Clinical relevance of the triple-negative breast cancer concept: Genetic basis and clinical utility of the concept

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Abstract

The beginning of microarray technology in the 1990s and the sequencing of the human genome in 2000 paved the way for the seminal paper of the Stanford group on the molecular portraits of human breast tumours in the same year. They described four distinct breast cancer subtypes, which they called 'luminal', 'basal', 'HER2-positive', and 'normal breast-like', based on unique gene expression patterns. This paper caused a paradigm shift. Breast cancer was no longer hormone receptor-positive or -negative, but rather luminal, basal or HER2-positive. Since then, numerous papers have appeared, trying to further characterise these subtypes on the DNA, RNA and protein level. Other groups have focussed on the epidemiology, prognosis and outcome after therapy of breast cancer patients according to these molecular subtypes. A promising prognostic marker within the subgroup of basal-like breast cancer is an up-regulated immune response, which is associated with favourable outcome. In addition, the majority of basal-like breast cancers harbour traits of a DNA damage repair defect. This feature can be exploited by the use of DNA damaging agents, and first exciting clinical results of the combination of carboplatin, gemcitabine and a poly (ADP-ribose) polymerase 1 (PARP-1) inhibitor have recently been reported. In this review, the molecular characterisation of triple-negative breast cancer, a proxy for basal-like breast cancer, is described and findings have been put into clinical context.

Introduction

Breast cancer is the most common malignancy in women worldwide and is one of the leading causes of cancer-related mortality [1]. More than 1.2 million cases are diagnosed each year, affecting 10–12% of the female population and accounting for almost 500,000 deaths per year worldwide [1,2]. Breast cancer mortality rates have been declining in the USA and several other countries since the early 1990s, for the most part due to the introduction of adjuvant systemic therapies [3]. However, the large majority of primary breast cancer patients who receive adjuvant systemic therapy do not benefit from this intervention [4]. Molecular biology has greatly enhanced our understanding of the heterogeneity of the disease [5], but only a few molecular tumour features (hormone receptor and HER2 status) are used in the clinic to guide the choice of a systemic treatment strategy [6]. For oestrogen receptor (ER)-positive disease, allocation to about 5 years of adjuvant endocrine therapy reduces the annual breast cancer death rate by approximately 30% [3]. The addition of trastuzumab to adjuvant chemotherapy has improved outcome of HER2-positive breast cancer patients considerably [7–9]. For ER-negative, progesterone receptor (PgR)-negative and HER2-negative breast cancer, so-called 'triple-negative breast cancer', only conventional chemotherapy, which lacks an established therapeutic target, is available as an effective treatment option [10]. Clearly, there is an urgent need for more rationally designed drugs to treat this subgroup of breast cancer patients [11]. This requires a coordinated effort from the research community to 1) molecularly characterise 'triple-negative breast cancer' and further subdivide this immunohistochemically defined subtype into distinct molecular entities; 2) identify existing drugs and develop new targeted drugs directed against those molecular aberrations that drive tumour progression and are indispensible for tumour cell survival – this includes functional *in vitro* studies with, for instance, RNA interference technology and/or mouse model studies; 3) run randomised phase II clinical trials in molecularly well-defined subgroups of breast cancer comparing standard treatment with the same regimen combined with a targeted approach. Here, we

summarise these coordinated research efforts and give a brief outlook on the future.

Triple-negative and basal-like breast cancer definitions

Triple-negative breast cancer (TNBC) is a phenotype based on a negative oestrogen, progesterone and HER2 receptor as assessed with immunohistochemistry (IHC). As there is a large inter-observer variation among pathologists on the ER, PgR and HER2 status of breast cancers [12–15], the composition of reported triple-negative breast cancer series may vary per study. In addition, ER and HER2 "double-negative" breast cancer can be used as a proxy for triple-negative breast cancer [16,17], as double-negative tumours with a positive PgR are considered a technical flaw by most pathologists.

Basal-like breast cancer (BLBC) was already described in the 1970s as a breast cancer subtype originating from the basal layer of mammary epithelium, also known as the myoepithelial cell [18], but only gained widespread popularity through its molecular definition coined by Perou and colleagues in their seminal paper on the molecular portraits of human breast tumours ([5] and reviewed in [19]). The early work from the Stanford group suggested that the basal-like subtype could be characterised immunohistochemically by staining for cytokeratin (CK) 5/6 [5]. Follow-up studies by the group of the University of British Columbia refined the immunohistochemical characterisation of the basal-like subtype [16]. Of the 115 breast cancers profiled by Sorlie and colleagues [20], 72 tumours had immunohistochemical data available. Eighteen HER2-negative and ER-negative (IHC) cases were selected from these 72 tumours. Fifteen tumours (15/18; 83%) appeared of the basal-like subtype by gene expression profiling. With their immunohistochemical definition of basal-like breast cancer as being ER and HER2 negative (neg), and cytokeratin (CK) 5/6 positive and/or epidermal growth factor receptor (EGFR) positive, Nielsen and colleagues could identify 17 of 21 basal-like breast cancers (based on microarray analysis) (81%) [16]. Several other studies compared microarray-based basal-like breast cancer (taken together $N \approx 240$ cases) [17,21–25] with triple (or double) negativity on IHC, and generally reported a concordance of circa 70–80%, with one exception: Calza and colleagues reported ~45% ER positivity (IHC) in a series of 59 microarray-based basal-like breast cancer cases [25].

Several studies, including three series of over 1000 patients [26–28], have demonstrated that triple-negative breast cancer and basal-like breast cancer as assessed by IHC (BLBC-IHC) overlap substantially (50–80%), but are not synonymous [16,21,26,27,29–32]. BLBC-IHC is a subgroup within TNBC and is biologically more homogeneous. It expresses more consistently markers indicative of a high proliferative profile, such as p53, p16, phospho-histone 3 and nuclear expression of c-Myc [27]. In addition, BLBC more often expresses luminal and basal cytokeratins, suggesting that BLBC may either have features of dual-lineage differentiation or a more "stem/progenitor" cell phenotype than the subgroup of TNBC that do not express basal cytokeratins and/or EGFR [27]. An informative illustration of the relationship and overlap between basal breast cancer subgroups can be found in a review by Schneider and colleagues [10].

In summary, three major definitions of basal-like breast cancer are in use: 1) based on gene expression profiling (considered by many as the "gold standard") [5,17,20–25,33]; 2) based on ER, PgR and HER2 status assessed with IHC [27,34–38]; 3) based on ER, HER2 and additional basal markers (e.g. CK5/6, CK14, CK17, EGFR) assessed with IHC [16,26,27,29,31,32]. Clearly, these definitions have substantial overlap, but they are not the same. As no rigorous staining and scoring protocols have been developed for the most commonly used additional basal-like breast cancer markers, in the interim most clinicians will use the triple-negative IHC definition for reasons of convenience. Table 1 summarises the "poor man's" IHC definitions of the molecular subtypes derived from the microarray-based intrinsic subtypes [5,33,34].

Table 1
"Poor man's" IHC definitions of microarray-based intrinsic subtypes of breast cancer [5,33,34]

Breast cancer subtype	ER	PgR	HER2
Luminal A	ER and/or PgR pos		neg
Luminal B*	ER and/or PgR pos		pos
HER2+/ER–	neg	neg	pos
Basal-like	neg	neg	neg

IHC = immunohistochemistry; ER = oestrogen receptor; PgR = progesterone receptor; HER2 = Human epidermal growth factor receptor 2; pos = positive; neg = negative.
*This definition comprises a minority of all luminal B tumours, and this method of subcategorising luminal B will misclassify a substantial fraction of the luminal B tumours into the luminal A category [34].

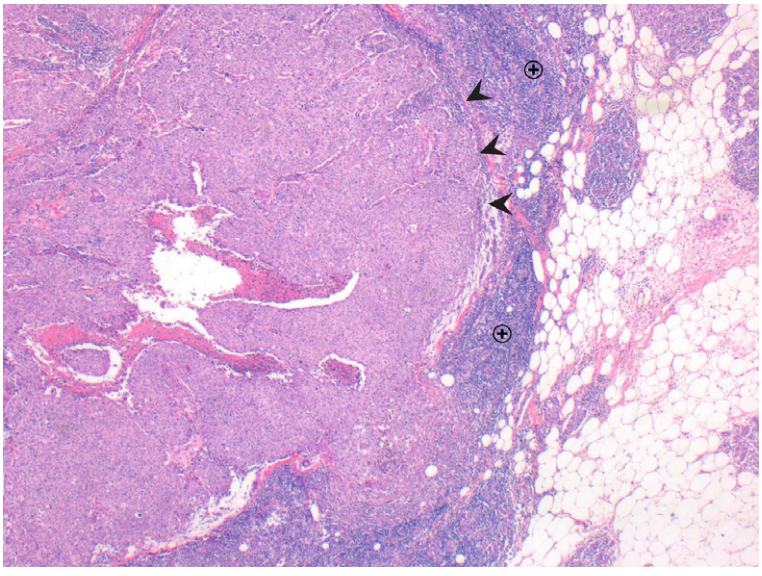

Fig. 1. Triple-negative breast cancer with an expansive growth pattern on histology. Arrowheads: 'pushing margins'. Target circles: Dense lymphocytic infiltrate.

Triple-negative / basal-like breast cancer and the BRCA1 pathway connection

The majority of *BRCA1*-associated breast cancers share many phenotypic features with triple-negative and BLBC (reviewed in [39]). *BRCA1*-associated tumours generally cluster with the basal-like subtype in gene expression profiling studies [20]. Around 80–90% of these breast cancers are ER negative, while less than 5% are HER2 positive [40,41]. The majority stain for one or more basal-like markers, e.g. CK5/6, CK14, CK17, EGFR [27,30,42,43]. The odds ratio for identifying a breast cancer with a germ-line *BRCA1* mutation within the BLBC group is ~10 when compared with non-BLBC [27], and ~9 within the group of TNBC [27]. On the other hand, within a randomly selected BLBC series ($N = 126$) diagnosed between 2000 and 2006 at Dana Farber/Harvard Cancer Center, only 15% appeared to be a *BRCA1* mutation carrier [30]. When restricted to age under 50 years, the prevalence increased to 23% [30]. Consequently, patients with BLBC, especially when diagnosed younger than 50 years of age, might be referred for *BRCA1* mutation screening.

BRCA1-associated tumours, like many TNBCs [27] and BLBCs [17,27], generally exhibit morphologic features such as 'pushing margins', also called an 'expansive growth pattern', a pathological description of growth characteristics at the tumour edge (Fig. 1), a dense lymphocytic infiltrate surrounding the tumour (Fig. 1), a high histological grade, and a high mitotic count [44,45]. In addition, medullary/atypical medullary features [17,45] and metaplastic characteristics [17] are more prevalent in BLBC and *BRCA1*-associated tumours than in other types of breast cancer. At the molecular level, *BRCA1*-associated tumours, similar to TNBCs and BLBCs, often harbour *TP53* mutations [20,46–48], c-*MYC* amplification [49], and display genomic instability [50,51].

BRCA1 has many functions in the normal cell, including maintenance of genomic integrity, chromatin remodelling, transcription regulation, and cell cycle checkpoint control (reviewed in [52]). BRCA1 is part of the BRCA/Fanconi anaemia DNA repair pathway involved in DNA repair of double-strand breaks (DSBs), stalled replication forks, and DNA crosslinks by homologous recombination [53]. Three different DNA repair systems for DSBs and stalled replication forks have so far been found in mammalian cells. These are non-homologous end joining (NHEJ), homologous recombination (HR) and single-strand annealing (SSA). HR is the mechanism of choice during the S and G2 phases of the cell cycle, is potentially error-free, and highly reliant on the BRCA1 and BRCA2 proteins [53]. When BRCA is absent, the cell cannot use HR, and turns to one of the alternative, error-prone DNA repair systems (NHEJ or SSA). Increased presence of mutations, translocations and chromosomal instability is the consequence. This trait has been called 'BRCAness' [53]. Recently, it has been estimated that this trait occurs in approximately one third of all breast cancers [54]. However, no clinical test exists to reliably identify this subgroup of breast cancers.

BRCA1 has also been implicated in regulation of breast epithelial cell differentiation [55,56]. Consequently, loss of *BRCA1* or a BRCA1 pathway disruption might lead to a differentiation block and a more pluripotent phenotype. Recently, an activated phosphatidylinositol 3-kinase (PI3K) pathway through molecular aberrations of the *PTEN* gene has been associated with BRCA1-dependent dysfunction in DNA repair [57]. An activated PI3K pathway is a potent oncogenic signalling cascade that promotes cell transformation, proliferation, migration, angiogenesis and genomic instability. Furthermore, it inhibits apoptosis, and maintains stem cell compartments ([57] and refs therein). The latter trait may contribute to the "stem/progenitor" cell phenotype observed in TNBC/BLBC.

In female mammals, one *X* chromosome is silenced in each cell to achieve equivalent *X*-linked gene dosage between females and males ([58] and refs therein). *X* inactivation is regulated by the *XIST* gene which is located on the *X* chromosome [58].

Conflicting evidence suggests that BRCA1 might influence, directly or indirectly, *XIST* coating of Xi in adult somatic cells [58,59]. Interestingly, X chromosome abnormalities were consistently found in BLBC, both sporadic and *BRCA1*-associated, while these abnormalities were rare in non-BLBC [60]. The sporadic BLBC in this study, where tested, were genetically wild-type for *BRCA1*, synthesised BRCA1 protein, and localised it in the nucleus normally [60]. These findings suggest that in sporadic BLBC, a BRCA1 pathway disruption might be responsible for the observed *X* chromosome abnormalities, and for the "pluripotent stem/progenitor" cell phenotype [60]. Alternatively, the more "pluripotent stem/progenitor" cell in normal breast tissue might be more vulnerable to BRCA1 pathway disruption and *X* chromosome abnormalities; while more differentiated cells might not tolerate such molecular aberrations [60].

Although several lines of evidence point at a disrupted BRCA1-pathway in a subgroup of TNBC/BLBC that closely resemble *BRCA1*-associated tumours [53], *BRCA1* mutations are very rare in sporadic breast cancer [61]. Methylation of the *BRCA1* promoter as an alternative mechanism for reduced *BRCA1* expression has been reported in BLBC, but appeared mainly present in medullary and metaplastic breast cancers [62,63]. Recently, upregulation of *ID4*, a helix-loop-helix transcription factor and a negative regulator of *BRCA1* [64], was demonstrated in BLBC [62], suggesting a potential mechanism for *BRCA1* downregulation. ID family proteins are key regulatory proteins in developmental processes, acting through negative regulation of gene transcription to block cell differentiation [65]. *ID4* upregulation might contribute to the undifferentiated phenotype observed in BLBC. In line with this, *XIST* expression appeared inversely correlated with *ID4* expression [62].

Tumour cells with a dysfunctional BRCA1-pathway have a defect in DNA DSB repair that could be exploited by using anticancer drugs that generate DNA DSBs [53]. This promising strategy will be discussed later in this review.

In contrast to *BRCA1*-associated breast cancers, *BRCA2* tumours are not associated with the TNBC or BLBC phenotype [40,42,44].

Clinical aspects of triple-negative and basal-like breast cancer

Epidemiology

Triple-negative breast cancer comprises 10–20% of all breast cancers [26,27,31,35,37,38] and its percentage varies with race, with the highest proportion reported in indigenous Africans and premenopausal African–Americans (up to 50% [28,37,38,66,67]; reviewed in [68]) and the lowest fraction occurring in Japanese (~10%) [69]. Similar but slightly lower percentages have been reported for BLBC (reviewed in [68]). The mean age at diagnosis for women with TNBC and BLBC is younger than for women with other breast cancer subtypes [28,35,37,43], and the odds ratio for TNBC under age 40 years is 1.53 when compared to non-TNBC [37]. Opposite to luminal breast cancer, risk factors for TNBC and BLBC include higher parity combined with lack of breast feeding, and young age at first full term pregnancy [28,31,66]. Similar to luminal breast cancer, no or short duration of breastfeeding, abdominal adiposity and early-onset menarche also appear to be risk factors for BLBC [28]. These risk factors suggest that a DNA damage repair defect may be an underlying cause in some of these TNBCs and BLBCs, as all risk factors are associated with a state of increased proliferation of breast epithelial cells. Why abdominal adiposity is a risk factor is unclear, but a connection with insulin resistance and increased mitotic activity in breast epithelial tissue has been made ([28] and refs therein). The protective effect of breast feeding after the high proliferative state of breast tissue during pregnancy is not well understood, but potential mechanisms include induction of terminal differentiation and/or removal of initiated breast epithelial cells, removal of oestrogens via breast fluid, delay in ovulation, and changes in breast pH ([28] and refs therein).

It has been estimated that >50% of BLBC could be prevented in younger African–American women by promoting breast feeding and reducing abdominal adiposity [32].

Clinical presentation

TNBCs/BLBCs are larger at presentation and more often poorly differentiated (~70% versus ~30%) than non-TNBCs/BLBCs [35,37,43]. They lack the well-known positive correlation between tumour size and nodal status, and between tumour size and prognosis, both present in non-TNBCs/BLBCs [35,70]. These findings point to early blood-borne dissemination in TNBC/BLBC and thus neither the tumour size nor the absence of involved axillary lymph nodes should be given the same prognostic weight as in other breast cancers [70].

In a screened population of age ⩾50 years, TNBCs were less often screen-detected by mammography or ultrasound than other breast cancers (~20% versus 35%) [35]. In a nested case-control study on interval cancers, BLBCs were more likely than other breast cancers to present in the 2-year interval between regular mammograms [71]. Dense breasts, positive p53 expression and young age were positively correlated with interval cancers in a logistic regression model [71]. These findings may reflect a more rapid growth rate of TNBC/BLBC in comparison with other breast cancers, or may be due to intrinsic differences in detectability [35], as has also been described for tumours with a *BRCA1/2* mutation [72]. Histologic characteristics of pushing margins and a high proliferation rate of TNBC/BLBC have been translated to imaging studies. Pushing margins translate to a smooth or circumscribed mass on mammography, generally without calcifications and/or a spiculated margin [73]. ER-negative and BLBCs usually lack an echogenic halo (thought to present spiculation) on ultrasound [74,75], while TNBCs have been associated with a mass lesion type, smooth mass margin, rim enhancement, persistent enhancement pattern and very high intratumoural signal intensity on T2-weighted magnetic resonance images [76]. TNBCs display a higher fluorine-18 fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET) than ER+/PgR+/HER2– breast cancers, and are easily discerned from the latter [77]. The enhanced glycolysis in TNBCs is probably due to their high proliferation rate and aggressive biology [77].

TNBC/BLBC patients are at increased risk of developing visceral [78] and brain metastases [79–81] when compared to non-TNBC/BLBC patients. In addition, these tumours tend to relapse early and have been associated with a short post-recurrence survival [35,78]. Similar findings have been reported for *BRCA1*-associated breast cancer patients [82,83].

Prognostic and predictive factors

High-quality prognostic and predictive factors are indispensible in order to make the right decision regarding systemic therapy for an individual breast cancer patient. Prognostic factors are most useful in the adjuvant setting and tell you *whether* the patient should be treated, but not *how*. Predictive factors are useful in any stage of the disease and tell you *how*, but not *whether,* the patient should be treated (Fig. 2) [84]. A very useful guideline has been published on how potential markers should be investigated in order to assess their value for clinical practice [85]. Currently, within the TNBC subtype, neither prognostic nor predictive factors are available to guide treatment decisions. Consequently, overtreatment in the adjuvant setting is common for this subtype [86], while the treatment given is only moderately effective, due to the lack of predictive factors, i.e. targeted therapy. Now the challenge is to identify "druggable" molecular changes driving tumourigenesis in TNBC, similar to how this has been accomplished for HER2-positive breast cancer with the identification of HER2 amplification (Fig. 3) [87]. Identification of such candidate targets should be followed by relatively small, hypothesis-driven studies within the breast cancer molecular subgroup of interest [88]. It is crucial to assess the impact of a new targeted drug in a study population with a tumour that harbours the target. If trastuzumab would have been assessed in an unselected breast cancer population (prevalence of HER2-positive breast cancer ~15–20%), probably over 1000 patients would have been needed to show a 100% extra benefit of trastuzumab added to chemotherapy (Table 2; Fig. 3),

Table 2
Number of patients required for a randomised clinical trial to demonstrate the extra benefit of a targeted drug in relation to the percentage of patients with the target present in the tumour [88]

% patients with target in tumour in study population	% extra benefit for patients with target in tumour		
	30%	50%	100%
10	32,000	11,000	3900
30	3600	1800	600
50	1700	780	280
70	900	400	150

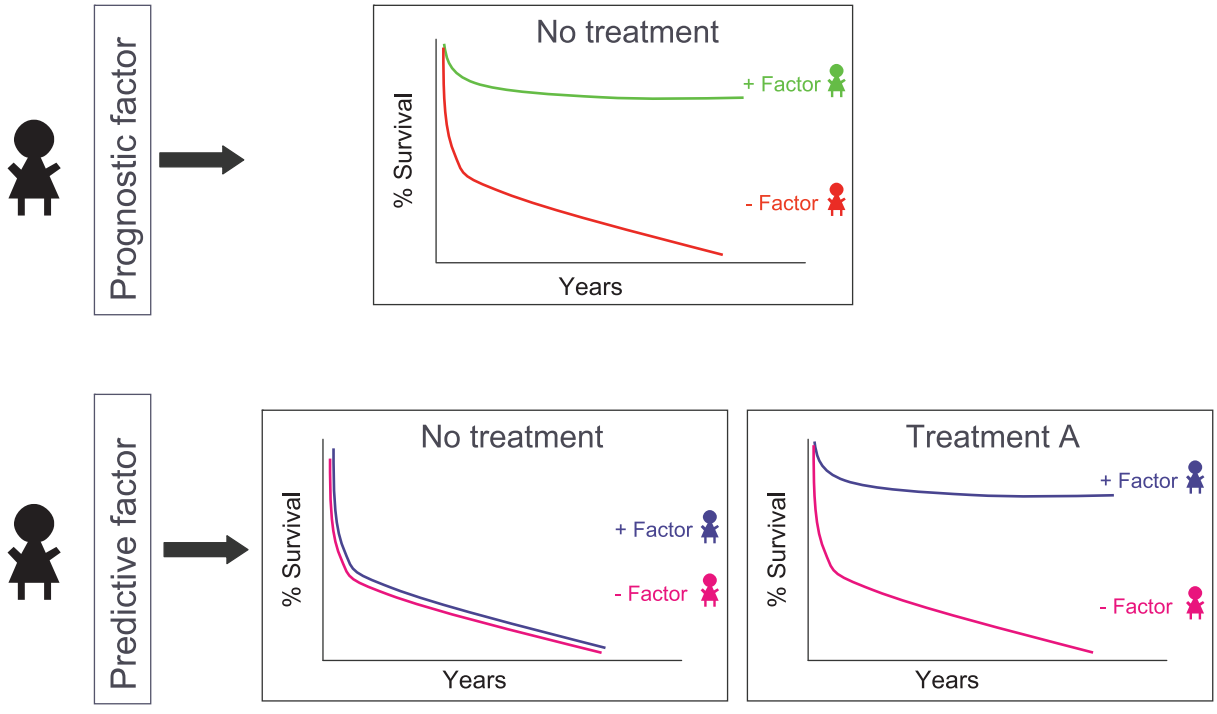

Fig. 2. A prognostic factor predicts outcome of the natural history of a disease. It can for instance predict outcome of breast cancer patients who have received local therapy, but no systemic therapy. It therefore tells you *whether* a patient should be treated, but not *how*. A predictive factor predicts outcome after a specific therapy, but not in the absence of that specific therapy. It therefore tells you *how* the patient should be treated, but not *whether* the patient should be treated.

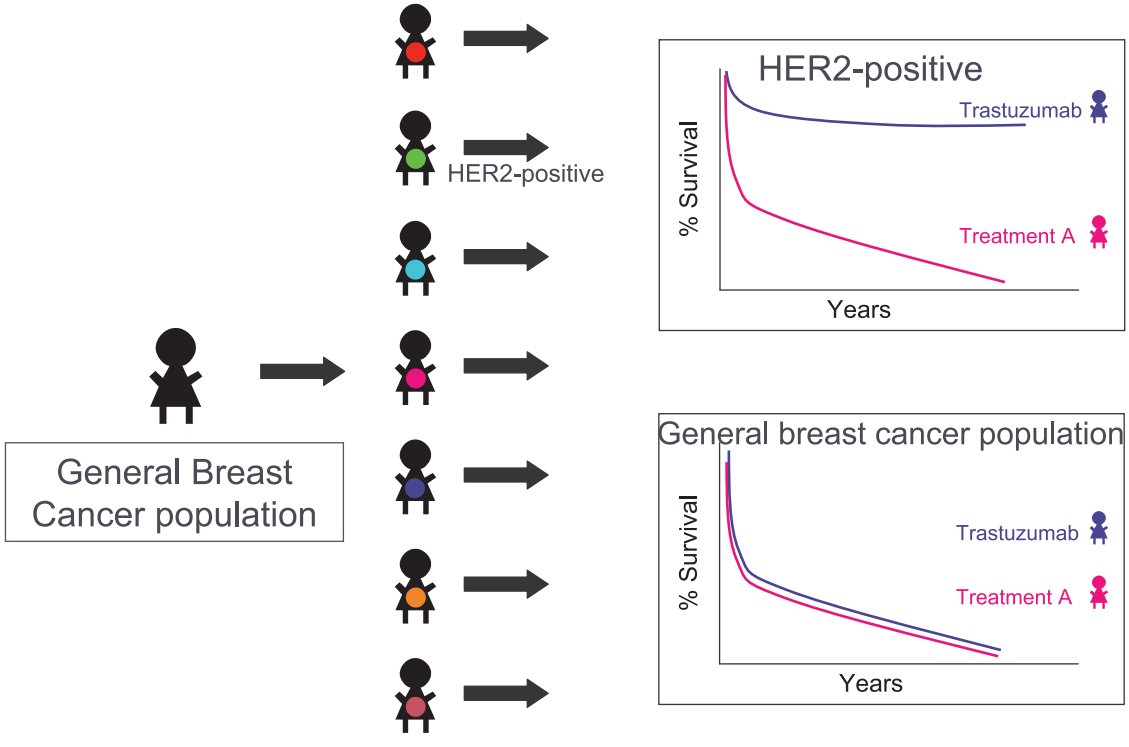

Fig. 3. It is crucial to study targeted therapies only in the population of interest, that is, the population who has the target present in their cancer. Had the addition of trastuzumab to standard chemotherapy been studied in the general breast cancer population, then the trial would have been negative, and trastuzumab discarded as a useless drug.

and the pivotal trial would have been negative with less than 500 patients randomised [87].

Prognosis of TNBC/BLBC in comparison with other breast cancer subtypes

The best way to study prognosis of TNBC/BLBC is in a cohort of conservatively managed breast cancer patients, that is, who only received local therapy without any adjuvant systemic treatment. Recently, Parker and colleagues translated the microarray-based intrinsic gene set [5,20] into a core intrinsic gene set of 50 genes (PAM50) that can be assessed with a quantitative real-time polymerase chain reaction (qRT-PCR) analysis suitable for archived material [89]. In a conservatively managed cohort of over 700 cases (~5% node positive; median follow-up time >7 years) luminal breast cancer patients had the best prognosis, followed by patients with the basal-like, luminal B and HER2-enriched subtypes, respectively. The 128 patients with a basal-like subtype had a 7-year relapse-free survival of over 60% [89], which is similar to the results reported by Desmedt and colleagues in a substantially overlapping publicly available dataset of systemic therapy-naïve patients [90]. Comparable results have been reported in patient series where a considerable fraction had been treated with adjuvant systemic therapy, thereby confounding prognostic information on outcome [20,24,91]. Several IHC studies have reported outcomes of TNBC/BLBC [26,35,37,43], but only one studied outcome in patients after adjuvant tamoxifen (which generally does not work in ER-negative breast cancer), who had not received adjuvant chemotherapy [22]. In this study of ~100 ER-negative patients, including ~25% HER2 amplified cases, with a median follow-up of 6 years, the 6-year distant disease-free survival was well above 60% [22]. Generally, TNBC/BLBC stage I/II patients who have been treated according to local practice guidelines at the time, have a 10-year breast cancer-specific survival of 60% or higher [26,35].

Although TNBC/BLBC appear to have a relatively poor prognosis in the first 5 years after diagnosis, a significant proportion of patients, even without adjuvant systemic therapy, do NOT relapse, and after approximately 8 years of follow-up, these patients have a high chance of being cured [22,26,35,89,90]. So, clearly, some TNBC/BLBC patients do not need adjuvant systemic therapy and useful prognostic markers are urgently needed to reduce overtreatment (see below). Currently available genomic prognostic tests (i.e. 70-gene prognostic profile [92,93] and 21-gene recurrence score [94]) do not meet this need.

Potential prognostic markers within TNBC/BLBC

In the clinic, to forego adjuvant chemotherapy in TNBC/BLBC patients, a prognostic marker needs to be associated with a 10-year breast cancer-specific survival of at least ~90–95%. Such a prognostic factor is not yet available for TNBC/BLBC. Several gene expression profiling studies, using partially overlapping datasets, have correlated an up regulated immune response with a favourable outcome in TNBC/BLBC [21,90] and in ER-negative breast cancer patients [95]. Interestingly, in one study, the presence of a moderate to extensive lymphocytic infiltrate on histology was associated with a median 5-year metastasis-free survival of ~90% [21]. When combining this with the absence of central fibrosis, a 100% 5-year metastasis-free survival was observed [21]. Although intriguing, these findings need to be confirmed and tested for inter-observer variation in an independent, preferably adjuvant systemic therapy-naïve TNBC/BLBC patient population.

Within the TNBC cancers, at least two subtypes have been described; 1) basal-like breast cancer [16]; 2) five-marker negative phenotype (negative for ER, PgR, HER2, CK5/6 and EGFR – nonbasal phenotype) [26,27,29]. Consistently, the basal-like breast cancers do worse than the other subgroup [26,27,29], even after adjuvant anthracycline-based [26,29] or cyclophosphamide-methotrexate-fluorouracil (CMF) chemotherapy [27].

Several markers have been associated with poor prognosis in TNBC/BLBC, such as P-cadherin [96], αB-crystallin [97] and stromal caveolin-1 expression [98]. None of these markers are suitable for the clinic. Rather, these markers may help to find new drug targets for TNBC/BLBC.

Response to chemotherapy of TNBC/BLBC in comparison with other breast cancer subtypes

Advantages of preoperative (neoadjuvant) systemic therapy include down-staging, increasing the breast-conserving therapy rate with ~15–20% in large operable breast cancer patients and allowing surgery in initially inoperable disease [99,100]. One of the theoretical advantages of preoperative (neoadjuvant) chemotherapy is that one can follow tumour chemosensitivity *in vivo*, thereby providing an ideal setting for translational research [101]. Higher pathological complete remission (pCR) rates have been associated with higher nuclear grade, smaller tumour size, ER-negative breast cancer, HER2-positive (irrelevant of hormone receptor status) breast cancer, and the presence of a *TP53* mutation [101–103]. A consistently higher pCR

rate has been reported for TNBC/BLBC in comparison with other subtypes, ranging from ~20–45%, with higher pCR rates for smaller tumours and regimens consisting of a combination of at least a taxane, an anthracycline and cyclophosphamide [23,34,78,104]. Among TNBC/BLBC patients treated in a single institute, black patients achieved equal pCR, 3-year recurrence-free survival (RFS) and 3-year overall survival (OS) rates as white/other patients, suggesting that the higher age-adjusted breast cancer mortality rates among African–American women compared to those of white women are at least partially due to the higher prevalence of TNBC/BLBC among African–American women [105]. pCR also appears to be an excellent surrogate marker for favourable long-term outcome in TNBC/BLBC patients corresponding to a 3-year OS of ~90–100%, while TNBC/BLBC patients with residual disease have a worse outcome than other subtypes with a 3-year OS of ~60–70%, the higher percentage being reserved for patients with smaller tumours [34,78,105]. The poor outcome after residual disease challenges the clinician to add a more rationally designed regimen after surgery in an attempt to improve survival. Several studies have been initiated and do allow the TNBC/BLBC group with residual disease to participate, e.g. the BEATRICE study (NCT00528567) randomises patients to standard (neo-)adjuvant chemotherapy with or without bevacizumab for 1 year; the NATAN study (NCT00512993) randomises patients with residual disease after neoadjuvant chemotherapy to 5 years of adjuvant zoledronic acid versus nil; the NSABP B-45 study will randomise HER2-negative breast cancer patients with residual disease after a neoadjuvant chemotherapy regimen containing at least a taxane, an anthracycline and cyclophosphamide to 51 weeks of sunitinib versus placebo.

TNBC/BLBC patients appear to derive substantial benefit in terms of improved RFS and OS after anthracycline-based chemotherapy with adjusted hazard ratios ranging between 0.35 (95% confidence interval [CI] 0.18–0.68) (nonbasal subtype) and 0.54 (95% CI 0.27–1.08) (basal subtype) when compared with a no treatment arm [29]. The addition of a taxane to an anthracycline-based adjuvant regimen leads to a further improved outcome of TNBC [106,107]. This is remarkable, as in preclinical studies relative resistance against taxanes has been demonstrated for breast cancer cells lacking functional BRCA1 [108]. Most *BRCA1*-associated breast cancers belong to the BLBC subtype and there are indications that a considerable fraction of BLBC has a dysfunctional BRCA1-pathway with a defect in DNA DSB repair [53]. There are two explanations for this apparent discrepancy of TNBC/BLBC – BRCAness – *in vitro* taxane resistance on the one hand, and clinical benefit of the addition of a taxane in TNBC/BLBC on the other hand; either the preclinical results do not translate to the clinical situation, or the nonbasal (or non-BRCAness) subgroup is exquisitely sensitive to taxanes, accounting for all of the benefit observed within the TNBC subtype. This controversy is currently being addressed in a randomised phase III trial of carboplatin versus docetaxel in first-line metastatic TNBC patients [109]. Interestingly, there are also indications that stage III TNBC/BLBC patients may selectively benefit from high dose, alkylating chemotherapy with autologous stem cell rescue [110,111]. This is especially fascinating in light of the assumed BRCA1-pathway disruption in many TNBC/BLBC and potential sensitivity to alkylating agents (high dose cyclophosphamide and thiotepa [110,111]) and cross-linking drugs, such as platinum salts [53,111].

Potential predictive markers within TNBC/BLBC

Within the TNBC subtype, using an array comparative genomic hybridisation (aCGH) classifier initially constructed to identify *BRCA1*-associated tumours [112–114] (Fig. 4), it appeared feasible to identify a group of metastatic breast cancer patients treated with platinum-based high dose chemotherapy with a high complete remission rate and long progression-free survival [115]. Moreover, this group contained all metastatic patients who were apparently cured by intensive alkylating chemotherapy ($N = 6$; continuous complete remission 56+ to 150+ months) [115]. This aCGH assay may represent an effective test for BRCAness and could be important to select patients with sporadic tumours for therapy targeted at cells deficient in DNA homologous recombination [115]. Additional studies are needed to confirm these results. Interestingly, in a follow-up study, using an expansive growth pattern ("pushing margins") as a surrogate read-out for the presence of a BRCA1-like aCGH profile, outcome was assessed of stage III HER2-negative and ER-negative or low (<25% tumour cells positive) breast cancer patients who had participated in a randomised trial of adjuvant 5 × 5-fluorouracil, epirubicin, cyclophosphamide (FEC) versus 4 × FEC followed by high-dose cyclophosphamide, thiotepa, carboplatin (CTC) with autologous stem cell rescue [116]. While RFS after 5 × FEC was similar for the 15 patients with an expansive growth pattern as compared to 38 without an expansive growth pattern (33%, and 31% 10-year RFS, respectively), 10-year

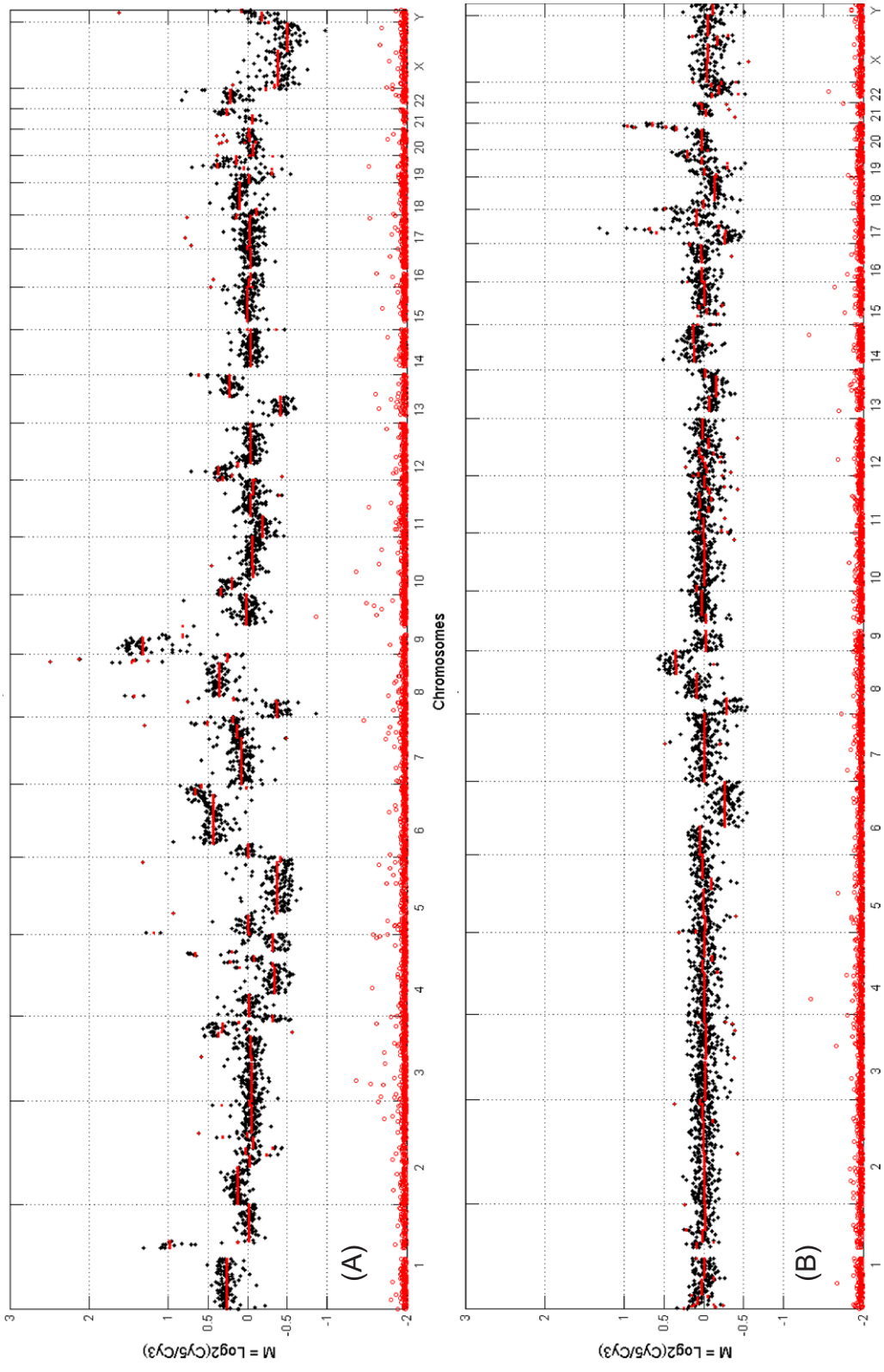

Fig. 4. Copy number aberrations over the whole genome of a 3.6K BAC Sanger platform (1 MB spacing) [114]. On the x-axis, chromosome 1 to 22 and X and Y are depicted. On the y-axis, the log2 ratio of tumour DNA versus reference pool (six healthy females) DNA is shown. A) A breast cancer of a patient with a BRCA1-like aCGH profile; note the high number of copy number aberrations (deletions and amplifications). B) A breast cancer of a patient with a sporadic-like (=non-BRCA1-like) aCGH profile; note the relatively unperturbed DNA profile.

RFS after high dose CTC was better for the 16 patients with an expansive growth pattern (81% [multivariate hazard rate (HR) of CTC versus FEC 0.11 (95% CI 0.02–0.53)]) as opposed to the 30 without an expansive growth pattern (53% [multivariate HR of CTC versus FEC 0.58 (95% CI 0.3–1.12)]); multivariate test for interaction $P = 0.056$. Using histological information, by determining the presence of an expansive growth pattern, it was possible to identify a group of stage III breast cancer patients who selectively benefited from platinum-containing, high-dose alkylating chemotherapy [117]. Whether the observed favourable outcome in the subtype with an expansive growth pattern was due to the alkylating regimen, the high dose, or the combination remains a subject of further investigation. All these observations are in line with the fact that the rates of DNA copy number aberrations [118] and loss of heterozygosity [119] are higher in TNBC/BLBC than in other subtypes, indicating a higher degree of genomic instability [120]. Of note, a consistent loss of the chromosome 5q arm has been reported for *BRCA1*-associated breast cancers [112–114,118,121], as well as for basal-like breast cancers [122,123]. Interestingly, many genes involved in DNA repair reside on the 5q11 locus, such as MSH3, RAD17, RAD50, and XRCC4 [120], questioning a possible relationship with the observed increased sensitivity to DNA damaging agents [108,124,125].

Targeted treatment strategies

Cell lines and mouse models

Potential activity of several targeted molecules and anticancer drugs in TNBC/BLBC or *BRCA1*-associated breast cancer has been suggested based on cell line and mouse model experiments (reviewed in [126]). These include anticancer drugs such as DNA interstrand cross-linking agents (platinum compounds and mitomycin-C), etoposide, bleomycin [108,124, 125] and inhibitors directed against src [127,128], checkpoint kinase-1 (chk1) [129], heat shock protein 90 (Hsp90) [130], c-KIT [120], EGFR [131], aberrations in glucose and fatty acid metabolism [132], androgen receptor [133,134], activated PI3K-pathway [57], and poly(ADP-ribose) polymerase 1 (PARP-1) [135–138]. PARP inhibitors, especially, are an exciting new class of drugs, as their efficacy is based on the principle of synthetic lethality [139,140]. Tumours with a DSB repair defect by a disruption of the BRCA1-pathway [138] are highly sensitive to blockade of the repair of DNA single-strand breaks (SSB) through the inhibition of PARP-1. When PARP-1 is inhibited, persistent DNA SSB can be turned into DNA DSBs by DNA replication fork stalling [140]. In a tumour cell with a BRCA1-pathway disruption, loss of PARP-1 function could result in the generation of replication-associated DSBs that are normally repaired by homologous recombination [140]. Of note, PARP-1 inhibition causes failure of SSB repair but hardly affects DSB repair in an experimental model using PARP-$1^{-/-}$ 3T3 fibroblasts [141]. Importantly, cells heterogeneous for *BRCA1* or *BRCA2* mutation were not sensitive to PARP-1 inhibition, which suggests that *BRCA1* or *2* mutation carriers, having only one copy of the wild-type *BRCA1* or *2* gene, would not experience increased toxicity with PARP-1 inhibitor treatment [135,136]. Following these successful preclinical studies, over 25 studies have now been initiated with PARP inhibitors in cancer patients, including breast, ovarian, colorectal, melanoma, glioma, and haematological malignancies (www.clinicaltrials.gov; www.clinicaltrials.gov/ct2/results?term=/Parp+inhibitor).

Unfortunately, two mechanisms of resistance have already been described using this synthetic lethal approach. One is caused by secondary intragenic mutations that (partly) restore BRCA1 or BRCA2 function, so-called 'genetic reversion' [142,143]. This mechanism has already been described for *FANCA* 10 years ago, where secondary mutations in the *FANCA* gene complemented the characteristic hypersensitivity of Fanconi Anaemia cells to crosslinking agents [144]. Another mechanism for PARP inhibitor resistance, as yet only described in mouse model systems, is up-regulation of Abcb1a/b genes encoding P-glycoprotein efflux pumps [137]. This resistance to PARP inhibition could be reversed by the P-glycoprotein inhibitor tariquidar [137]. These findings may have implications for clinical resistance to DNA damaging agents and/or PARP inhibition.

Phase I/II trials

First exciting results of PARP inhibition in *BRCA1*-associated [145] and TNBC patients [146] have been reported at ASCO 2009. The oral PARP inhibitor olaparib (400 mg bid continuously), administered as a single agent to 27 heavily pretreated BRCA-deficient advanced breast cancer patients, resulted in a 40% overall response rate and a median progression-free survival of 6 months. Toxicity was low, with only grade 3–4 fatigue, nausea and vomiting in less than 20% of patients. Haematological toxicity was mild, and only one patient with grade 3 anaemia was reported. A second study assessed the addition of

BSI-201, a PARP inhibitor administered intravenously (5.6 mg/kg; days 1, 4, 8, 11 q 3 weeks), to a combination of carboplatin (AUC2; days 1, 8 q 3 weeks) and gemcitabine (1000 mg/m2; days 1, 8 q 3 weeks) (CG), administered to 116 metastatic TNBC patients in a randomised phase II design (1:1) [146]. Approximately 62% of patients receiving BSI-201 in combination with CG showed clinical benefit, compared with 21% in the group receiving chemotherapy alone ($P = 0.0002$). Overall response rate was 48% for patients who received BSI-201 combined with chemotherapy, as compared with 16% for the CG only arm. Women who received BSI-201 had a median progression-free survival of 6.9 months and OS of 9.2 months compared with 3.3 and 5.7 months, respectively, for women who received chemotherapy alone. The hazard ratios for progression-free survival and OS were 0.342 ($P < 0.0001$) and 0.348 ($P = 0.0005$), respectively [146]. The most common grade 3 and 4 side effects included neutropenia (42% in BSI-201/CG arm versus 53% in CG only arm), thrombocytopenia (12% in BSI-201/CG arm versus 10% in CG only arm) and anaemia. No febrile neutropenia was observed in patients receiving BSI-201 combined with chemotherapy. BSI-201 did not add to the frequency or severity of adverse events associated with chemotherapy [146]. The next steps in the further development of PARP inhibitors include research on the best schedule of administration (continuously versus intermittent), the best route of administration (intravenous versus oral), the most efficacious dose and how to monitor this (pharmacokinetics versus pharmacodynamics), the most accurate biomarker for the presence of a DNA damage repair defect in the tumour, and the most promising combination of conventional DNA damaging cytotoxic agents with PARP inhibition.

Several other phase I/II studies are currently running, but mature results are not available yet. Most studies explore the efficacy of crosslinking agents, PARP inhibitors, angiogenesis inhibitors (bevacizumab, sunitinib), src inhibitors (dasatinib), EGFR inhibitors (erlotinib, panitumumab, cetuximab), mTOR inhibitors (everolimus; targeting an activated PI3K pathway) and Chk1 inhibitors (UCN-01; 7-hydroxystaurosporine; *in vitro* potentiating cytotoxicity of DNA damaging agents in TP53 mutant cells [126,129]), alone or in combination with conventional chemotherapy (www.clinicaltrials.gov; www.clinicaltrials.gov/ct2/results?term=triple-negative+breast+cancer).

Critical for the success of targeted drugs is to study patients with tumours having the right molecular make-up (Table 2). If no biomarker is available, then inclusion criteria should be as broad as possible. As soon as responders are identified, then molecular tumour studies can be initiated for biomarker discovery. Randomised phase II studies should follow in well-defined patient populations in order to quickly identify promising new drugs. Finally, randomised phase III trials in a well-defined population should then establish the new standard for clinical practice. Meanwhile, randomised phase II trials in the neoadjuvant setting can be started to explore the potential of the new drug in the adjuvant setting.

Conclusion

Triple-negative breast cancer, a substitute for basal-like breast cancer, comprises a heterogeneous subgroup of breast cancers that can be divided into at least two subcategories. One is characterised by the presence of a DNA damage repair defect, supposedly caused by a disruption in the BRCA1-pathway, causing genomic instability and sensitivity to DNA damaging agents. In addition, these tumours are highly sensitive to a combination of a DNA damaging agent, such as a platinum compound, and a PARP inhibitor. Potential biomarkers that may enrich for this subcategory include: I) a BRCA1-like aCGH classifier; II) basal-like IHC markers; III) an expansive growth pattern on histology; IV) a *TP53* mutation. It is very likely that this subcategory of TNBC patients will receive a completely different adjuvant systemic treatment in the near future, resulting in a markedly improved outcome, based on the strategy of synthetic lethality. The other subcategory is less well-defined and might be more sensitive to taxanes than the first subcategory. This subcategory is sometimes called the nonbasal group, or the multiple marker negative group. More molecular studies are needed to further characterise this latter subcategory. Meanwhile, phase I/II studies directed against many targets, such as an activated PI3K pathway, src, and the androgen receptor are ongoing and their results are eagerly awaited. Finally, clinically useful prognostic markers are currently lacking for TNBC/BLBC, leading to unnecessary exposure to toxic adjuvant chemotherapy of a substantial number of TNBC/BLBC patients. However, promising results have been reported for an upregulated immune response as a favourable prognostic marker, and a further characterisation of this phenotype may ultimately lead to the identification of a TNBC/BLBC subgroup for which adjuvant chemotherapy can be replaced by a milder adjuvant systemic therapy, or even no systemic therapy.

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Conflict of interest statement

None declared.

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