BreastCare

Review Article

Breast Care 2018;13:251–262
DOI: 10.1159/000492122

Published online: August 14, 2018

Neoadjuvant Model as a Platform for Research in Breast Cancer and Novel Targets under Development in this Field

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Keywords

Biomarker · Breast cancer · Chemotherapy · Endocrine therapy · Neoadjuvant · Translational research

Summary

For decades, the neoadjuvant setting has provided a useful scenario for research in breast cancer. Historically, neoadjuvant clinical trials, either hormone therapy-based or chemotherapy-based, have tried to recapitulate the results of their counterpart adjuvant studies, but with smaller patient numbers, more rapid outcomes (clinical response and/or pathologic complete response (pCR)), together with additional biologic information. As for neoadjuvant chemotherapy trials, the increase in pCR rates has been recently accepted as an appropriate surrogate marker to accelerate drug approval in high-risk breast cancer patients. In this setting, with the exception of luminal A tumors, pCR has been associated with improved long-term outcomes, particularly when the analysis is based on specific trials for each breast cancer subtype. For luminal tumors receiving neoadjuvant endocrine therapy, Ki67 at 2–4 weeks and the preoperative endocrine prognostic index score are the most accepted intermediate markers of efficacy, which will be validated in ongoing larger trials. In this review, we describe the different neoadjuvant designs: from the classical randomized trials in which treatment is delivered for 6 or more months to short non-therapeutic presurgical studies lasting just 2 or 3 weeks. We also review the main neoadjuvant trials, either ongoing or completed, for luminal, triple-negative, and HER2-positive breast cancer. The translational effort and research of biomarkers conducted in these studies will be particularly addressed.

Introduction

The neoadjuvant (NA) setting has provided a well-recognized scenario for research in breast cancer (BC) for nearly 20 years. From a historic point of view, NA clinical trials, either hormone therapy-based or chemotherapy(CT)-based have tried to recapitulate the results of their counterpart adjuvant studies, but with fewer patients, additional biologic information, and faster results since endpoints in the NA studies are evaluated in a few months (e.g., clinical response rate (cRR), conservative surgery (CS) or pathologic complete response (pCR) rates) or even in just a few days (e.g., Ki67 on day +15 from treatment initiation in endocrine trials). Main completed studies exploring new drugs or combinations including neoadjuvant chemotherapy (NACT) or endocrine therapy (ET) with/without biologic agents have been summarized elsewhere in this issue of Breast Care [1, 2].

In the last years, neoadjuvant therapy (NAT) has become an even more attractive strategy for drug development and translational research. This is the result of several factors: i) Food and Drug Administration (FDA) acceptance of NA clinical trials for new drug approval; ii) development of NA platforms such as I-SPY 2 that test multiple drugs in each specific BC subtype to speed up drug approval; iii) emergence of the 'window of opportunity' trials, which takes advantage of the usual period between diagnostic biopsy and surgery date to deliver a short course of drug/s just for translational purposes; and iv) development of specific adjuvant trials for those patients having a poor response to NAT, such as those not obtaining pCR to NACT.

Regarding the first point, it was in 2012 that the FDA published a guide for the industry that recognized pCR as an appropriate surrogate endpoint to support accelerated approval of investigational drugs. This guidance, updated in 2014, recommended that approval in the NA setting would be contingent on the demonstration of an improvement in disease-free survival (DFS) by future or

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Table 1. Drugs graduated in the I-SPY 2 trials

Drug/combination	Mechanism of action	Subtype	Patients accrued, n	Predicted probability of success in phase III, %	Date of graduation
Veliparib-carboplatin	poly-ADP ribose polymerase inhibitor	HER2–/hormone receptor(HR)–MammaPrint® high-risk	72	88	December 2013
Neratinib	tyrosine kinase inhibitor	HER2+/HR–	115	79	December 2013
MK-2206	AKT inhibitor	HER2+/HR–	93	87	May 2015
T-DM1-pertuzumab	HER2 dimerization inhibitor	HER2+	52	94	April 2016
Pertuzumab-paclitaxel-trastuzumab	HER2 dimerization inhibitor	HER2+	44	90	April 2016
Pembrolizumab-paclitaxel-doxorubicin and cyclophosphamide	PD1 inhibitor	HER2–	69	99	November 2016

concomitant studies [3]. Pertuzumab, in combination with trastuzumab in human epidermal growth factor receptor 2-positive (HER2+) disease, was the first drug approved under this new program based on the results of 2 NA studies [4, 5]. The FDA guide was developed in accordance with the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) working group. The CTNeoBC carried out a pooled meta-analysis including the main NA trials with at least 3 years of median follow-up [6]. They found that individual patients who attain a pCR have a 64% reduction in the risk of death, compared with patients with residual tumor at surgery. However, no association between the magnitude of difference in pCR rate by treatment arms and the differences in long-term survival was found. This fact was probably due to several factors, including the low rate of pCR in the trials analyzed, the population heterogeneity, and the scarcity of studies using targeted therapies. Hence, the pooled analysis could not validate pCR as an established surrogate endpoint for improved DFS and overall survival (OS); however, given that individual patients who achieve a pCR have a substantial improvement in OS, an agent that produces a marked improvement in pCR rate may be reasonably likely to ultimately improve outcomes and can be considered a candidate for FDA-accelerated approval. This program assumes the risk that post-marketing trials may fail to confirm long-term clinical benefit, but that risk is considered to be acceptable.

Also the *Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging and Molecular Analysis 2* (I-SPY 2) platform was launched in 2010 with the aim of speeding up the access to potentially effective drugs for high-risk early BC patients. The designs of the I-SPY 2 trials take into account BC subtype, thus avoiding the heterogeneity of previous NA trials. Low-risk luminal A tumors identified by MammaPrint® (Agendia, Amsterdam, The Netherlands) are excluded. Magnetic resonance imaging (MRI) of the breast, with/without other imaging techniques, is performed to evaluate cRR and correlation with histopathologic findings. For each BC subtype, the studies applied an adaptive design and a Bayesian probabilistic model based on pCR rate 'graduating' only those drugs/combinations with a high probability to succeed in a hypothetic phase III trial. By this model, several agents have been declared 'graduated' so far (table 1), such as pembrolizumab added to standard CT in triple-negative BC (TNBC) and hormone receptor-positive (HR+)/HER2-negative (HER2–) disease [7]. Interestingly, with this novel subtype approach, pCR after NACT has now proven to be a strong predictor of event-free survival (EFS) and distant recurrence-free survival (RFS), and pCR appears to be equally predictive in all tested tumor subsets [8]. With these reassuring results, the platform serves as a robust scenario in which to test predictive biomarkers both in tumor and blood samples. This article reviews the main studies using NAT as a platform to accelerate new drug approval or for translational research. Both recent completed trials and ongoing trials for each BC subtype are included.

Luminal Breast Cancer

Background

Luminal breast cancer, as defined by estrogen receptor (ER) and/or progesterone receptor (PR) expression (HR+) by immunohistochemistry (IHC), comprises up to 70% of all BC cases. Historically, neoadjuvant endocrine treatment (NET) was firstly delivered to older women with locally advanced luminal tumors unfit for CT or even surgery. Over time, it appeared that cRR and even substantial CS rates could be achieved in postmenopausal women harboring operable tumors, especially if aromatase inhibitors (AI) were used for 3–4 months instead of tamoxifen [9–11]. In those trials, rates of progressive disease during NET were about 10%, while pCR was rarely achieved.

Table 2. Main ongoing neoadjuvant endocrine trials

Study	Profile	Initial treatment	Randomization	Primary endpoint(s)
NCT019553588 ALTERNATE	phase IIb–III n = 2,820 T2–T4 Allred score 6–8	A: anastrozole B: fulvestrant C: fulvestrant + anastrozole	at 4 & 12 weeks Ki67 < 10% → ET ×24 weeks Ki67 > 10% → CT ×24 weeks after surgery PEPI 0 → ET ×5 years	modified PEPI + Ki67 at 4 & 12 weeks
NCT00265759 ACOSOG Z1031-B	phase III n = 610 Allred score 6–8	anastrozole vs. letrozole	2–4 weeks Ki67 < 10% → ET Ki67 > 10% → CT or surgery after surgery PEPI 0 → ET alone	ORR pCR for CT arm
NCT01779206 ADAPT HR+/HER2–	phase III n = 4,000 N0-1 and Oncotype DX® RS: 12–25	ET	3 weeks Ki67 < 10% → ET Ki67 > 10% → CT	RFS
NCT02592083 PREDIX-A	phase II n = 200 pre- and postmenopausal luminal A: ER > 50% and Ki67 < 20%	ET	4 weeks Ki67 decrease >20%: A: ET ×10 weeks B: ET + palbociclib ×10 weeks Ki67 decrease <20%: C: ET + palbociclib ×10 weeks	ORR at 16 weeks
NCT02603679 PREDIX-B	phase II n = 200 luminal B or A (Ki67 > 20%) and age < 40 years or N1	A: paclitaxel B: ET + palbociclib	12 weeks if not progressive disease: A → B ×12 weeks B → A ×12 weeks	ORR at 24 weeks
NCT01613560	phase II n = 404 T2–T3 ER or PR > 50%	ET	16–20 weeks PEPI 0–1: ET ×5 years PEPI 2–4: randomized to: A: ET ×5 years B: CT + ET ×5 years	RFS[a]

[a]Adjuvant trial based on PEPI score results.
CT = Chemotherapy; ET = endocrine therapy; ORR = overall response rate; pCR = pathologic complete response; RFS = recurrence-free survival; PEPI = preoperative endocrine prognostic index; RS = recurrence score.

NET with AI in luminal tumors was compared to NA anthracycline and taxane-based CT in 2 studies [12, 13]. The first was conducted exclusively in the postmenopausal setting, and the AI (anastrozole or exemestane) was administered for 3 months [13]. In the second study, postmenopausal (or premenopausal under luteinizing hormone-releasing hormone analogs) patients received exemestane for 24 weeks [12]. Both trials showed a similar cRR and pCR rate with NET and CT, although in the second trial there was a trend for worse outcome in the endocrine arm for premenopausal patients and those with high Ki67. The longer NET in the second trial was consistent with data from non-randomized series [14, 15] suggesting that longer treatment could yield increased CR and higher CS rates beyond those achieved by 3 or 4 months of therapy; therefore, 6 months is now the minimum period recommended for NET and the standard approach in current trials. In light of all the above, NET for at least 6 months appears to be a reasonable alternative to CT for postmenopausal women with stage II and III BC with luminal A characteristics, while for premenopausal women NET still remains investigational.

As mentioned earlier in this monograph, Ki67 value, measured at 2–4 weeks of treatment initiation or at surgery, and the preoperative endocrine prognostic index (PEPI) score at surgery have emerged as the most accepted surrogate markers for NET [16–18]. This is due to the fact that NET mainly induces cell cycle arrest and because of the low frequency of pCR in the luminal tumor context.

The prognostic validation of both Ki67 percentage and PEPI score have shown substantial flaws up to now, including Ki67 reproducibility issues, impracticality (biopsy at day +15 is rarely performed in the clinical setting), and, above all, a limited number of patients on whom data is based, in contrast to the overwhelming evidence for pCR and CT. Focusing on Ki67, recent data from the POETIC trial validates the prognostic role of both basal and Ki67 at day +15 [19], and, luckily, large trials are ongoing that will provide further validation. In the meantime, a Ki67 score > 10% after 2

Table 3. Clinical response rate by Oncotype DX® breast cancer recurrence score (RS) in the TransNEOS trial [29]

Response	RS < 18, n	RS 18–30, n	RS ≥ 31, n	Total, n
CR + PR	85	35	12	132
SD	70	46	33	149
PD	1	4	9	13
Total	*156*	*84*	*54*	*294*

CR = Complete response; PR = partial response; PD = progressive disease.

or 4 weeks of ET has been suggested as a cutoff for the early identification of non-responders who are therefore suitable for other strategies including investigational agents and/or CT. As for the PEPI score which was developed as a prognostic tool to guide indication for adjuvant CT, its definitive validation must also await the results of ongoing phase II–III clinical trials (table 2).

The third-generation AIs (letrozole, anastrozole, exemestane) are equally effective compared to NET, as shown by the ACOSOG Z1031 trial (Cohort A) [20]. In this study, similar efficacy in terms of cRR, CS rate, Ki67 percentage decrease at day +15, and incidence of PEPI score equal to 0 was demonstrated for the 3 compounds. However, based on a numerically inferior cRR for exemestane, only letrozole and anastrozole are used in the ongoing second part of the trial (Cohort B).

Interestingly, genomic signatures commonly used to evaluate prognosis and/or benefit of adding CT for luminal tumors in the adjuvant setting have been recently investigated as predictive factors for NAT. MammaPrint, for example, has been used in the I-SPY 1 and 2 trials to identify low-risk luminal tumors not eligible for investigational strategies that include CT or ET with new biologic agents [21, 22]. Oncotype DX® (Genomic Health, Redwood City, CA, USA) has been tested in the NA setting, both with ET and with CT, in several studies [23–28]. Overall, these studies show that patients with a low recurrence score (RS) experience higher cRR with ET, while patients with high RS achieve higher cRR and pCR rates with CT. Probably the largest series yet to address the role of RS in NET is the TransNEOS trial [29] which included postmenopausal patients with ER+/HER2– primary tumors, clinically N0, that received NET for 6 months. In a recent communication from this study, including 291 women, RS was an independent variable associated with cRR (table 3). Furthermore, an increase in CS rate after NET was mainly seen in patients with low RS (from 62% before ET to 79% for RS < 18; and from 63% to 60% for RS > 31). The authors concluded that Oncotype DX RS was a valid biomarker to predict cRR to NET.

More recently, a combined 4-gene signature able to predict cRR to NET with an accuracy of 96% has been described. The study was conducted by Turnbull et al. [30] who analyzed pre- and on-treatment biopsies from 89 postmenopausal women receiving NA letrozole. This signature was based on the pre-treatment level of 2 genes (*IL6ST*, associated with immune signaling, and *NGFRAP1*, related to apoptosis) and the on-treatment (2-week) level of 2 proliferation genes (*ASPM* and *MCM4*). Together with its association to cRR, the 4-gene expression correlated significantly with DFS (p = 0.029) and BC-specific survival (p = 0.009). Of note, the authors could validate the signature in an independent data set (accuracy of 91%) and demonstrated that it can even be performed using IHC, which greatly facilitates its further implementation. In spite of these promising results, it must be remarked that the development of this biomarker is based on a small retrospective study, and its role should be prospectively confirmed in larger cohorts.

Neoadjuvant Endocrine Therapy as a Platform for New Therapies

NET not only offers clinical benefit for selected patients with early HR+ BC; it also provides an excellent platform for drug development, triage of novel combinations, biomarker validation, and discovery of mechanisms of drug resistance. This section reviews the different designs of NET trials and the main groups of drugs currently tested.

NET Trial Designs

Based on design, we can differentiate several types of NET trials.

Firstly, in *classic* NET trials, such as IMPACT or P024, patients receive the treatment for 3–6 months before surgery. A biopsy for research purposes is incorporated at 2–3 weeks to assess drug-induced cellular activity and/or pharmacodynamic biomarkers, but there is no therapy modification at this time. The NA trial RADFEMARA that compared letrozole plus everolimus/placebo constitutes an example of this kind of design [31] and one of the first studies to use the complete cell cycle arrest rate (CCC = Ki67 < 2.7%) as a biologic endpoint [31].

Another study type, as mentioned earlier, has an *enrichment adaptive* design, where all patients receive ET for 2–3 weeks, and those whose tumors do not show Ki67 suppression below a pre-established threshold are switched to alternative therapies, e.g., CT or addition of a new drug; otherwise, patients continue on ET alone. This design enriches for drug-'resistant' tumors, where additional benefit from a new drug can be more easily identified. The ongoing ALTERNATE, ACOSOG Z1031-B, and ADAPT HR+/HER2– trials (table 1) illustrate this second strategy.

A third type of NET trial has a *multi-arm lead-in phase II* design. This kind of study, such as LORELEI [32] or PALLET (NCT02296801), compares head-to-head ET ± investigational drug using the on-treatment Ki67 value at 2 weeks as endpoint. Afterwards, all patients receive ET plus the investigational drug for 4–6 months before surgery.

Another design for NET trials is that of *a single-arm* where *multiple biopsies* are obtained. The NeoMONARCH study is one example [33]. In this trial, patients received anastrozole for 28 days, at which time a biopsy was performed and the CDK4/6 inhibitor palbociclib was added. After 2 additional weeks of the combination, a second biopsy was obtained, and those patients with a Ki67

> 10% were taken off the study, whereas those with a Ki67 < 10% continued treatment with the combination until surgery. This design evaluated the effect of CDK4/6 inhibition in patients in whom the AI did not optimally suppress tumor cell proliferation, with each tumor serving as its own control.

Finally, an additional design increasingly used at present is that of the '*window of opportunity*'. In these non-therapeutic trials, patients are treated for 2–3 weeks immediately after their diagnostic biopsy and before breast surgery. Pharmacodynamic and pharmacokinetic biomarkers are analyzed to provide additional knowledge of molecular mechanisms of action of a new agent or combination, to confirm the molecular efficacy of the drug dose chosen, or to test the performance of candidate predictive biomarkers. The Queen Mary trial, which is testing enzalutamide alone or in combination with exemestane (NCT02676986), is an example of this type of short presurgical trial.

Targeting CDK4/6 Inhibitors in NET Trials

The addition of CDK inhibitors (palbociclib, ribociclib, abemaciclib) to ET therapy has shown to improve progression-free survival (PFS) significantly in ER+/HER2– metastatic patients, compared to ET alone, with a favorable toxicity profile. Currently, several randomized clinical trials have been initiated to assess the role of CDK inhibitors in the adjuvant setting for luminal HER2– patients with higher risk or relapse, but their results will not be available for several years. In contrast, the NA setting provides an opportunity to rapidly assess their clinical and biologic effect in patients with tumors >2 cm.

Two trials testing NA palbociclib have so far reported results. The NeoPalAna [34] was an NA phase II trial that involved 50 stage II/III patients. All were initially treated with anastrozole alone for 4 weeks, followed by the addition of palbociclib for 4 cycles (3 weeks on/1 week off), followed by breast surgery. Ki67 score, gene expression, and mutational profiles were performed in biopsies taken on cycle 1 day 1 (C1D1), C1D15, and on the day of surgery. The CCC arrest rate (primary endpoint of the study) was significantly higher at C1D15 with palbociclib plus anastrozole than at C1D1 with anastrozole alone (87 vs. 26%). Notably, the patients who stopped palbociclib 4 weeks before surgery experienced a rebound in Ki67 level, which was abrogated in those continuing the drug until surgery. The impact of adding palbociclib was similar regardless of luminal A or B subtype or PIK3CA status. Non-luminal subtypes were associated with palbociclib resistance. The second study, named NeoPAL [35], compared palbociclib + letrozole with CT (5-fluorouracil, epirubicin, and cyclophosphamide (FEC) followed by docetaxel). Patients were required to have PAM50-determined luminal B subtype or luminal A disease with lymph node involvement. The primary endpoint was locally assessed residual cancer burden (RCB). At final analysis, the RCB was not different between the 2 treatment arms.

A third NET trial with palbociclib, the phase II PALLET study, is still ongoing. This study evaluates palbociclib and/or letrozole as NET. Patients (n = 306) are being randomized in a 3:2:2:2 design to 1 of 4 treatment arms: i) letrozole alone; ii) letrozole for 2 weeks followed by palbociclib plus letrozole for 12 weeks; iii) palbociclib for 2 weeks followed by palbociclib plus letrozole for 12 weeks; and iv) letrozole plus palbociclib for 14 weeks. Patients receive letrozole until surgery, planned 15–18 weeks after randomization. The study has 2 co-primary endpoints: change in Ki67 from baseline to week 1 and cRR.

Ribociclib has also been evaluated in the NA setting. The ongoing trial CORALLEEN (NCT03248427) compares ribociclib + letrozole versus a standard anthracycline- and taxane-based CT in a design similar to that of the NeoPAL trial. To be eligible, patients must harbor luminal B tumors defined by PAM50. The primary objective is to explore the clinical benefit of ribociclib + letrozole versus CT. The phase II FELINE study (NCT02712723) is also ongoing. In this trial, patients are being randomized 1:1:1 to letrozole 2.5 mg daily + placebo, letrozole 2.5 mg daily + ribociclib 600 mg daily on days 1–21 of a 28-day cycle (intermittent dosing) or letrozole 2.5 mg daily + ribociclib 400 mg daily (continuous dosing). Treatment is delivered for 6 months before surgery. The primary endpoint is the rate of PEPI score equal to 0 with ribociclib + letrozole versus letrozol alone. A third study, the MONALEESA-1 (NCT01919229), has a window-of-opportunity design and aims to assess the biological activity of ribociclib + letrozole versus single-agent letrozole in primary BC, with CCC as the primary outcome.

As for abemaciclib, we have previously mentioned the phase II trial NeoMONARCH [33]. In this study, 173 women were randomized to receive abemaciclib plus anastrozole, abemaciclib in monotherapy, or anastrozole in monotherapy for the first 2 weeks. At that time, all patients underwent a second biopsy and subsequently received the abemaciclib/anastrozole combination for 14 weeks. The rate of Ki67 responders (those achieving CCC at week 2) was higher with the combination (69.9%) and with abemaciclib in monotherapy (68.4%) than with anastrozole alone (22.7%). cRR by caliper, radiologic response, and pCR was seen in 53.6, 46, and 3.7%, respectively. As a translational component of this study, the authors examined the tumor microenvironment. With abemaciclib, enhanced tumor differentiation together with an increase in total CD3+ T cells and CD8+ cytotoxic T cells were observed, without upregulation of immunosuppressive T-regulatory cells. These immune changes constitute a good rationale for investigating abemaciclib in combination with immunotherapies.

Targeting the PI3K/AKT/mTOR Pathway in NET Trials

The PI3K/AKT/mTOR pathway has been shown to be the most frequently altered signaling pathway in BC. Robust data have shown significant crosstalk between the ER and PI3K/AKT/mTOR pathways, leading to endocrine resistance.

The first study in this context was the RAD-FEMARA trial [31]. In this study, letrozole was given for 16 weeks in combination with placebo/everolimus 10 mg/day. cRR by palpation and CCC at 2

weeks were significantly superior in the letrozole-everolimus arm. The safety profile was consistent with previous results with everolimus in monotherapy.

Regarding PIK3CA inhibitors, the phase II clinical trial LORELEI [32] randomized postmenopausal women with stage I–III BC and evaluable tissue for *PIK3CA* genotyping to receive either taselisib (an α-isoform PIK3CA inhibitor) plus letrozole or placebo and letrozole for 16 weeks prior to surgery. The co-primary endpoints of the study included ORR by MRI and pCR rate. The addition of taselisib to letrozole improved cRR in both the *PIK3CA*-mutant and the general patient population, while pCR rates were not significantly different between the 2 treatment arms in any patient subgroup. Grade 3–4 toxicities were infrequent in the taselisib arm and included gastrointestinal disorders (7.8%), infections (4.8%), skin/subcutaneous tissue disorders (4.8%), and hyperglycemia (1.2%). These findings, together with the upcoming results of the SANDPIPER phase III study (fulvestrant ± taselisib, NCT02340221) in the metastatic setting will help to define the further development of taselisib. Another reported trial in the NA context is the phase II single-arm trial with MK-2206, a pan-AKT inhibitor [36]. Potential eligible patients with clinical stage II/III were preregistered and received anastrozole (plus goserelin if premenopausal) for 28 days in cycle 0, pending tumor *PIK3CA* analysis. *PIK3CA*-mutant patients started MK-2206 (150 mg orally weekly, with prophylactic prednisone) on C1D2 and received up to 4 28-day cycles of combined therapy before surgery. Serial biopsies were collected (baseline, C1D1, and C1D17). 16 of 22 *PIK3CA*-mutant patients received the study drug. 3 patients were withdrawn from the study due to C1D17 Ki67 > 10% (n = 2) and toxicity (n = 1); 13 patients completed NAT and surgery. No pCRs were observed. Rash was common. MK-2206 did not further suppress cell proliferation and did not induce apoptosis on C1D17 biopsies and PRAS40 phosphorylation at C1D17 after MK-2206 persisted. In their conclusion, the authors discourage further studies in this target population.

Immunotherapies in NET Trials

Overall, luminal BC is not considered an appropriate setting for immunotherapies. In the metastatic setting, treatment with anti-programmed cell death protein ligand 1 (PD-L1) drugs such as pembrolizumab or avelumab has shown poor efficacy [37, 38]. The ULTIMATE study is trying to challenge these concepts (NCT02997995); the study includes 2 therapeutic sequences: in the first part, patients receive a single infusion of tremelimumab as an immune-attractant plus exemestane; and in the second part, CD8+ patients (tumor CD8 infiltration > 10%) receive 6 months of durvalumab, another anti-PD-L1 agent, also with exemestane. The primary objective is pCR. Finally, an ongoing trial is studying the combination of pembrolizumab with paclitaxel in both TNBC and HR+/HER2– BC (NCT01042379).

Triple-Negative Breast Cancer

Background

Nowadays, TNBC constitutes the poorest prognostic BC subtype. The term TNBC actually includes several tumor subsets with different biology and sensitivity to therapies [39]. Systemic CT based on anthracycline and taxanes (mostly delivered sequentially) is the cornerstone treatment. The addition of carboplatin to increase pCR rate has been proposed (mainly in *BRCA1/2* mutant carriers, see later), but is not yet recommended as a standard of care [40]. In a recent trial, nab-paclitaxel demonstrated superiority to conventional paclitaxel in all BC subtypes [41, 42], but is not actually approved by regulatory agencies. The aim of NAT in TNBC is to test tumor drug sensitivity and, above all, to achieve a pCR, which represents an optimal surrogate for survival outcome [43].

Preclinical data have identified 6 different molecular subtypes by gene expression profiling: 2 basal-like (BL1 and BL2) characterized by a high frequency of chromosomal rearrangements, *BRCA1* or *BRCA2* mutations, and genomic instability; mesenchymal (M), mesenchymal stem-like (MSL), immunomodulatory (IM), and luminal androgen receptor (LAR). This classification seems to have predictive and prognostic value, with different sensitivity to targeted therapies and a significant difference in RFS [44].

The triple-negative basal-like subtypes are frequently represented in tumors arising in *BRCA1* mutation carriers or with gene expression profiles similar to *BRCA1*-deficient tumors that result in an inefficient repair mechanism. BRCA status is considered a predictive factor of response to CT, principally to DNA double-strand break agents, and poly (ADP-ribose) polymerase (PARP) inhibitory agents [45].

In the last decade, the immune system has been under investigation as a possible target in cancer. The published data relating to tumor-infiltrating lymphocytes (TILs) in BC have been based on NA and adjuvant trials. TNBC have the highest tumor TIL expression compared to other BC subtypes, mainly due to the IM subtype which is characterized by activation of immune pathways, antigen presentation, and high presence of immune cells [44, 46]. Clinical data have suggested a predictive role of TILs as a strong marker for response in the NA scenario, mainly with platinum regimens [47, 48], and the presence of TILs in residual tumor disease after NACT seems to be related to more favorable long-term outcomes [49]. There are 2 different functional subsets of TILs: cytotoxic CD8+ T cells which lead to cancer cell death via linking foreign antigens on tumor cells, and FOXP3+ TILs that have a critical role in suppressing antitumor immunity. Retrospective data showed that patients with high CD8+ TILs had smaller residual tumors (≤2 cm) than patients with low TILs ($p = 0.005$) after NAT, and that both increased CD8+ levels and a higher CD8/FOXP3 ratio were associated with improved RFS and BC-specific survival ($p < 0.0001$) [50]. Regarding PD-L1, its expression has been reported in 50% of all BC subtypes, mainly associated with HR negativity and high histologic grade [51, 52]; however, it constitutes a dynamic marker not yet ready to select BC patients for immunotherapies [53].

Neoadjuvant Therapy as a Platform for New Therapies in TNBC

Antiandrogens

The LAR tumors are triple-negative tumors characterized by high androgen receptor (AR) expression, and are associated with low histologic grade, postmenopausal status, and better prognosis [54, 55]. Several reports have noted an overlap between AR positivity and apocrine histologic features or apocrine gene expression signature in TNBC [56]. The AR is expressed in 12–36% of all TNBC and could be considered a predictive biomarker for response to antiandrogen therapies in the metastatic setting [57, 58]. Based on this data, a NA phase II study (NTC02689427) is being conducted in AR-positive TNBCs treated with enzalutamide plus paclitaxel.

PARP Inhibitors

Some recent/ongoing studies test PARP inhibitors in the NA setting. One of them is a single-arm phase II study of gemcitabine combined with carboplatin and iniparib. This study showed a higher cRR in the presence of homologous recombination deficiency regardless of *BRCA1/2* mutational status [59]. Another study was carried out in the context of the I-SPY platform. In this trial, the veliparib and carboplatin arm achieved a 51% pCR compared to 26% with the standard regimen, but at the expense of increased hematologic toxicity [60]. In contrast, no difference in pCR rate was found with the addition of velaparib to NACT in a phase III trial recently presented by the German Breast Group [61]. Finally, there is a phase II study of NA talazoparib monotherapy in *BRCA*-related BC ongoing at the MD Anderson Cancer Center (NCT02282345). Taking into account all this controversial data, PARP inhibitors in this setting remain investigational.

Anti-Angiogenic Agents

Focusing on anti-angiogenic agents, different studies of combinations with bevacizumab have been conducted in the early-stage BC setting, showing overall an increased cRR in TNBC patients (GeparQuinto, ARTemis, CALGB 40603/Alliance, Ca.Pa.Be, and KCSG BR-0905). The pCR rate achieved ranged from 40 to 59% and was associated with an increase in postoperative complications, neutropenia, and hypertension. Therefore, the use of bevacizumab in this context is still controversial and not recommended.

PI3K/AKT/mTOR-Targeting Agents

Agents targeting the PI3K/AKT/mTOR pathway have been explored in TNBC both in advanced disease and in the NA setting. The preliminary results of a phase II study with the AKT inhibitor ipatasertib (FAIRLANE trial, NCT02301988) were presented at the 2018 American Association for Cancer Research (AACR) meeting [62]. This trial was a hypothesis-generating study to provide information about ipatasertib in combination with weekly paclitaxel as neoadjuvant therapy for patients with early TNBC. The addition of this agent showed a numerical difference in pCR rate favoring the combination treatment, with a higher anti-tumor effect observed in biomarker-selected patients with alterations in the PIKCA3/AKT1/PTEN pathway. The safety profile was consistent with previous experience of the combination in the metastatic setting, with gastrointestinal disorders (particularly diarrhea), asthenia/fatigue, peripheral neuropathy, and mucosal inflammation reported as the more common adverse events.

Immunotherapy

Cytotoxic drugs are able to modify the tumor microenvironment by inducing dendritic cell activation, enhancing specific cytotoxic T-cell populations, and favoring cross-presentation of new peptide antigens. These facts reinforce the idea of a potential synergic combination of CT and immunotherapy. At the 2017 American Society of Clinical Oncology (ASCO) meeting, 2 NAT studies testing this hypothesis were reported. As mentioned earlier, the I-SPY 2 tested pembrolizumab plus paclitaxel followed by anthracyclines in high-risk BC patients and showed an absolute increase in pCR rate in the TNBC subgroup [7]. The second study was a phase I trial that evaluated pembrolizumab with NACT for locally advanced TNBC (KEYNOTE-173) [63]. The addition of pembrolizumab correlated with an increased pCR rate that was higher in the carboplatin cohort (90 vs. 60%) and was associated with a manageable toxicity profile.

As for the introduction of PD1/PDL1 agents as NAT in TNBC, 2 different strategies are currently being evaluated: The first is the addition of PD1/PD-L1 inhibitors to different NACT. This is the case in the KEYNOTE-522 trial (NCT03036488), a phase III study in which pembrolizumab or placebo are added to NACT with carboplatin-paclitaxel followed by an AC regimen (doxorubicin/cyclophosphamide). Other examples of this strategy are the phase III NeoTRIPaPDL1 trial (NCT02620280) and the GeparNuevo trial (NCT02685059). All of these studies include an important translational research to increase the knowledge about potential predictive biomarkers and the tumor microenvironment. The second strategy is the administration of checkpoint inhibitors in the adjuvant setting in patients with residual BC disease after NAT. 2 ongoing trials randomize patients in this situation to receive 1 year of adjuvant pembrolizumab or avelumab versus observation (NCT02954874, NCT02926196) (table 4).

Despite the fact that upcoming results from all these studies are eagerly awaited, anthracycline-taxane-based CT remains the standard of care for NAT in all TNBC subtypes. As a result of the heterogeneity of this disease, it seems necessary to design biology-driven clinical trials wherein patients may be treated on the basis of their particular tumor molecular profile.

HER2-Positive Breast Cancer

Background

About 15–20% of all BC are considered HER2+ because of the overexpression of this receptor, and about 50% of these will also have HR expression [64]. The HER2+ subtype has an aggressive behavior that makes NAT an appealing therapeutic strategy to con-

Table 4. Main ongoing neoadjuvant trials in triple-negative breast cancer

Trial	Phase	n	Schedule	Endpoint
KEYNOTE-522	III	855	*neoadjuvant phase* arm 1: pembrolizumab q3W + CT[a] arm 2: placebo q3W+ CT[a] *adjuvant phase* arm 1: pembrolizumab ×9 C arm 2: placebo ×9 C	pCR EFS
NeoTRIPaPDL1	III	272	*neoadjuvant phase* control arm: carboplatin + nab-paclitaxel[b] experimental arm: carboplatin+nab-paclitaxel[b] + atezolizumab 1,200 mg q3W ×8 C *adjuvant phase:* AC/EC/FEC ×4 C	EFS
GeparNUEVO	II	174	arm 1: durvalumab 0.75 g → durvalumab 1.5 g q4w + CT[c] arm 2: placebo → placebo + CT[c]	pCR
NCT02954874	III	1,000	no intervention: observation experimental: pembrolizumab 200 mg iv days 1 and 22 & q42 days for 52 weeks	IDFS
NCT02926196	III	335	no intervention: observation experimental: avelumab 10 mg/kg iv q2w for 52 weeks	DFS

[a]CT: paclitaxel weekly + carboplatin q3w or weekly × 4 cycles followed by doxorubicin or epirubicin + cyclophosphamide (AC or EC) q3w × 4 cycles.
[b]Carboplatin area under the curve 2 on days 1 and 8 q3w, nab-paclitaxel 125 mg/m^2 on days 1 and 8 q3w.
[c]CT: nab-paclitaxel 125 mg weekly × 12 cycles → EC q2w × 4 cycles.
CT = Chemotherapy; pCR = pathologic complete response; EFS = event-free survival; IDFS = invasive disease-free survival; DFS = disease-free survival; q3/2w = every 2/3 weeks; iv = intravenous; C = cycles; FEC = 5-fluorouracil, epirubicin, cyclophosphamide.

sider, especially in tumors > 2 cm and/or axillary positive lymph nodes. Since the development of anti-HER2-targeted therapies, the prognosis and survival of HER2+ patients has substantially improved. Trastuzumab + CT has improved pCR rates compared to CT alone [65, 66]. Other anti-HER2 therapies, such as the tyrosine kinase inhibitor lapatinib or the antibody pertuzumab, in association with trastuzumab, increased pCR rates even more than trastuzumab alone when combined with CT [67, 68]. In this setting, double blockade with trastuzumab and pertuzumab combined with CT is considered the standard approach regardless of HR status [4, 5].

NAT as a Platform for New Therapies in HER2+ Disease

NACT Combinations with Trastuzumab and Pertuzumab

Different taxane-based CT schedules including or not including anthracyclines, or comparing dose-dense versus triweekly regimens in combination with trastuzumab and pertuzumab are being tested to determine the best therapeutic strategy. BERENICE is a 2-cohort non-randomized trial in which patients in cohort A received 4 cycles of dose-dense AC followed by 12 weekly doses of standard paclitaxel together with 4 trastuzumab and pertuzumab cycles. In contrast, treatment in Cohort B consisted of 4 FEC cycles, followed by 4 cycles of docetaxel, trastuzumab, and pertuzumab. The primary endpoint was cardiac safety during NAT, which was consistent with prior studies and similar in both arms. pCR rates were 61.8% in cohort A and 60.7% in cohort B. Of note, the highest pCR rates were found in the HER2-enriched PAM50 subtype (75 and 73.7%, respectively) [69]. Another trial, the TRAIN-2 study, compared 6 cycles of weekly paclitaxel, trastuzumab, carboplatin (PTC) plus pertuzumab preceded either by 3 cycles of FEC, trastuzumab plus pertuzumab or by 3 cycles of PTC plus pertuzumab. The pCR rate did not differ between arms (arm A 68% vs. arm B 67%; p = 0.75), whereas there was more left ventricular ejection fraction decline in the anthracycline-containing arm (18 vs. 29%; p = 0.007). However, symptomatic left ventricular systolic dysfunction was rare (<1%) in both treatment groups [70, 71].

GeparOcto investigated 2 different anthracycline and taxane regimens in combination with dual blockade; in this trial, sequential treatment with high-dose epirubicin, taxane, and cyclophosphamide (EPC) was compared to weekly treatment with paclitaxel and non-pegylated liposomal doxorubicin (PM(Cb)), with the dual blockade administered from the beginning of NAT in both arms. No significant differences in pCR were found between EPC (62%) and PM(Cb) (57.4%) in the HER2+ population [72].

Finally, and based on the recognized crosstalk between the HER2 and ER pathways, the NSABP-52 trial is testing whether the addition of estrogen deprivation to docetaxel, carboplatin, trastuzumab, and pertuzumab (TCHP) might significantly increase the pCR rate in pre- and postmenopausal patients with luminal HER2+ tumors. The pCR for the TCHP alone arm and for the TCHP plus estrogen deprivation arm were 40.9 and 46.1%, respectively (p = 0.36). Estrogen deprivation combined with CT was unable to significantly increase pCR but was not antagonistic [73].

NAT with Antibody-Drug Conjugates

The KRISTINE trial addresses the role of antibody-drug conjugates (ADCs) such as trastuzumab-emtansine (T-DM1) in the NA setting. This is based on the proven activity of T-DM1 in metastatic patients, but with a better toxicity profile compared to conventional CT plus dual blockade [74–76]. The KRISTINE study randomized patients to T-DM1 plus pertuzumab (n = 223) versus TCHP (n = 221). A pCR rate of 44.4 versus 55.7% was found (p = 0.016). Taking into account the lower pCR associated with the experimental arm, the approach of TDM1 with pertuzumab, although less toxic, is not preferred to CT plus double blockade [77].

Another related study is the phase III trial KATHERINE which evaluates the efficacy and safety of T-DM1 versus trastuzumab as adjuvant therapy for HER2+ patients who have residual tumor in the breast or axillary lymph nodes following preoperative therapy (NCT01772472).

De-Escalating Chemotherapy in the Presence of Dual Anti-HER2 Blockade and Use of Positron Emission Tomography-Based Strategies to Guide Treatment or Predict Treatment Response

Other investigational approaches are considering that HER2+ tumors might achieve pCR when treated with anti-HER2 therapies even in the absence of CT. In the NeoSphere trial, there was an exclusively biologic treatment arm with pertuzumab and trastuzumab for 4 cycles. Interestingly, in this arm, 16.8% of patients achieved pCR [4]. Hence, a subset of HER2+ BC tumors are indeed addicted to HER2 itself for the maintenance of their malignant phenotype [78]. It is of key importance to be able to identify such tumors in order to properly select those patients who might be treated with anti-HER2 therapies exclusively, therefore avoiding the toxicity related to CT [79]. In this context, the mentioned crosstalk between the HER2 and ER pathways that may favor acquired resistance to anti-HER2 treatment must be particularly considered [80, 81]. The PAMELA and TBRC006 trials combined double anti-HER2 blockade with trastuzumab and lapatinib plus ET therapy in the case of HR+ patients. High pCR rates in the breast of 27 and 30%, respectively, were achieved without CT [82, 83]. Similarly to data reported from the BERENICE trial combining trastuzumab and pertuzumab, PAMELA demonstrates that HER2-enriched patients according to PAM50 are more likely to achieve a pCR (41%) with double blockade in the absence of CT than other HER2+ intrinsic molecular subtypes (10%).

Positron emission tomography (PET) imaging adaptive strategies are also under investigation to identify HER2+ patients who might be safely spared from CT. The PHERGain trial compares an experimental trastuzumab-pertuzumab non-CT arm, plus ET for HR+ patients, versus the non-anthracycline conventional arm TCHP. A baseline F-18 fluorodeoxyglucose(FDG)-PET computed tomography scan, and another after 2 cycles of NAT, are performed in all patients. If there is a significant reduction in FDG uptake, patients in the experimental arm continue on the same treatment for a total of 8 cycles before surgery; otherwise, for PET 'non-responders', CT plus double blockade is immediately initiated for 6 cycles before surgery. Moreover, PET responders that do not achieve pCR are treated with adjuvant CT and double blockade. All patients continue adjuvant trastuzumab and pertuzumab for a total of 18 cycles (NCT03161353). An additional trial (NCT02827877) is investigating the role of Cu64-DOTA-trastuzumab-PET to predict response to treatment with trastuzumab and pertuzumab before surgery.

Other Investigational Strategies in the NA Setting: Neratinib and CDK4/6 Inhibitors

Neratinib added to weekly paclitaxel followed by AC was tested in the I-SPY 2 trial compared with the same CT backbone and trastuzumab. In the HER2+/HR– population, an estimated pCR rate of 56% for neratinib versus 33% for trastuzumab was found, which predicted a 79% probability of success in a phase III trial [84]. Another 3-arm randomized trial, NSABP FB-7, explored the combination of neratinib and trastuzumab with paclitaxel, which resulted in a higher pCR rate (50%) compared with the single blockade (trastuzumab or neratinib) with paclitaxel (38.1 and 33.3%, respectively) [85].

The pan-PIK3CA inhibitor buparlisib or placebo was added to taxane and trastuzumab-based therapy in the NeoPHOEBE trial. Recruitment was halted early due to liver toxicity in the buparlisib group. Both arms showed similar pCR rates. Of note, the ER+ subgroup receiving buparlisib experienced a significant decrease in Ki67 (75 vs. 26.7%; p = 0.021) and tended to have higher cRR (68.8 vs. 33.3%; p = 0.053) [86].

There is preclinical evidence of the synergism of anti-HER2 therapies and CDK4/6 inhibitors in luminal HER2+ BC [87]. NAPHER2 is an exploratory phase II NAT that has investigated the activity of trastuzumab, pertuzumab, fulvestrant, and palbociclib in HER2+/HR+ BC patients. This CT-free combination showed significant reduction in Ki67 expression at 2 weeks and at surgery compared to baseline. Remarkably, 97% of patients obtained a clinical objective response with a 27% pCR in the breast and axillary nodes [88].

Biomarkers

An enormous translational effort in identifying predictive biomarkers of response or resistance to HER2-directed therapies beyond HER2 itself has been made in previous studies and constitutes an important component of most ongoing clinical trials. *PIK3CA* mutations were analyzed in individual patient data from 5 NA trials including 967 patients with HER2+ tumors. Among them, *PICK3CA* mutations were found in 21.7% and were associated with a significantly lower rate of pCR (29.6 vs. 16.2%) that did not translate into an inferior DFS [89]. PTEN negatively regulates PIK3CA and its loss might be found in 20–25% of HER2+ tumors. In the GeparQuattro trial, PTEN-high levels were significantly associated with increased pCR compared to PTEN-low levels, whereas this correlation was not found in the NeoALTTO study [90, 91]. Growing evidence regarding the crucial role of the immune system for the outcome of HER+ BC has been seen in both the preclinical and

clinical setting [92]. TILs have been analyzed in a meta-analysis of 6 NA clinical trials including 1,369 patients with HER2+ disease. A significant difference in terms of pCR, DFS, and OS favoring increased TILS was reported [93]. However, this association was not seen in the adjuvant setting in the N9831 trial. Long-term data showed that the presence of stromal TILs correlates with an improvement in RFS in patients treated with CT alone but not among patients treated with CT plus trastuzumab [94]. Therefore, further research is needed to clarify the role of TILS in this setting. The biomarker panel of the TRYPHAENA trial included *HER2*, *HER3*, *EGFR*, and *PTEN* PCR-based mutational analyses. No additional biomarker correlated to pCR other than HER2 expression status [95]. Results from the NeoSphere study did not show biomarkers predictive of pCR across all groups; however, significant associations were observed for 2 markers in certain subsets: HER2 protein levels correlated with sensitivity to pertuzumab, while *PIK3CA* exon 9 mutation was associated with a lack of sensitivity to HER2-targeted monoclonal antibody treatment. In contrast to previous reports, truncated forms of HER2 could not be correlated with resistance to HER2-targeted therapy [96].

Conclusion

In BC, the NA setting has long constituted an excellent scenario for testing the efficacy of new treatments as clinical and biological information is obtained in a faster way and with fewer patients compared to adjuvant trials.

In the last decade, regulatory agencies have accepted pCR as a valid endpoint in the NA setting to accelerate drug approval for high-risk BC patients, provided that the positive results in terms of DFS/OS are ultimately confirmed. pCR has been associated with EFS/OS when specific NACT for each subtype (except for luminal A) is conducted, such as in the trials of the I-SPY 2 platform. The Bayesian probabilistic approach in the I-SPY 2 trials allows disregarding drugs with a low probability of success in a putative phase III study.

Several designs in the NA setting have been described, from the classic NAT randomized trials with treatments lasting 3–6 months to short presurgical trials of 2–3 weeks. The spectrum also includes trials in which a modification of treatment is planned after obtaining samples for biological information, and others in which treatment is precisely modified based on the clinical (e.g. PET imaging) or biological (Ki67 percentage) information obtained.

In luminal BC, where pCR are anecdotic, Ki67 percentage (basal and at 2–4 weeks) and PEPI score emerge as the most accepted surrogate markers for NET benefit, and they will be validated in larger ongoing trials. Genomic signatures to predict clinical benefit of NET (and also NACT) are now intensively studied, with some promising results. In TNBC, NA studies are aimed at increasing pCR rates, and the new anti-PD1/PD-L1 agents appear to be the most interesting group of drugs to be tested in this setting. Regarding HER2+ disease, new strategies aim to either add drugs to a CT + trastuzumab/pertuzumab backbone or de-escalate CT in the presence of anti-HER2 dual blockade.

Regardless of BC subtype, intensive translational research is needed. This research must be aimed at: delving into the drug mechanism of action (specific pharmacodynamic analyses); describing prognosis-related factors; and describing early biomarkers of response/resistance to the treatment delivered. To achieve this goal, genomic profiling of serial tumor or blood samples appears to be crucial. This information will allow to tailor therapies by differentiating poor-prognosis patients eligible for other adjuvant/NA treatments from those with a predicted good outcome who could be spared toxicities from needless additional therapies.

Disclosure Statement

No disclosures to declare.

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