2000 breast tumours reveals novel subgroups. Nature 2012;486:346–52.
[25] Prat A, Bianchini G, Thomas M, Belousov A, Cheang MC, Koehler A, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. Clin Cancer Res 2014;20:511–21.
[26] Carey L, Berry D, Ollila D, Harris L, Krop I, Weckstein D, et al. Clinical and translational results of CALGB 40601: a neoadjuvant phase III trial of weekly paclitaxel and trastuzumab with or without lapatinib for HER2-positive breast cancer. Proc Am Soc Clin Oncol 2013;a500.
[27] Prat A, Ellis MJ, Perou CM. Practical implications of gene-expression-based assays for breast oncologists. Nat Rev Clin Oncol 2012;9:48–57.
[28] Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, et al. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in Breast Cancer Working Group. J Natl Cancer Inst 2011 Nov 16;103(22): 1656–64.
[29] Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010;28:2784–95.
[30] Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31: 3997–4013.
[31] McCullough A, Dell'Orto P, Reinholz M, Gelber R, Dueck A, Russo L, et al. Central pathology laboratory review of HER2 and ER in early breast cancer: an ALTTO trial [BIG 2-06/NCCTG N063D (Alliance)] ring study. Breast Cancer Res Treat 2014;143:485–92.
[32] Prat A, Cheang MCU, Martín M, Parker JS, Carrasco E, Caballero R, et al. Prognostic significance of progesterone receptor–positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol 2013;31:203–9.
[33] Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, et al. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet 2013;45:970–6.
[34] Kuong KJ, Loeb LA. APOBEC3B mutagenesis in cancer. Nat Genet 2013;45: 964–5.
[35] Prat A, Cruz C, Hoadley K, Díez O, Perou C, Balmaña J. Molecular features of the basal-like breast cancer subtype based on BRCA1 mutation status. Breast Cancer Res Treat 2014;147:185–91.
[36] Foulkes WD, Stefansson IM, Chappuis PO, Bégin LR, Goffin JR, Wong N, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast Cancer. J Natl Cancer Inst 2003;95:1482–5.
[37] Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, et al. Triple-negative breast cancer: risk factors to potential targets. Clin Cancer Res 2008;14:8010–8.
[38] Prat A, Adamo B, Fan C, Peg V, Vidal M, Galvan P, et al. Genomic analyses across six cancer types identify basal-like breast cancer as a unique molecular entity. Sci Rep 2013;3:3544.
[39] Hoadley KA, Yau C, Wolf DM, Cherniack AD, Tamborero D, Ng S, et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. Cell 2014;158:929–44.
[40] Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med 2009;15:907–13.
[41] Molyneux G, Geyer FC, Magnay F-A, McCarthy A, Kendrick H, Natrajan R, et al. BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. Cell Stem Cell 2010;7:403–17.
[42] Keller PJ, Arendt LM, Skibinski A, Logvinenko T, Klebba I, Dong S, et al. Defining the cellular precursors to human breast cancer. Proc Natl Acad Sci 2012;109:2772–7.
[43] Anderson WF, Rosenberg PS, Prat A, Perou CM, Sherman ME. How many etiological subtypes of breast cancer: two, three, four, or more? J Natl Cancer Inst 2014;106.
[44] Millikan R, Newman B, Tse C-K, Moorman P, Conway K, Smith L, et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008;109: 123–39.
[45] Liu M, Pitcher B, Mardis E, Davies S, Snider J, Vickery T, et al. PAM50 gene signature is prognostic for breast cancer patients treated with adjuvant anthracycline and taxane based chemotherapy. San Ant Breast Cancer Symp 2012;2012. P2-10-01.
[46] Martín M, Prat A, Rodríguez-Lescure Á, Caballero R, Ebbert MW, Munárriz B, et al. PAM50 proliferation score as a predictor of weekly paclitaxel benefit in breast cancer. Breast Cancer Res Treat 2013;138:457–66.
[47] Cheang MC, Voduc KD, Tu D, Jiang S, Leung S, Chia SK, et al. Responsiveness of intrinsic subtypes to adjuvant anthracycline substitution in the NCIC.CTG MA.5 randomized trial. Clin Cancer Res 2012;18:2402–12.
[48] Filipits M, Nielsen TO, Rudas M, Greil R, Stöger H, Jakesz R, et al. The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with endocrine-responsive early breast cancer. Clin Cancer Res 2014 Mar 1;20(5):1298–305.
[49] Sestak I, Cuzick J, Dowsett M, Lopez-Knowles E, Filipits M, Dubsky P, et al. Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian Breast and Colorectal Cancer Study Group 8 and arimidex, tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score. J Clin Oncol 2015;33:916–22.
[50] Sestak I, Dowsett M, Zabaglo L, Lopez-Knowles E, Ferree S, Cowens JW, et al. Factors predicting late recurrence for estrogen receptor–positive breast cancer. J Natl Cancer Inst 2013 Oct 2;105(19):1504–11.
[51] Usary J, Zhao W, Darr D, Roberts PJ, Liu M, Balletta L, et al. Predicting drug responsiveness in human cancers using genetically engineered mice. Clin Cancer Res 2013;19:4889–99.
[52] von Minckwitz G, Blohmer JU, Costa SD, Denkert C, Eidtmann H, Eiermann W, et al. Response-guided neoadjuvant chemotherapy for breast cancer. J Clin Oncol 2013 Oct 10;31(29):3623–30.
[53] von Minckwitz G, Untch M, Blohmer J-U, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796–804.
[54] Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13:2329–34.
[55] Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al., Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 384:164–172.
[56] Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol 2006;24:3726–34.
[57] Albain KS, Barlow WE, Shak S, Hortobagyi GN, Livingston RB, Yeh IT, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol 2010;11:55–65.
[58] Early Breast Cancer Trialists' Collaborative G. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet 2012;379:432–44.
[59] Coates AS, Colleoni M, Goldhirsch A. Is adjuvant chemotherapy useful for women with luminal A breast cancer? J Clin Oncol 2012;30:1260–3.
[60] Hayes DF. Targeting adjuvant chemotherapy: a good idea that needs to be proven! J Clin Oncol 2012;30:1264–7.
[62] Mackey JR, Martin M, Pienkowski T, Rolski J, Guastalla J-P, Sami A, et al. Adjuvant docetaxel, doxorubicin, and cyclophosphamide in node-positive breast cancer: 10-year follow-up of the phase 3 randomised BCIRG 001 trial. Lancet Oncol 2013;14:72–80.
[63] Ellis MJ, Tao Y, Luo J, A'Hern R, Evans DB, Bhatnagar AS, et al. Outcome prediction for estrogen receptor–positive breast cancer based on post-neoadjuvant endocrine therapy tumor characteristics. J Natl Cancer Inst 2008;100:1380–8.
[64] Hugh J, Hanson J, Cheang MCU, Nielsen TO, Perou CM, Dumontet C, et al. Breast cancer subtypes and response to docetaxel in node-positive breast cancer: use of an immunohistochemical definition in the BCIRG 001 trial. J Clin Oncol 2009;27:1168–76.
[65] Iwamoto T, Booser D, Valero V, Murray JL, Koenig K, Esteva FJ, et al. Estrogen receptor (ER) mRNA and ER-related gene expression in breast cancers that are 1% to 10% ER-positive by immunohistochemistry. J Clin Oncol 2012;30: 729–34.
[66] Cheang M, Martin M, Nielsen T, Prat A, Rodriguez-Lescure A, Ruiz A, et al. Quantitative hormone receptors, triple-negative breast cancer (TNBC), and molecular subtypes: a collaborative effort of the BIG-NCI NABCG. Proc Am Soc Clin Oncol 2012;a1008.
[67] Prat A, Parker JS, Fan C, Cheang MCU, Miller LD, Bergh J, et al. Concordance among gene expression-based predictors for ER-positive breast cancer treated with adjuvant tamoxifen. Ann Oncol 2012;23:2866–73.
[68] Vaz-Luis I, Ottesen R, Hughes M, Marcom PK, Moy B, Rugo H, et al. Impact of hormone receptor status on patterns of recurrence and clinical outcomes among patients with human epidermal growth factor-2-positive breast cancer in the National Comprehensive Cancer Network: a prospective cohort study. Breast Cancer Res 2012;14:R129.
[69] Perez EA, Romond EH, Suman VJ, Jeong J-H, Davidson NE, Geyer CE, et al. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2–positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 2011;29: 3366–73.
[70] de Azambuja E, Holmes AP, Piccart-Gebhart M, Holmes E, Di Cosimo S, Swaby RF, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): survival outcomes of a randomised, open-label, multicentre, phase 3 trial and their association with pathological complete response. Lancet Oncol 2014;15:1137–46.
[71] Piccart-Gebhart M, Holmes A, Baselga J, De Azambuja D, Dueck A, Viale G, et al. First results from the phase III ALTTO trial (BIG 2–06; NCCTG [Alliance] N063D) comparing one year of anti-HER2 therapy with lapatinib alone (L), trastuzumab alone (T), their sequence (T → L), or their combination (T + L) in the adjuvant treatment of HER2-positive early breast cancer (EBC). Proc Am Soc Clin Oncol 2014;LBA4.
[72] Tolaney SM, Barry WT, Dang CT, Yardley DA, Moy B, Marcom PK, et al. Adjuvant paclitaxel and trastuzumab for node-negative, HER2-positive breast cancer. N Engl J Med 2015;372:134–41.

[73] Eiermann W, Baselga J, Semiglazov V, Byakhov M, Ciruelos E, Ojeda B, et al. Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 375: 377–384.
[74] Buzdar AU, Ibrahim NK, Francis D, Booser DJ, Thomas ES, Theriault RL, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2–positive operable breast cancer. J Clin Oncol 2005;23:3676–85.
[75] Eiermann W, Baselga J, Semiglazov V, Byakhov M, Ciruelos E, Ojeda B, et al. Hormone-receptor status and likelihood of predicting pathological complete response (pCR) in the NOAH trial of neoadjuvant trastuzumab in patients (pts) with HER2-positive locally advanced breast cancer (LABC). In: EBCC-6, European breast cancer conference, Poster: 228; 2008.
[76] Gianni L, Eiermann W, Semiglazov V, Lluch A, Tjulandin S, Zambetti M, et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet Oncol 2014;15: 640–7.
[77] Untch M, Gelber RD, Jackisch C, Procter M, Baselga J, Bell R, et al. Estimating the magnitude of trastuzumab effects within patient subgroups in the HERA trial. Ann Oncol 2008;19:1090–6.
[78] Perez EA, Romond EH, Suman VJ, Jeong J-H, Sledge G, Geyer CE, et al. Trastuzumab plus adjuvant chemotherapy for human epidermal growth factor receptor 2–positive breast cancer: planned joint analysis of overall survival from NSABP B-31 and NCCTG N9831. J Clin Oncol 2014;32:3744–52.
[79] Pogue-Geile KL, Song N, Jeong J-H, Gavin PG, Kim S-R, Blackmon NL, et al. Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in the NSABP B-31 trial. J Clin Oncol 2015 Apr 20;33(12):1340–7.
[80] Gianni L, Pienkowski T, Im Y-H, Roman L, Tseng L-M, Liu M-C, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25–32.
[81] Rimawi MF, Mayer IA, Forero A, Nanda R, Goetz MP, Rodriguez AA, et al. Multicenter phase II study of neoadjuvant lapatinib and trastuzumab with hormonal therapy and without chemotherapy in patients with human epidermal growth factor receptor 2–overexpressing breast cancer: TBCRC 006. J Clin Oncol 2013;31:1726–31.
[82] Prat A, Baselga J. Dual human epidermal growth factor receptor 2 (HER2) blockade and hormonal therapy for the treatment of primary HER2-positive breast cancer: one more step toward chemotherapy-free therapy. J Clin Oncol 2013;31:1703–6.
[83] Rimawi M, Niravath P, Wang T, Rexer B, Forero A, Wolff A, et al. TBCRC023: a randomized multicenter phase II neoadjuvant trial of lapatinib plus trastuzumab, with endcorine therapy and without chemotherapy, for 12 vs. 24 weeks in patients with HER2 overexpressing breast cancer. San Ant Breast Cancer Symp 2014;2012. S6-02.
[84] Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750–67.
[85] Lehmann BD, Pietenpol JA. Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. J Pathol 2014;232: 142–50.
[86] Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua S, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 2015 Apr 1;21(7):1688–98.
[87] Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 2013;19: 5533–40.
[88] Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 2014;25:1544–50.
[89] Ali HR, Provenzano E, Dawson S-J, Blows FM, Liu B, Shah M, et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12 439 patients. Ann Oncol 2014;25:1536–43.
[90] Liedtke C, Mazouni C, Hess KR, André F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008;26:1275–81.
[91] Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol 2014.
[92] von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol 2014;15:747–56.
[93] Tutt A, Ellis P, Kilburn L, Gilett C, Pinder S, Abraham J, et al. The TNT trial: a randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or BRCA1/2 breast cancer (CRUK/07/012). San Ant Breast Cancer Symp 2014;2012. S3-01.
[94] Gucalp A, Tolaney S, Isakoff SJ, Ingle JN, Liu MC, Carey LA, et al. Phase II trial of bicalutamide in patients with androgen receptor–positive, estrogen receptor–negative metastatic breast cancer. Clin Cancer Res 2013;19:5505–12.