Surrogate endpoints for early-stage breast cancer: a review of the state of the art, controversies, and future prospects

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***Abstract*:** Drug approval for early-stage breast cancer (EBC) has been historically granted in the context of registration trials based on adequate outcomes such as disease-free survival and overall survival. Improvements in long-term outcomes have made it more difficult to demonstrate the clinical benefit of a new cancer drug in large, randomized, comparative clinical trials. Therefore, the use of surrogate endpoints rather than traditional measures allows for cancer drug trials to proceed with smaller sample sizes and shorter follow-up periods, which reduces drug development time. Among surrogate endpoints for breast cancer, the increase in pathological complete response (pCR) rates was considered appropriate for accelerated drug approval. The association between pCR and long-term outcomes was strongest in patients with aggressive tumor subtypes, such as triple-negative and human epidermal growth factor receptor 2 (HER2)-positive/hormone receptor-negative breast cancers. Whereas in hormone receptor-positive/HER2-negative EBC, the most accepted surrogate markers for endocrine therapy–based trials include changes in Ki67 and the preoperative endocrine prognostic index. Beyond the classic endpoints, further prognostic tools are required to provide EBC patients with individualized and effective therapies, and the neoadjuvant setting provides an excellent platform for drug development and biomarker discovery. Nowadays, the availability of multigene signatures is offering a standardized quantitative and reproducible tool to potentiate the efficacy of standard treatment for high-risk patients and develop de-escalated treatments for patients at lower risk of relapse. In this article, we first evaluate the surrogacies used for long-term outcomes and the underlying evidence supporting the use of each surrogate endpoint for the accelerated or regular drug approval process in EBC. Next, we provide an overview of the most recent studies and innovative strategies in a (neo)adjuvant setting as a platform to accelerate new drug approval. Finally, we highlight some clinical trials aimed at tailoring systemic treatment of EBC using prognosis-related factors or early biomarkers of drug sensitivity or resistance.

Keywords: breast cancer subtypes, early breast cancer, intermediate endpoints, neoadjuvant therapy, pathological complete response, surrogate markers

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Introduction

Breast cancer is the most common cancer among women globally, accounting for one in four cancer cases, and is the leading cause of cancer-related deaths in women worldwide responsible for an estimated 684,996 deaths in 2020.[1] The introduction of service screening with mammography has been shown to reduce mortality from breast cancer, and the incidence of invasive breast cancer substantially increases as screening starts and continues to grow with the aging of the population.[2,3]

Neoadjuvant therapy has become a reasonable treatment option for patients with early-stage breast cancer (EBC) who candidates for systemic

treatment. Downstaging primary breast tumors and improving breast conservation rates represent the primary aims for using preoperative systemic therapy, although, in recent years, investigators have recognized that its use additionally provides a research tool for developing new therapies. In addition to the established advantages of allowing for a less aggressive surgical approach, neoadjuvant therapy for breast cancer enables the early assessment of clinical and molecular activities in a treatment-naïve population without affecting the risk of distant recurrence.[4] A neoadjuvant strategy might also have important consequences on long-term outcomes offering further systemic therapies to patients with triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2 (HER2)-positive breast cancers that do not achieve pathologic complete response (pCR). Thus, tumor response, pCR, and residual cancer burden evaluated after preoperative therapy can be considered suitable intermediate endpoints in the neoadjuvant setting.

The use of surrogate endpoints rather than traditional measures allows for cancer drug trials with smaller sample sizes and shorter follow-up periods, which can reduce drug development time by approximately 11–19 months,[5] and thus resulting attractive to study sponsors or manufacturers alike. Nevertheless, despite early surrogates of response offer the advantage of potentially shortening the time needed to identify effective adjuvant therapies, integrating this strategy into the standard-of-care results historically challenging. Their use in oncology remains controversial because the approval of new products by centralized agencies, including the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), seeks to assess the combined benefit–risk profile of emergent medicine and a broader value-for-money evaluation by health care policymakers as well.

The challenges introduced in the last decade continue to escalate as the use of preoperative therapies and our understanding of the biology of breast cancer subtypes improve. Given the societal pressure for accelerated approval for therapies, the use of surrogate endpoints in health care policy as well as their controversies are likely to step up. In the present article, we discuss the appropriate surrogate endpoints for clinical trials in EBC, giving an overview of the methods used to validate the selection of surrogates by each breast cancer subtype. We also review the primary studies in a (neo)adjuvant setting as a platform to accelerate new drug approval or for translational research.

Definition of intermediate endpoint

Historically, drug approval for breast cancer has been granted in the context of registration trials based on outcomes such as disease-free survival (DFS) and overall survival (OS). Advancements in DFS for the adjuvant setting and event-free survival (EFS) in the neoadjuvant setting have led to difficulties in demonstrating the clinical benefit of new cancer drugs in large, randomized, comparative clinical trials, which requires longer follow-up time, larger sample size, and higher costs. Due to this unsustainable scenario, in the preapproval research and development outlay, the FDA can approve new cancer drugs based on intermediate endpoints that reasonably predict clinical benefit and arrive sooner than OS and, thereby, accelerate drug approval.

A surrogate endpoint is a substitute for a direct measure of how long patients live, function, or feel. In a randomized clinical trial, a surrogate endpoint represents a clinical or laboratory outcome that predicts the effect of an independent variable – such as an experimental medicinal product – on a dependent variable – such as OS or quality of life.[6] Surrogates are also identified as intermediate measures evaluated earlier than the true outcome of interest because it is speculated to be in the middle of the causal sequence that relates the independent variable to the dependent variable.[7]

The use of surrogate measures instead of clinical outcomes for regulatory approval in some clinical trials ignited a heated debate within the scientific community, health care policymakers, and regulatory communities. In July 2018, the FDA first shared a table listing all surrogate endpoints used to date and which they will accept going forward for regulatory approval under the condition that '… clinical trials are needed to show that surrogate endpoints can be relied upon to predict, or correlate with, clinical benefit'.[8] Surrogate endpoints adopted for new cancer drug approvals must be validated by compelling evidence relevant to the tumor indication and setting.[9] In August 2019, the Agency published a table of surrogate endpoints as a reference guide to help clarify the FDA's approach and to help inform discussions with the Center for Biologics

Evaluation and Research and the Center for Drug Evaluation and Research.[10] The most recent Surrogate Endpoint Table was published on 23 July 2018, and updated versions will be made available every 6 months going forward to reflect the current thinking as established by Section 507 of the Federal Food, Drug, and Cosmetic Act.

In the FDA's Surrogate Endpoint Table, the surrogate measures listed for breast cancer include (1) pCR, (2) EFS, (3) DFS, (4) objective response rate, and (5) progression-free survival (PFS) (Table 1).

Pathological complete response

The preferred definition of pathological complete response (pCR) is the absence of residual invasive cancer within both the breast and lymph nodes through hematoxylin and eosin staining of the complete resected breast specimen and all sampled regional lymph nodes.[11] No standard definition of pCR exists across different studies, and the FDA has proposed two different definitions: (1) no invasive and non-invasive residual cancer in the breast and lymph nodes (ypT0 ypN0)[11,12] and (2) no invasive residual cancer in the breast and lymph nodes irrespective of residual non-invasive disease (ypT0/is ypN0).[13–15] Despite uncertainty about the exact definition and its prognostic impact on survival, pCR is included in the FDA's Surrogate Endpoint Table only for breast cancer and is used as an intermediate measure in clinical trials for patients with EBC who complete systemic neoadjuvant therapy. For high-risk EBC, pCR has been used as a surrogate endpoint reflective of treatment effect to support accelerated approval and a surrogate endpoint to support traditional approval.[16]

A critical aspect that remains unanswered is whether the inclusion of patients with residual ductal carcinoma *in situ* (DCIS) among patients with pCR results in decreased recurrence-free survival (RFS) or OS. Pooled analyses with a large enough sample size have been carried out from different expert panels to draw a definitive conclusion on the best definition of pCR in terms of prognostic discrimination.

In 2007, an analysis of a US study group investigated whether the inclusion of patients with residual DCIS among patients with pCR results in decreased RFS or OS after preoperative chemotherapy. The retrospective review of a database of 2302 breast cancer patients who received neoadjuvant chemotherapy and attained either pCR (ypT0 ypN0) or pCR plus DCIS (ypT0/is ypN0) showed that both the DFS at 5 (87.1% in both groups) and 10 years (81.3% *versus* 81.7%, respectively) and the OS at 5 (91.9% *versus* 92.5%, respectively) and 10 years (91.8% *versus* 92.5%, respectively), as well as the 5-year locoregional RFS (92.8% *versus* 90.9%, respectively) did not differ significantly between the groups of patients. The authors concluded that the inclusion of residual DCIS did not confer any significant adverse prognostic effect for patients who experience a complete eradication of invasive cancer from the breast and lymph nodes after preoperative chemotherapy. Thus, considering patients with residual DCIS in the definition of pCR is justified when this outcome is used as an early surrogate for long-term survival.[15]

In contrast, a more recently pooled analysis conducted by two German study groups examined the impact on survival of different pCR definitions on 6377 patients with primary breast cancer receiving neoadjuvant chemotherapy and revealed that patients who experienced ypT0 ypN0 had better DFS [hazard ratio (HR) = 1.74, 95% confidence interval (95% CI) = 1.28–2.36, $p < 0.001$] and a trend in better OS (HR = 1.41, 95% CI = 0.87–2.29, $p = 0.166$) compared with patients who attained ypT0/is ypN0. Thus, ypTis, ypT1mic, and ypN+ residuals are associated with increased relapse risk and should therefore no longer be considered in the definition of pCR.[11]

Thus, the definition of pCR associated with the most favorable outcome seems to be ypT0 ypN0.

pCR by breast cancer subtypes

The inability to demonstrate a clear correlation between early response to neoadjuvant therapy and prognosis of patients with breast cancer could be explained by heterogeneity across breast cancer patients with different tumor subtypes who received different treatment regimens and contributing with different improvements of pCR.

Houssami and colleagues performed a meta-analysis on 11,695 breast cancer subjects from 30 eligible studies, aiming to report summary estimates of pCR by tumor subtype, and to compare pCR rates according to different subtypes, using methods that allow for heterogeneity across studies. The overall pooled pCR (ypT0 ypN0) rate was

Table 1. List of surrogate endpoints in adult patients with breast cancer that may be considered and discussed with FDA for individual development programs of a drug or a biological product under both accelerated and traditional approval pathways.

Patient population	Disease status	Treatment setting	Surrogate endpoint	Type of approval	Drug mechanism of action
Patients with breast cancer	Early stage	Preoperative neoadjuvant therapy	pCR	Accelerated	Agnostic[a]
Patients with breast cancer and neuroblastoma	Early stage	Preoperative neoadjuvant therapy	EFS[b]	Accelerated/ traditional[c]	Agnostic[a]
Patients receiving adjuvant therapy following complete surgical resection of colon cancer, colorectal cancer, melanoma, renal cell cancer, gastrointestinal stromal tumor, breast cancer, and adjuvant therapy for stage III non-small cell lung cancer	Early stage	Postoperative adjuvant therapy	DFS	Accelerated/ traditional[c]	Agnostic[a]
Patients with breast cancer, ovarian cancer, renal cell carcinoma, pancreatic neuroendocrine cancer, colorectal cancer, head and neck cancer, non-small cell lung cancer, melanoma, tuberous sclerosis complex-associated subependymal giant cell astrocytoma and renal angiomyolipoma, Merkel cell carcinoma, unresectable or metastatic cutaneous basal cell carcinoma, urothelial carcinoma, cervical cancer, endometrial cancer, hepatocellular carcinoma, fallopian tube cancer, microsatellite instability-high cancer, gastric cancer, thyroid cancer, astrocytoma, Kaposi's sarcoma, unresectable or metastatic cutaneous squamous cell carcinoma, *NTRK* gene fusion without a known acquired resistance mutation, prostate cancer, esophageal cancer, tumor mutational burden high solid tumors, cholangiocarcinoma, bladder cancer, and neuroblastoma	Unresectable locally advanced or metastatic stage	Therapy in metastatic setting	Durable objective ORR	Accelerated/ traditional[c]	Agnostic[a]
Patients with breast cancer; renal cell carcinoma; pancreatic neuroendocrine tumor; soft tissue sarcoma; ovarian, fallopian tube, or primary peritoneal cancer; prostate cancer; thyroid cancer; colorectal cancer; non-small cell lung cancer; head and neck cancer; tuberous sclerosis complex; Merkel cell carcinoma; basal cell carcinoma; urothelial carcinoma; cervical cancer; endometrial cancer; hepatocellular carcinoma; melanoma; astrocytoma; and gastrointestinal stromal tumors	Unresectable locally advanced or metastatic stage	Therapy for metastatic setting	PFS	Accelerated/ traditional[c]	Agnostic[a]

Recreated from the FDA's adult Surrogate Endpoint Table at https://www.fda.gov/drugs/development-resources/table-surrogate-endpoints-were-basis-drug-approval-or-licensure.
BLA, Biologic License Application; DFS, disease-free survival; EFS, event-free survival; FDA, US Food and Drug Administration; NDA, New Drug Application; NTRK, neurotrophic tyrosine receptor kinase; ORR, overall response rate; pCR, pathological complete response; PFS, progression-free survival.
[a]Since disparate mechanisms of action could be involved, mechanism agnostic refers to the absence of a causal pathway which is directly related to a surrogate endpoint.
[b]Despite not yet used to support an approved NDA or BLA, this surrogate endpoint could be appropriate for use as a primary efficacy clinical trial endpoint for a drug or biologic approval.
[c]Endpoints based on changes in tumor burden may be used for both traditional and accelerated approval depending on context of use, including factors such as disease, effect size, effect duration, residual uncertainty, and benefits of other available therapy.

18.9% (16.6–21.5%) with a great difference among breast cancer subtypes: the highest odds of pCR were observed in the HER2-positive/hormone receptor-negative subtype (38.9%), followed by the triple-negative subtype (31.1%), the HER2-positive/hormone receptor-positive subtype (18.7%), and the hormone receptor-positive/HER2-negative subtype (8.3%).[17]

In another pooled analysis on 6377 patients with primary breast cancer receiving neoadjuvant chemotherapy, von Minckwitz and colleagues demonstrated that pCR (ypT0 ypN0) is not associated with improved DFS in subgroups having slowly proliferating tumors (luminal A, $p = 0.39$, or luminal B/HER2-positive, $p = 0.45$), whereas pCR is a suitable surrogate endpoint for patients with HER2-positive/non-luminal, triple-negative (both ps < 0.001), and luminal B/HER2-negative ($p = 0.005$) tumors.[11]

The confirmation that the prognostic relevance of pCR differs across breast cancer subtypes emerged from the meta-analysis funded by the FDA and conducted by the CTNeoBC on 11,955 patients who received neoadjuvant treatment of breast cancer. Cortazar and colleagues reported that the association between pCR (ypT0/is ypN0) and long-term EFS or OS outcomes was strongest in patients with TNBC (EFS: HR = 0.24, 95% CI = 0.18–0.33; OS: HR = 0.16, 95% CI = 0.11–0.25) and in those with HER2-positive/hormone receptor-negative tumors who received trastuzumab (EFS: HR = 0.15, 95% CI = 0.09–0.27; OS: HR = 0.08, 95% CI = 0.03–0.22).[18]

Taken together, the results of both analyses[11,18] suggest that pCR's association with survival is greatest in aggressive tumor subtypes, such as triple-negative and HER2-positive/hormone receptor-negative breast cancers, whereas less obvious in patients with hormone receptor-positive/HER2-negative tumors.

pCR as a trial-level surrogate for long-term survival outcomes

A surrogate endpoint must be validated opportunely to be considered as a substitute for the reference endpoint. Meta-analysis of randomized controlled trials constitutes a widely accepted approach for surrogacy evaluation in oncology, assessing the correlation between the surrogate endpoint and the final endpoint.[19] The standard meta-analytical approach considers two levels of validation for a surrogate endpoint: the individual level and the trial level. If an individual-level surrogate will correlate to the final endpoint means that, for each patient, it reliably predicts who will have relatively good outcomes from those who will have relatively poor outcomes. Whereas an intermediate endpoint will be considered a trial-level surrogate if findings of a between-arm comparison trial using an intermediate endpoint accurately predict the possible findings of a between-arm comparison trial using the definitive endpoint.

Different measures of validation have been proposed at individual level for pCR and time-to-event surrogates of long-term survival outcomes: (1) the adjusted HR between patients with and without pCR, (2) the coefficient of determination (R^2) between endpoints at patient level, and the (3) Kendall's τ or Spearman's rank correlation coefficient (r_s) using a copula model. Alternatively, the usual measure of validation at the trial level was the coefficient of determination (R^2) of the trial computed *via* a linear regression of the estimated treatment effects (R^2 of 0 indicates no association and an R^2 of 1 is a perfect association).[18,20–22]

Although the lower risk of death among patients who experienced a pCR in breast and/or lymph nodes compared with patients with residual tumor at the end of neoadjuvant therapy, the statistically significant association between increased pCR rate and longer survival is not so obvious and does not automatically mean that treatment response also represents a surrogate measure for long-term survival.

In 2012, the FDA distributed its first draft guidelines to the industry stating that a new neoadjuvant treatment for high-risk patients with EBC could receive accelerated FDA approval if sufficient improvement in pCR rates over a standard treatment in a randomized controlled trial was demonstrated.[23] The FDA guidelines were finalized in 2014.[24] When first released, investigators commissioned by the FDA were performing the first large meta-analysis to assess pCR surrogacy. In the CTNeoBC pooled analysis of 12 international neoadjuvant randomized controlled trials, the HR for improved EFS was 0.44 (95% CI = 0.39–0.51) and improved OS was 0.36 (95% CI = 0.30–0.44) in patients with breast cancer who obtained pCR, and this effect was more marked in patients with more aggressive tumor subtypes. However, in the trial-level analysis,

increased pCR frequency was not predictive of the improved clinical outcome by the same treatment in terms of both EFS ($R^2 = 0.03$, 95% CI = 0–0.25) and OS ($R^2 = 0.24$, 95% CI = 0–0.70); thus, the authors were not able to demonstrate pCR's validity as a surrogate endpoint for improved survival.[18] This was probably due to several factors, including the overall low rates of pCR in the trials analyzed, population heterogeneity, and that only one trial out of 12 used targeted therapies.

The aforementioned lone trial was the NeOAdjuvant Herceptin (NOAH) phase 3 study, which randomized 235 patients with HER2-positive, locally advanced breast cancer to receive preoperative anthracycline/taxane regimen alone or in combination with trastuzumab. Gianni and colleagues showed that chemotherapy plus trastuzumab significantly improved pCR rates and, subsequently, that 5-year EFS was strongly associated with pathological complete remission (ypT0/is ypN0) in patients in the trastuzumab group compared with those in the chemotherapy group (58%; 95% CI = 48–66 *versus* 43%; 95% CI = 34–52, respectively), resulting in a 36% reduction in risk of death or recurrence (unadjusted HR = 0.64, 95% CI = 0.44–0.93, $p = 0.016$).[25] In addition, the benefit of adjuvant trastuzumab in combination with chemotherapy was confirmed in the combined National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31/North Central Cancer Treatment Group (NCCTG) N9831 trials reporting an absolute improvement in RFS during the first 5 years (HR = 0.65, 95% CI = 0.56–0.77, $p < 0.001$).[26] Taken together, the findings from these studies suggest that a correlation between a complete response and long-term survival outcomes can be identified in randomized, controlled trials in selected subjects with specific breast cancer subtypes treated with more homogeneous therapeutic regimens.

Berruti and colleagues performed a trial-based meta-regression of 29 randomized studies that compared different systemic neoadjuvant treatments in a total of 14,641 unselected patients with breast cancer. A minimal association between the effect of the treatment on pCR and the effect on both DFS ($R^2 = 0.08$, 95% CI = 0–0.4) and OS ($R^2 = 0.09$, 95% CI = 0.01–0.41) was observed, thereby not supporting the use of pCR (regardless of the definition) as a surrogate endpoint for long survival.[27] Intriguingly, an exploratory analysis of this meta-regression focusing on a subset of trials comparing intensified/dose-dense chemotherapy with standard-dose regimens revealed stronger associations between DFS ($R^2 = 0.79$, 95% CI = 0.26–0.95, $p = 0.003$) and OS ($R^2 = 0.57$, 95% CI = 0.19–0.93, $p = 0.03$). Besides the prognostic heterogeneity among breast cancer subtypes and differential responses to neoadjuvant chemotherapy, as observed in the CTNeoBC pooled analysis, the distinct pCR definitions used in addition to the disparate treatment regimens administered before and after surgery are confounders that potentially underlie the inability to demonstrate a clear pCR surrogacy.

By closely re-examining the evidence from Cortazar and Berruti's previously published meta-analyses, Korn and colleagues found no evidence that pCR is a trial-level surrogate for EFS or OS.[28]

In contrast, a larger meta-analysis of 36 studies representing 5768 patients with stages I to III HER2-positive breast cancer found that improved pCR rates with the neoadjuvant therapy were associated with substantially longer times until recurrence ($R^2 = 0.63$) and death ($R^2 = 0.29$).[29]

EFS

EFS is defined as the time from randomization to the progression of the disease precluding surgery, local or distant recurrence, and death due to any cause.[16] EFS is considered an endpoint similar to DFS except that EFS is used in the neoadjuvant setting where the randomization takes place before definitive surgery or radiotherapy, while DFS occurs in the adjuvant setting where randomization happens after surgery or radiation. Therefore, failure to undergo surgery represents an event in EFS but not in DFS.

Although EFS has been interchanged (incorrectly) with DFS in prior trials in the adjuvant setting, in recent years, the term EFS has become preferred to DFS because the patient is not technically 'disease-free' at the time of randomization for neoadjuvant therapy.

In a cross-sectional analysis evaluating the correlation of five surrogate measures in breast cancer with treatment effects on OS, no validated studies were found to support that the treatment effects on EFS predicted treatment effects on OS.[30] Among the intermediate endpoints for breast cancer

included in the FDA's table, EFS is a newer surrogate adopted in neoadjuvant studies, which may explain the lack of any surrogacy studies.

Just recently, Gyawali and colleagues performed a correlation analysis to assess EFS as a trial-level surrogate for OS in seven randomized controlled trials involving 2211 patients with EBC, and five of these seven trials included patients with the HER2-positive subtype. The authors found a moderate but not significant trial-level association between the HRs for OS and EFS with wide confidence intervals ($R^2 = 0.76$, 95% CI = 0.34–1.00).[31] Hence, EFS may be suitable as a surrogate primary efficacy endpoint for accelerated approval of breast cancer drugs, but its validity as a regulatory endpoint for traditional approval remains to be demonstrated.

DFS

DFS is defined as the time from randomization until disease recurrence or death from any cause[16] and is frequently employed as a surrogate endpoint in the adjuvant setting for evaluating postoperative treatment – definitive surgery or radiotherapy – for stages I, II, and IIIA breast cancer. DFS is a combined measure that typically comprises locoregional and distant recurrences, new contralateral breast cancers, second cancers, as well as death from any cause. If recurrence predicts death over a longer period, DFS may represent an early indicator of improved survival. DFS can be a surrogate endpoint to support both accelerated and traditional approvals, and it has been the primary basis of approval for adjuvant endocrine therapy and cytotoxic treatment for patients with breast cancer.

Ng and colleagues conducted a systematic review of 126 randomized trials in adjuvant settings to ascertain whether changes in DFS accurately predict OS changes. The authors concluded that, while a statistically significant correlation of moderate strength exists ($R^2 = 0.38$, full model, $p < 0.001$), the correlation between the 2-year DFS difference and the difference in 5-year OS was not strong enough for DFS to be used as a predictor of OS.[32] However, as highlighted by the authors, most of the trials included in the systematic review were prior to the anti-HER2 therapies and obtained only small improvements in DFS and OS when compared with their respective control arms. Thus, the resultant limited variability in the predictor variable diminishes the power to detect the correlation between changes in DFS and OS.[32]

A systematic review and meta-analysis identified eight randomized controlled trials assessing the correlation of treatment effects on DFS with those on OS in 21,480 patients with HER2-positive EBC who received up to 1 year of adjuvant trastuzumab. Saad and colleagues found strong associations between DFS and OS both at trial level for the full set ($R^2 = 0.75$, 95% CI = 0.50–1.00) and patient level ($r_s = 0.90$, 95% CI = 0.89–0.90), arguing for the suitability of DFS as a surrogate measure for long-term outcome OS in adjuvant studies for HER2-positive breast cancer.[20]

The inclusion of both trial-level and patient-level correlations constitutes a major strength of Saad and colleagues' analysis,[20] providing robust support for the adequate surrogacy of DFS and OS. Nevertheless, a concerning aspect is that the DFS surrogacy results are less strong in studies with small numbers of events. Over the last decade, the development of effective HER2-targeted therapeutic approaches continued to evolve with a positive impact on the survival of HER2-positive breast cancer. Hence, clinical trials for EBC have registered an impressive decrease in the frequency of events, from the 3-year DFS of 87.1% in the trastuzumab group of the 2005 combined analysis of the NSABP B31 and NCCTG N9831 trials[33] to 3-year invasive DFS of 93.2% in the 2017 report of the Adjuvant Pertuzumab and Herceptin IN Initial TherapY in Breast Cancer (APHINITY) trial.[34] Thus, whether DFS will maintain robust surrogacy in contemporary randomized controlled trials with an undeniable beneficial impact on the long-term outcomes and the concomitant low event frequencies is unpredictable.

Intermediate markers of efficacy for luminal tumors

About 70% of all invasive breast carcinomas are characterized by their hormone receptor-positive/HER2-negative status, and these are responsible for most breast cancer–related deaths worldwide.[35]

Both the endocrine- and chemotherapy-based neoadjuvant trials have historically tried to recapitulate the findings from larger adjuvant studies, requiring considerably smaller numbers of patients with inherent reduced costs, assessing

additional molecular/biologic biomarkers, and providing preliminary results in a relatively shorter time frame due to endpoints that are evaluated in a few months – for example, pCR, conservative surgery, clinical response rate – or even in just a few days – for example, Ki67 proliferative marker. Although adjuvant chemotherapy has consistently shown long-term clinical benefits in terms of DFS and OS,[36] patients with hormone receptor-positive/HER2-negative breast cancers present with great heterogeneity in prognosis and treatment benefit. Historically, hormone receptor-positive breast cancer, especially luminal A subtype, represents a considerable therapeutic challenge because of the minimal response to neoadjuvant systemic chemotherapy.[11,18,37]

Due to the lack of benefit, less toxic alternatives to neoadjuvant chemotherapy have been investigated in patients with hormone receptor-positive disease. Preoperative endocrine therapies with or without targeted agents can facilitate breast-conserving surgery in patients destined for mastectomy.[38]

Although most trials gave neoadjuvant endocrine therapy for 3–4 months and the majority of beneficial responses were observed at 4 months,[39] the optimal duration has not yet been unequivocally established. In this scenario, patients with hormone receptor-positive/HER2-negative disease rarely achieve pCR, limiting the value of pCR as a surrogate for the effectiveness of endocrine therapy in early settings.

Instead, there has been significant interest in evaluating several biologic endpoints that may be analyzed during treatment in serial biopsies of the primary tumor or at the end of treatment in surgical specimens. The most accepted surrogate markers for endocrine therapy–based trials include changes from baseline in Ki67, which is measured at 2–4 weeks from treatment initiation or at surgery, and the preoperative endocrine prognostic index (PEPI) score.[40,41] Nowadays, the availability of multigene signatures is a consistently changing paradigm for EBC treatment, offering a standardized quantitative and reproducible tool to define the risk of distant recurrence as a major determinant of recommendations for or against chemotherapy[42] and the identification of the molecular phenotype beyond routinely obtained pathological features[43] (please refer to the 'Genomic signatures' section).

Nevertheless, because of the lack of definitive data indicating the situations in which a multigene assay should be preferred, more clinical evidence is needed to guide clinicians' decisions to extend endocrine therapy and select patients who could avoid overtreatment while maintaining a late recurrence risk and improve the development of de-escalation and escalation treatment strategies.

Ki67 proliferation index

Ki67 represents a protein encoded by the *MKI67* gene associated with cellular proliferation as it identifies cells in the G1/S and M phases of the cell cycle. High levels of Ki67 expression – which is examined as a continuous variable – routinely indicate increased cellular proliferation and, in breast cancer, elevated Ki67 is associated with impaired prognosis. In addition to being a surrogate for proliferative capacity and a useful breast cancer prognosticator, the Ki67 proliferation index has been proposed as a predictor of therapeutic benefit among patients with hormone receptor-positive breast cancer under treatment with neoadjuvant aromatase inhibitors. This is due to neoadjuvant endocrine treatment mainly inducing cell cycle arrest, hence the suppression of the proliferative marker Ki67, among other effects.

In the IMPACT trial comparing the preoperative use of tamoxifen with anastrozole alone or both combined in 330 postmenopausal women with primary hormone receptor-positive breast cancer, a decrease in the proliferative marker Ki67 occurred in the majority of patients. Intriguingly, significantly greater suppression of Ki67 was observed after 2 weeks in the anastrozole-treated group than in the tamoxifen- or combination-treated groups.[44] Interestingly, in a multivariable analysis of the same trial, Dowsett and colleagues found that higher Ki67 expression after 2 weeks of presurgical endocrine therapy for primary breast cancer was statistically significantly associated with lower RFS ($p = 0.004$) and, thus, predicted outcome more faithfully than Ki67 levels at baseline.[40]

The POETIC phase 3 randomized controlled trial was designed to assess whether tumor Ki67 values after 2 weeks of perioperative aromatase inhibitor predict individual patient outcome better than baseline Ki67.[45] Recent data indicate

that women with low tumor levels of Ki67 both at baseline and 2 weeks have less 5-year recurrence risk (4.3%, 95% CI = 2.9–6.3) than women with high Ki67 levels at baseline and low at 2 weeks (8.4%, 95% CI = 6.8–10.5), or high both at baseline and 2 weeks (21.5%, 95% CI = 17.1–27.0).[46]

While the ALTERNATE phase 3 study is ongoing to provide the definitive clinical evidence to inform future practice, a Ki67 score >10% after 2 or 4 weeks of preoperative endocrine therapy has been suggested as the cutoff for accurately selecting individual non-responders who are, therefore, suitable for other treatment strategies, including investigational agents and/or chemotherapy.

PEPI score

PEPI is an algorithm combining anatomy and biology, to estimate the amount of residual cancer in a surgical specimen following preoperative therapy. It includes features from the initial diagnosis prior to neoadjuvant endocrine therapy and treatment responses to separate patients into prognostic groups, thus representing the major drawback because the final score cannot be obtained until patients have completed 4 months of endocrine therapy and undergone surgery. Specifically, the PEPI score takes into account pathological stage (tumor size and nodal status), Ki67 expression, and the Allred estrogen receptor (ER) score measured on the primary tumor during uninterrupted endocrine therapy. The scoring process produces three groups (risk groups 0, 1–3, and ⩾4) that were associated with different relapse risks (risk score 0 associated with a low risk of relapse/death and risk score ⩾4 associated with a high risk of relapse/death).

Data from the neoadjuvant P024 study comparing letrozole and tamoxifen in postmenopausal women with ER-positive breast cancer[38,47,48] were used to generate a PEPI score that was subsequently validated in an independent study of 203 postmenopausal women enrolled in the IMPACT trial, which compared treatment with anastrozole, tamoxifen, and the combination of anastrozole and tamoxifen for 3 months before surgery.[49] Women from the P024 trial with PEPI scores of 0 (pT1 or pT2, pN0, Ki67 ⩽ 2.7%, Allred score > 2) after neoadjuvant endocrine therapy had an extremely low risk of relapse (10% of PEPI score 0 *versus* 23% of PEPI scores 1–3 *versus* 48% of PEPI score ⩾4) and, as such, are unlikely to benefit from adjuvant chemotherapy.[49] The PEPI model based on these factors effectively predicted RFS in the IMPACT trial ($p = 0.002$).

Subsequently, the ACOSOG Z1031A randomized phase 2 trial was designed to determine which aromatase inhibitor – anastrozole, letrozole, or exemestane – should be used in future testing against chemotherapy in the neoadjuvant setting. When enrollment in ACOSOG Z1031A was complete, an amendment was introduced (ACOSOG Z1031B) that triaged patients with tumors exhibiting a Ki67 > 10% upon biopsy 2–4 weeks after starting aromatase inhibitors to standard chemotherapy. The hypothesis being tested was that the pCR rate would be at least 20% in this aromatase inhibitor-resistant population. For the 3.7% of patients (4 of 109) with PEPI score of 0, the relapse risk over 5 years was only 3.6% without chemotherapy compared with 14.4% of patients (49 of 341) with PEPI scores ⩾0 (recurrence HR = 0.27, $p = 0.014$), supporting the study of adjuvant endocrine monotherapy in this group.[41]

As for the Ki67 proliferation score, the PEPI triage approach is being definitively investigated in the ALTERNATE trial, which will provide an indication for adjuvant chemotherapy.

Genomic signatures

The introduction of innovative, advanced molecular technologies such as RNA sequencing and DNA microarray analysis has allowed us to decipher the molecular subtypes of each tumor, with distinct biological features that lead to differences in response patterns and clinical outcomes, shedding light on the heterogeneous complexity of luminal breast tumors. Global gene expression profiling analyses have provided evidence for classifying breast cancer into the following five distinct molecular classes according to the hierarchical clustering of thousands of genes simultaneously expressed: (1) Luminal A [representing the immunohistochemically defined tumors with ER-/Progesterone receptor (PR)-positive, HER2-negative, histological grade I/II, low Ki67 index], (2) Luminal B (ER-/PR-positive, HER2-negative/positive, histological grade II/III, high Ki67 index), (3) HER2-enriched (ER-/PR-negative, HER2-positive, histological grade II/III), (4) Basal-like (ER-/PR-/HER2-negative, histological grade III), and (5) Normal-like (ER-/PR-positive, HER2-negative, histological grades I–III, low Ki67 index).[50] In the context of the

luminal-like subclasses, the low-proliferating luminal A breast tumors – accounting for 50–60% of all breast cancers – present a more favorable clinical than the luminal B subtype and seem to benefit more from endocrine therapy alone *versus* the combination of chemotherapy and endocrine therapy.[51,52] While the highly proliferative and more aggressive luminal B breast tumors – comprising 15–20% of all breast cancers – may derive more benefit from the combined therapeutic strategy of chemotherapy and endocrine therapy.[53] In luminal EBC, additional prognostic indicators are required to identify patients with a late recurrence risk and provide them with a reliable and effective therapeutic plan. In addition, several gene-expression-based prognostic signatures describe the unique molecular portrait associated with a tumor, and estimate the residual risk of recurrence (ROR) and post-surgery survival. The availability of these multigene signatures is nowadays paving the way for the development of de-escalation and escalation treatment strategies.

The Oncotype DX® Recurrence Score (RS),[54] MammaPrint®,[55] EndoPredict (EP/EPclin),[56] Prosigna® Risk of Recurrence Score,[57] and Breast Cancer Index^{SM} (BCI) are the five commercially available multigene assays and the most widely used prognostic signatures in early luminal breast cancer to be endorsed by clinical practice guidelines. In addition, these multiparameter prognostic signatures can be used to guide the treatment of patients with ER-positive/HER2-negative primary breast cancer, thus helping to de-escalate systemic therapy.

The Oncotype DX® RS (Genomic Health) based on the 21-gene breast cancer assay consists of a molecular signature of 16 prognostic genes, 13 of which are grouped into modules of proliferation, with the ER, HER2, and invasion pathways weighted differentially in a final signature algorithm.[54] The 5- or 10-year risk of distant relapse is stratified by RS into three risk groups that classify patients as low ($RS < 18$), intermediate ($18 \leqslant RS \leqslant 31$), and high risk ($RS > 31$). The cutoff points for the expression of the individual genes were based on tumor samples from the NSABP B-20 trial,[54] and the final validation was performed on ER-positive/HER2-negative and HER2-positive, node-negative patients treated with tamoxifen from the NSABP B-14 trial. The RS has been validated in NSABP B-20,[58] TransATAC,[59] and Southwest Oncology Group (SWOG) 8814 studies.[51] Recent findings from the prospective Trial Assigning Individualized Options for Treatment (TAILORx) study on 10,273 women with hormone receptor-positive/HER2-negative, node-negative breast cancer showed that adjuvant endocrine therapy was non-inferior to chemoendocrine therapy in the analysis of invasive DFS for patients aged >50 years with midrange RS (11–25).[60]

The ongoing randomized Rx for Positive Node, Endocrine Responsive breast cancer (RxPONDER) (ClinicalTrials.gov identifier: NCT01272037) and West German Study Group-Adjuvant Dynamic marker-Adjusted Personalized Therapy (WSG-ADAPT) trials (ClinicalTrials.gov identifier: NCT01817452) will contribute to defining the optimal RS cutoff values for patients with hormone receptor-positive/HER2-positive EBC who may be spared adjuvant chemotherapy. Finally, the prospective phase 3 WSG-ADAPTcycle trial (ClinicalTrials.gov identifier: NCT04055493) will provide evidence for chemotherapy omission in patients with intermediate RS who can be treated with cyclin-dependent kinase (CDK) 4/6 inhibitor ribociclib combined with endocrine therapy.

MammaPrint® (Agendia NV) is a microarray-RNA 70-gene prognostic test that provides a numerical index ranging from −1 to +1. This value is used to classify patients with breast cancer into high- and low-risk groups estimating the risk of relapse within 10 years for both untreated patients and patients treated with endocrine therapy alone.[55]

The microarRAy-prognoSTics-in-breast-cancER (RASTER) trial was the first study designed to prospectively evaluate the performance of the MammaPrint® signature in patients with node-negative EBC,[61] and the randomized European Organisation for Research and Treatment of Cancer (EORTC) 10041/BIG3-04 phase 3 trial confirmed its clinical utility in 6693 women with node-negative and 1–3 node-positive EBC thereafter.[62] After having determined the genomic risk of relapse by the 70-gene signature and clinical risk of relapse using a modified version of Adjuvant! Online, patients were divided into four groups: women at low clinical and genomic risk did not receive chemotherapy, women at high clinical and genomic risk did receive such therapy, women with a discordant genomic risk or clinical risk were randomized to receive endocrine therapy alone, and women with a discordant

genomic risk or clinical risk were randomized to receive combined chemo- and endocrine therapy in an adjuvant setting.

The first aim of Microarray In Node negative Disease may Avoid ChemoTherapy (MINDACT) was to assess the lower boundary of the 95% CI for the rate of the 5-year survival without distant metastasis would be equal to or higher than 92%. At 5 years, among the 1550 patients (23.2%) at high clinical risk and low genomic risk who did not receive chemotherapy, the rate of survival without distant metastasis was 94.7% (95% CI = 92.5–96.2), thereby achieving the study's primary endpoint. The absolute difference in this survival rate among patients at high clinical risk and low genomic risk who did not receive chemotherapy relative to those who received chemotherapy was 1.5% lower with no clear difference according to nodal status. Thus, these findings suggested that approximately 46% of women with breast cancer who are at high clinical risk could safely omit adjuvant chemotherapy and its toxic effects at a cost of a rate of survival without distant metastasis that was 1.5 percentage points lower than the rate with chemotherapy.[62] Long-term results from 5-year[63] and 8-year[64] estimates for distant metastasis-free survival showed the intact ability of a 70-gene signature to identify women with high clinical risk and low genomic risk when treated with adjuvant endocrine therapy alone, confirming MINDACT as a positive de-escalation study. Interestingly, an underpowered exploratory analysis confined to the subgroup of patients with luminal EBC revealed an age-dependent benefit with women over 50 years old at clinical high but genomic low risk who benefit the most from the chemotherapy-free strategy.[64] On the contrary, however, for women younger than 50 years, the benefit of chemotherapy is present, perhaps due to ovarian function suppression induced by chemotherapy. Further research is needed to better understand the role of genomic information in younger women who can continue to be spared adjuvant chemotherapy and to encourage future investigations into de-escalation strategies.

The EndoPredict® (Myriad Genetics, Inc.) is a 12-gene signature-based risk score, which ranges between 0 and 15 according to the expression of eight prognostic genes. The more comprehensive risk score EPclin could be obtained combining the EP score with tumor size and nodal status to provide decision-making results on the benefit of chemotherapy and extended endocrine therapy in postmenopausal patients with ER-positive/HER2-negative, node-negative breast tumors.[56]

EP and EPclin scores were evaluated as independent prognostic parameters in 664 patients with ER-positive/HER2-negative breast cancer with both node-negative and node-positive forms for adjuvant chemotherapy and endocrine therapy.[65] Thereafter, EP and EPclin were validated in the ABCSG-6 and ABCSG-8 trials[56] and TransATAC.[66] This multigene signature was based on the prognostic genes mainly associated with ER signaling pathways, suggesting its ability to predict the benefit of an extended adjuvant endocrine therapy in the low-risk group. However, prospective randomized trials are needed to confirm this assumption.

The Prosigna®/PAM50/Risk of ROR test (NanoString Technologies) is a 50-gene signature based on NanoString nCounter® technology, which, in addition to distinguishing between the intrinsic molecular subtypes of a breast tumor, provides the probability of distant recurrence. The clinically applied Prosigna® score integrated with a proliferation score, and tumor size information generates the ROR score. Depending on the nodal status, the ROR is categorized differently on a scale from 0 to 100 through its association with the 10-year probability of distant recurrence: in node-negative cancers, ROR is classified as low (0–40), intermediate (41–60), or high (61–100); in 1–3 node-positive cancers, ROR is classified as low (0–15), intermediate (16–40), or high (41–100); and in >4 node-positive cancers, ROR is classified as high risk. The ROR score was validated in the TransATAC,[42] ABCSG-8,[67] and Danish Breast Cancer Cooperative Group (DBCG) cohorts[68] to reliably predict the risk of relapse for postmenopausal women with hormone receptor-positive, node-negative (stage I or II), or node-positive (stage II or IIIA) EBC to be treated with adjuvant endocrine therapy. Despite the well-known prognostic value of the Prosigna® test, the large, prospective Optimal Personalised Treatment of early breast cancer usIng Multiparameter Analysis (OPTIMA) trial is validating the predictive value and cost-effectiveness of the Prosigna® PAM50-based test-guided chemotherapy decisions in node-positive patients with EBC patients.[69]

BCI (Biotheranostics, Inc.) represents a combined signature also known as the molecular grade index that combines a ratio of the *HOXB13* and *IL17BR* genes with five proliferation genes

measured by reverse-transcription polymerase chain reaction. This test determines the risk of both early and late (>5 years) distant recurrences and the likelihood of a benefit from endocrine therapy in postmenopausal patients with node-negative, ER-positive breast cancer receiving adjuvant endocrine therapy.[70] The prognostic utility of the test was first proved in recurrences matched to non-recurrences tumor samples from patients within NCIC CTG MA.17 trial who was investigated for late recurrence and treatment benefits from extended adjuvant letrozole. A subgroup of patients who were ER-positive and disease-free after 5 years of tamoxifen, yet still at risk for late recurrence, were characterized by a high ratio of *HOXB13* and *IL17BR* genes; when an extended adjuvant endocrine therapeutic course with letrozole is prescribed, this gene ratio reveals a subgroup of patients with a 16.5% reduction in their absolute ROR at 5 years.[70]

Although its prognostic ability for risk of both early and late distant recurrence was further validated in the TransATAC[71] and Stockholm[72] trials, the definitive clinical evidence of the extension of adjuvant endocrine therapy beyond 5 years and the use of the BCI in luminal, node-positive EBC is still missing.

In summary, the deep genetic characterization of EBC is providing a uniquely useful tool in treatment decision making by algorithms based on the combination of biological, clinical, and genomic features. Only the Oncotype DX® and MammaPrint® multigene signatures have been validated by prospective, randomized phase 3 trials. The prognostic and predictive role of the other multigene signatures should be prospectively confirmed in large cohorts of patients with hormone receptor-positive/HER2-negative EBC before they are implemented in the clinic for a better individualization of systemic treatments.

(NEO)adjuvant model as a platform for research and innovative strategies

The preoperative setting has become more widespread over the last decade, providing a well-recognized scenario for research in breast cancer. The emerging cancer genomic data led to the development of new treatment management, targeting specific molecular drivers. In this scenario, the neoadjuvant setting provides an excellent platform for drug development, biomarker discovery and validation, and the characterization of mechanisms of drug sensitivity or resistance.[73]

HER2-positive early breast cancer

Although the arrival of anti-HER2 therapies has substantially improved the prognosis of HER2-positive breast cancer patients,[74] several questions on the appropriate treatment strategies remain unsolved. One year of adjuvant trastuzumab combined with chemotherapy represents the current standard of care. However, some patients will relapse despite receiving the optimal treatment.

On one hand, the addition of trastuzumab to adjuvant anthracycline and taxane-based chemotherapy increases the risk of cardiac events, and trastuzumab-associated cardiotoxicity remains the main cause of its discontinuation.[75,76] On the other hand, patients observed in clinical practice compared with patients enrolled in randomized trials often present histological characteristics associated with a lower risk of relapses, such as small tumors, negative nodes, or hormone receptor-positive status.[77]

Thus, recent clinical research efforts have focused on improving treatment individualization and the risk/benefit ratio for patients with HER2-positive EBC using two distinct strategies: potentiating the efficacy of standard treatments for high-risk patients and developing de-escalated treatments for low-risk patients.

Escalated treatment. Extending the duration of adjuvant therapy and combining HER2-targeted agents represent the main treatment strategies for HER2-positive EBC.

The randomized phase 3 HERceptin Adjuvant (HERA) (BIG 1-01) trial focused on the duration of trastuzumab and discovered that 1 year of adjuvant trastuzumab after locoregional therapy and neoadjuvant or adjuvant chemotherapy significantly improved long-term DFS, with a 10-year DFS estimation of 69% with no additional statistically significant benefit from the completion of a second year of trastuzumab.[78]

APHINITY trial assessed whether pertuzumab added to adjuvant trastuzumab and chemotherapy significantly improved invasive DFS in patients with node-positive or high-risk node-negative HER2-positive breast cancer. At 74 months median follow-up, despite the lack of OS benefit with fewer deaths in the pertuzumab arm, the 6-year rate of invasive DFS was 88% in patients with node-positive disease who received pertuzumab with adjuvant chemotherapy and

trastuzumab compared with 83% in those who were treated with placebo (HR = 0.72, 95% CI = 0.59–0.87).[79]

The phase 3 ExteNET trial assessed the administration of the second-generation HER2-targeted tyrosine kinase inhibitor neratinib after the completion of chemotherapy and adjuvant trastuzumab. At 5 years, invasive DFS rates were 90.8% in the neratinib arm compared with 85.7% in the placebo arm (absolute benefit = 5.1%; HR = 0.58, 95% CI = 0.41–0.82) starting 1 year or sooner after neoadjuvant/adjuvant trastuzumab-based therapy in patients with hormone receptor-positive/HER2-positive EBC. Although data from this study are not yet mature, estimated 8-year OS rates were 91.5% *versus* 89.4% in the neratinib and placebo group, respectively (HR = 0.79, 95% CI = 0.55–1.13, $p = 0.203$),[80] and based on these findings, adjuvant neratinib is recommended in patients with HER2-positive breast cancer with a high ROR, and limiting the indication to hormone receptor-positive patients only according to the European approval.

Residual disease after neoadjuvant therapy is substantially associated with a worse prognosis than the achievement of pCR. Thus, the randomized phase 3, KATHERINE study evaluated whether escalation therapy with adjuvant ado-trastuzumab emtansine (T-DM1) could obtain better outcomes than standard treatment with trastuzumab in patients with HER2-positive EBC and residual invasive disease in the breast or axillary lymph nodes after receiving neoadjuvant taxane and trastuzumab.[81] Invasive DFS was significantly higher with T-DM1 than trastuzumab (88.3% *versus* 77.0%, respectively; HR = 0.50, 95% CI = 0.39–0.64, $p < 0.001$) with a 50% decreased ROR of invasive breast cancer or death without an unacceptable increase in toxicity. These findings indicated T-DM1 as the new standard of care for patients with HER2-positive residual disease after neoadjuvant therapy and a higher ROR.

Additional studies investigating promising drugs for the residual disease setting are in development. In particular, given the high rate of brain metastases in HER2-positive breast cancer and the efficacy of both tucatinib[82] and the antibody-drug conjugate trastuzumab deruxtecan[83] on preventing metastases at this site, CompassHER2 RD trial (ClinicalTrials.gov identifier: NCT04457596) is testing tucatinib combined with T-DM1 and DESTINY-Breast05 trial (ClinicalTrials.gov identifier: NCT04622319) trastuzumab deruxtecan *versus* T-DM1 in a high-risk residual disease setting.

De-escalated treatments. An increasing number of de-escalation studies have evaluated the possibility of avoiding overtreatment and improving the quality of life of patients with breast cancer. Several studies have also evaluated possible predictive factors of pCR to neoadjuvant treatment. Imaging tools that could guide the response to preoperative therapy are of particular interest, especially the potential usefulness of ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET). Relationships between early treatment response on ^{18}F-FDG PET and clinical outcomes have been evaluated in patients with HER2-positive breast cancer in both neoadjuvant[84] and metastatic[85] settings, indicating that early metabolic evaluation using ^{18}F-FDG PET may identify HER2-positive tumors with high anti-HER2 sensitivity and an increased likelihood of pCR to neoadjuvant HER2 blockade.

PHERGain is a randomized, non-comparative phase 2 study, aiming at assessing pCR and 3-year invasive DFS of HER2-positive, stage I–IIIA invasive operable breast cancer patients who receive dual HER2 blockade with trastuzumab and pertuzumab and are ^{18}F-FDG PET-responders. Of the 376 enrolled patients, 76% were randomized to receive trastuzumab and pertuzumab (with endocrine therapy if hormone receptor-positive) and 19% to receive trastuzumab and pertuzumab alone. At surgery, 37.9% (95% CI = 31.6–44.5; $p < 0.001$) of ^{18}F-FDG-PET-responders receiving dual HER2 blockade achieved pCR and reduced toxicity and impact on global health status compared with patients receiving chemotherapy.[86] In contrast to the previous de-escalation studies, the PHERGain trial is powered to evaluate invasive DFS and depending on the 3-year invasive DFS results, this ^{18}F-FDG-PET-based, pathologic response–adapted strategy may provide useful information to identify patients who may not require chemotherapy, thus offering a new therapeutic option enabling an improvement in quality of life for this patient population.

The impressive activity and DFS results upon adding trastuzumab and pertuzumab to chemotherapy in HER2-positive EBC have initiated a debate regarding the necessity, duration, and intensity of (neo)adjuvant chemotherapy in these patients. Validation of pCR as a strong surrogate endpoint

for DFS and OS in HER2-positive EBC has led to exploration and validation of de-escalation strategies in the neoadjuvant setting, and several groups have evaluated dual HER2 blockade without chemotherapy in this setting. In the NeoSphere trial, four cycles of trastuzumab and pertuzumab resulted in a 16.8% breast pCR rate,[25] a result that improved with the addition of endocrine therapy in the WSG-Triple Positive II (TP-II) study.[87] The PAMELA study demonstrated that trastuzumab and lapatinib, with or without endocrine therapy, achieved a 30.0% pCR in the breast, which increased to 41.0% among HER2-enriched patients.[88] In the WSG-ADAPT phase 2 trial, as a part of the ADAPT-umbrella protocol, 12 weeks of trastuzumab and pertuzumab in patients with HER2-positive/hormone receptor-negative EBC were associated with a total pCR rate of 34.4%, which increased to 44.7% among early responders (defined as low cellularity and/or Ki67 decrease >30% after 3 weeks).[89] Despite these promising results, the trial was stopped early due to the observed pCR superiority in the neoadjuvant paclitaxel plus pertuzumab and trastuzumab arm. Among patients with pCR who received no further chemotherapy (29% in the trastuzumab and pertuzumab arm *versus* 79% in the paclitaxel plus pertuzumab and trastuzumab arm), only one distant relapse was observed. Intriguingly, pCR and high HER2 expression (immunohistochemical score 3+) were strongly associated with improved distant DFS and invasive DFS, thus representing a predictive clinical marker for further treatment de-escalation.[90]

Further de-escalating approaches have evaluated either T-DM1 in the KRISTINE trial[91] or the addition of endocrine therapy and palbociclib[92] to trastuzumab and pertuzumab in patients with HER2-positive/ER-positive EBC. However, many of these studies had design limitations, such as the use of surrogate endpoints without statistical power to evaluate the strong endpoint of invasive DFS, so the possibility of implementing these strategies in clinical practice remains unclear.

Previous trials of de-escalation strategies in HER2-positive EBC have highlighted the importance of establishing innovative clinical designs, together with optimal selection of treatment and study population. The single-arm, phase 2 APT trial is the only study that has successfully evaluated a de-escalation strategy using a less toxic chemotherapy regimen in appropriately selected patients with stage I, HER2-positive breast cancer.[93,94] Based on the results of the Adjuvant Paclitaxel and Trastuzumab (APT) trial, current management for small, node-negative, HER2-positive EBC is upfront surgery followed by adjuvant systemic therapy with weekly paclitaxel and 12 months of trastuzumab, achieving a 5-year and 7-year DFS of 98.5%[93] and 93.3%,[94] respectively.

In the randomized phase 2 ATEMPT trial evaluating 1 year of T-DM1 compared with paclitaxel plus trastuzumab as adjuvant therapy in patients with stage I HER2-positive breast cancer, although 3-year DFS was superior to the standard of care (97.7% with two distant recurrences *versus* 93.2% with seven distant recurrences), the second co-primary endpoint was not met, thus failing to demonstrate that T-DM1 improved safety relative to paclitaxel plus trastuzumab (clinically relevant toxicities were experienced by 25% and 36% of patients receiving T-DM1 and paclitaxel plus trastuzumab, respectively, with a relative reduction of less than 40%).[95]

Nevertheless, it is worth highlighting that both APT and ATEMPT included almost entirely T1N0 EBC, which limits the generalizability of these treatment strategies to higher risk subsets only. PHERGAIN-2 (ClinicalTrials.gov identifier: NCT04733118) is a phase 2 trial aiming to assess the feasibility of chemotherapy de-escalation with neoadjuvant trastuzumab and pertuzumab followed by adjuvant trastuzumab and pertuzumab or T-DM1 using a pathological response-adapted strategy in low-risk HER2-positive EBC. This study, whose primary objective is the analysis of 3-year invasive DFS, will provide a chemotherapy-free alternative for patients with small [>5–25 mm by magnetic resonance imaging (MRI)], node-negative, HER2-positive (immunohistochemical score 3+) breast cancer.

On the contrary, most adjuvant trials evaluating de-escalation approaches (e.g. shorter trastuzumab duration) have been prolonged and used a non-inferiority design; consequently, they have been time-consuming, expensive, and difficult to conduct. However, despite a large number of patients included in various studies with this standard design, these studies demonstrated that shorter trastuzumab duration is not an optimal de-escalation strategy in patients with HER2-positive EBC.[96–99] Therefore, more efficient clinical trials are required to bring effective therapies to patients in the shortest amount of time and to ensure that time and resources are properly expended to address the most relevant and meaningful issues

for improving cancer care. Moreover, when outcomes are expected to be excellent, strategy-based phase 2 studies should be conducted.

Triple-negative early breast cancer

TNBC, accounting for approximately 15% of all invasive breast cancers, constitutes the poorest prognostic breast cancer subtype, due mainly to the lack of targeted treatments. Systemic chemotherapy with sequential anthracycline and taxanes is the mainstay treatment. Despite not yet being recommended as a standard of care, platinum-containing regimens showed to increase pCR rate in germinal *BRCA1/2* mutated patients. About 33% of patients with TNBC achieve a pCR following neoadjuvant chemotherapy,[18] which extends up to 53.2% or 54% for the GeparSixto[100] and CALGB 40603[101] trials, respectively, following platinum-based neoadjuvant regimens.

The term TNBC actually shows a remarkable diversity of prognosis and clinical response to cancer treatment. Analysis of TNBC gene expression profiling unveiled a highly diverse group of cancers, each displaying unique biology. The TNBC molecular subtypes consist of two basal-like, a mesenchymal, and a luminal androgen receptor (AR) subtype.[102] The four molecular subtypes present specific somatic alterations and distinct clinicopathological characteristics, such as age at diagnosis, histopathology, tumor grade, and disease progression; however, the transcriptional profiling is not able to predict pCR for neoadjuvant chemotherapy in patients with TNBC.

DNA-targeted agents. The triple-negative basal-like subtype is characterized by high genomic instability and mutations in the breast cancer (*BRCA1*) or *BRCA2* susceptibility genes functionally associated with ineffective repair mechanisms. Germline *BRCA1/2* mutational status is predictive of response to therapies that target DNA repair pathways, such as DNA cross-linking platinum salts, DNA-damaging anthracycline, and poly (ADP-ribose) polymerase (PARP) inhibitors.[103–105] Two clinical trials (ClinicalTrials.gov identifiers: NCT01630226 and NCT00148694) of neoadjuvant single-agent cisplatin in women with TNBC and a germinal *BRCA1* mutation showed pCR rates of 21%[106] and 61%.[107] In addition, in *BRCA1* carriers treated with anthracycline with or without taxane regimens, the pCR rate was 57.1% compared with 29.0% for non-carriers.[108]

A study investigating a neoadjuvant PARP inhibitor in the context of the I-SPY2 platform reported that veliparib in combination with carboplatin achieved a 51% pCR compared with 26% from the standard regimen, with an increased but manageable hematologic toxicity.[109] However, in the phase 3 BrighTNess trial, an improved rate of pCR was obtained by the addition of carboplatin plus paclitaxel, with or without veliparib, when compared with paclitaxel alone in previously untreated high-risk TNBC patients (53% in the paclitaxel, carboplatin, and veliparib arm *versus* 58% in paclitaxel plus carboplatin arm *versus* 31% in paclitaxel alone arm).[110] At a median follow-up of 4.5 years, a continued significant benefit in terms of EFS was observed in the arm with all three agents compared with paclitaxel alone (HR = 0.63; $p = 0.02$) but not in the arm of paclitaxel plus carboplatin over paclitaxel alone (HR = 1.12; $p = 0.62$).[111]

A non-randomized, single-arm, phase 2 study assessing the efficacy of neoadjuvant single-agent talazoparib (a PARP inhibitor with the highest catalytic activity and the most efficient trapping mechanism) for 24 weeks without chemotherapy produced a 45.8% pCR rate compared with those obtained with combined anthracycline- and taxane-based regimens.[112] This promising antitumor activity and the generally well-tolerated profile support further investigations in larger neoadjuvant trials.

In the phase 3 OlympiA trial, patients with high-risk HER2-negative breast cancer and germinal *BRCA1/2* mutations were randomized to receive either olaparib or placebo for 12 months as adjuvant therapy after having completed local treatment and (neo)adjuvant chemotherapy.[113] At a median follow-up of 2.5 years, 85.9% of patients treated with adjuvant olaparib were alive and free of recurrent invasive cancer and new second cancer compared with 77.1% of placebo-treated patients. The estimated 3-year distant DFS rate was 87.5% for olaparib compared with 80.4% for placebo. These practice-changing findings could have a huge impact on treatment management of this population, remarking the need for genetic testing for *BRCA* mutations in subjects diagnosed with high-risk EBC.[114]

Sporadic TNBCs (germline wild-type *BRCA1/2*) are sensitive to DNA-damaging therapeutics as a result of a homologous recombination deficiency (HRD) similar to that underlying *BRCA1/2*

mutated cancers. Three independent, DNA-based measures of genomic instability have distinguished deficient from non-deficient tumors, and the unweighted numeric sum of the three metrics seems best at distinguishing HRD: loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions.[115] Recently, different genomic scars associated with HRD have been identified and are being investigated to determine which sporadic patients are most likely to benefit from DNA-damaging agents and PARP inhibitors, and spare others the added toxicity, such as genomic alterations and mutational signatures,[116–118] genome instability,[119–121] and epigenetic modification.[122–124]

A pooled analysis of six platinum-based neoadjuvant studies revealed HRD as a predictor of the likelihood of a pathological response regardless of *BRCA* mutation status, and TNBC patients with an HRD score ⩾42 and/or *BRCA1/2* mutation had an increased pCR rate compared with non-deficient patients (53% *versus* 18%, respectively).[125]

Current genetic and genomic tests detecting HRD have limited predictive value for treatment optimization, thus paving the way to additional indicators of the homologous recombination repair. An alternative functional marker called RAD51 was recently generated to accurately detect HRD and predict patient response to carboplatin or PARP inhibitors in patients with TNBC. In the phase 2 PErsonalized TREatment of High-risk MAmmary Cancer (PETREMAC) trial, 18 of 32 patients with primary TNBC treated with olaparib for up to 10 weeks before chemotherapy obtained an objective response. Of 18 responders, 10 patients had somatic or germline mutations in homologous recombination repair pathway and 6 patients had a *BRCA1* hypermethylation. In addition, low RAD51 scores, high tumor-infiltrating lymphocytes (TILs), and high programmed death–ligand 1 (PD-L1) expression levels correlate to the olaparib response.[126] An exploratory study of the RIO trial (EudraCT 2014-003319-12) in treatment-naïve patients with early-stage TNBC who received the PARP inhibitor rucaparib showed that 75% of tumors with known homologous recombination repair alterations had low RAD51 foci at baseline. Of the HRD-positive tumors, 70% had low RAD51 foci, while none of the HRD-negative tumors had low RAD51 foci.[127] Recently, in a retrospective analysis from the GeparSixto trial, the RAD51 assay showed a high concordance with tumor *BRCA* status or genomic HRD score of TNBC patients who received neoadjuvant paclitaxel plus non-pegylated liposomal doxorubicin and bevacizumab with or without carboplatin, supporting further development to incorporate the RAD51 analysis in clinical decision making.[128]

The international guidelines highlight the importance of identifying carriers of *BRCA1/2* mutations at risk of an inherited disease as early as possible. However, prospective clinical trials and larger retrospective meta-analyses are needed to assess the clinical utility of innovative markers of deficient homologous recombination repair in decision-making processes.

Immunotherapy. TNBCs have the highest levels of TILs, expression of immune evasion molecules in a tumor microenvironment such as PD-L1, and the most genomic instability compared with other breast cancer subtypes, which consequently makes it an immunogenic disease. Recently, results from several clinical studies showed that immune checkpoint inhibitors alone or in combination with a backbone (chemotherapy-induced) had strong clinical activity in patients with metastatic TNBC, supporting their use in early settings.

The Keynote-522 phase 3 trial was the first to demonstrate the effectiveness of an immunotherapy-based regimen in a neoadjuvant setting. In this study, the addition of pembrolizumab to standard neoadjuvant chemotherapy resulted in a significant increase in pCR rate compared with placebo plus neoadjuvant chemotherapy (64.8% *versus* 51.2%). At 36 months, patients who were alive without disease progression, local or distant recurrence, and without a second primary tumor were 84.5% and 76.8% in the pembrolizumab plus chemotherapy group and the placebo plus chemotherapy group, respectively, not reaching the median in either group.[129]

In the open-label, adaptively randomized, I-SPY2 platform, phase 2 trial, patients with high-risk, stage II/III breast cancer achieved a 40% improvement in the probability of pCR with pembrolizumab plus standard neoadjuvant chemotherapy compared with the control arm (60% *versus* 22%).[130]

The phase 2 randomized GeparNUEVO study showed that the addition of anti-PD-L1 durvalumab to neoadjuvant anthracycline- and taxane-based regimen did not increase the pCR rate

(53.4% in the durvalumab arm *versus* 44.2% in the placebo arm), raising the question of whether pCR represents an appropriate endpoint to catch the long-term benefit of the immune response.[131] After a median follow-up of 42.2 months, 3-year invasive DFS was 84.9% *versus* 76.9% ($p = 0.0559$) and 3-year OS was 95.1% *versus* 83.1% ($p = 0.0076$) for durvalumab *versus* placebo, respectively. Despite the improved long-term outcome, further investigations are required to clarify whether adjuvant therapy with durvalumab is needed at all.[132]

The double-blind, randomized, phase 3 study IMpassion031 trial achieved improved pCR rates with the addition of atezolizumab to sequential nab-paclitaxel and anthracycline-based neoadjuvant chemotherapy in patients with previously untreated stage II or III TNBC. pCR response was significantly documented both in the intent-to-treat population (58% of the atezolizumab group *versus* 41% of the control group) and the PD-L1-positive population (69% of the atezolizumab group *versus* 49% of the control group), with toxicity balanced among treatment groups. Although the IMpassion031 is not powered for EFS and OS, trends in long-term efficacy endpoints seem to be suggestive of benefit for the atezolizumab plus chemotherapy combination.[133]

The recently released data from the primary analysis of the IMpassion050 study showed no benefit from adding atezolizumab to preoperative atezolizumab plus pertuzumab, trastuzumab, and chemotherapy in terms of pCR both in the overall population and PD-L1-positive tumors (62.4% in the atezolizumab arm *versus* 62.7% in the placebo arm).[126]

Given the high heterogeneity of TNBC, it is opportune to conceive biology-driven clinical trials wherein patients may be treated based on their specific tumor molecular profile.

Targeting cell surface targets by antibody-drug conjugates. The AR is expressed in 12–36% of all TNBC patients and could represent a promising strategy for the treatment of TNBC expressing AR on immunochemistry. Endocrine therapy, such as enzalutamide, prevents the androgen from binding to the AR, and hence blocks cell proliferation and induces tumor cell death. A neoadjuvant trial (ClinicalTrials.gov identifier: NCT02689427) is currently ongoing to determine the efficacy and safety of enzalutamide plus paclitaxel in patients with stages I–III AR-positive TNBC.

Different studies (GeparQuinto,[134] ARTemis,[135] CALGB 40603/Alliance)[101] have evaluated the anti-angiogenic agent bevacizumab in combination with neoadjuvant chemotherapy in early TNBC. In two large, randomized studies (BEATRICE[136] and E5103),[137] bevacizumab with standard adjuvant chemotherapy failed to indicate a favorable effect in terms of DFS in patients with TNBC. Despite the pCR rate achieved ranged from 40% to 59%, bevacizumab-containing regimens were associated with an increase in postoperative complications, a reason why its use as neoadjuvant treatment is still controversial and not recommended.

Sacituzumab govitecan is a novel antibody-drug conjugate with the topoisomerase 1 inhibitor SN-38 coupled to an anti-Trop-2 antibody that was recently for the treatment of patients with advanced TNBC.[138] The NeoSTAR phase 2 trial is evaluating sacituzumab govitecan as a response-guided neoadjuvant treatment for patients with localized TNBC in terms of pCR. After four cycles, patients with a pCR may proceed directly to surgery, while patients with any residual disease could receive additional standard neoadjuvant therapy, then proceed to surgery.[139]

The sequencing of EBC has been enabling the molecular characterization of the tumors by identifying multiple potentially actionable targets. In this context, several biomarker-based neoadjuvant trials are currently ongoing to evaluate the efficacy of targeted therapies according to distinct gene signatures aiming to identify a specific population to include in future confirmatory phase 3 trials.

Hormone receptor-positive/HER2-negative, EBC

Assessing response by imaging. Clinical exploration, imaging (mammography, ultrasound, and/or MRI), and pathological examinations of sectioned surgical tissue samples are the usual means of assessing tumor response to presurgical treatment. However, because of changes within the tumor such as post-therapy fibrosis or undefined tumor margins, conventional tumor measurements *via* physical examination are 20% less accurate than mammography and ultrasound in assessing pCR in patients with locally advanced breast cancer after neoadjuvant endocrine or systemic treatment.[140] Despite the lack of confirmatory data from large, prospective phase 3 trials,

combining mammography and ultrasound is a reliable way to predict the presence of a disease from the final pathology of patients with primary breast cancer with an 80% of prediction likelihood.[141]

Dynamic contrast-enhanced MRI remains the most sensitive available imaging modalities in differentiating residual tumor from pCR after neoadjuvant therapy by identifying changes in tumor vascularity.[142,143] In addition, MRI could also predict that patients with minimal background parenchymal enhancement would benefit most from neoadjuvant endocrine therapy compared with patients with a higher degree of baseline parenchymal enhancement.[144]

^{18}F-FDG PET imaging has proven to assess metabolic changes in response to neoadjuvant endocrine therapy with a marked correlation between the maximum standardized uptake volume (SUVmax) and Ki67 levels in the tumor,[145,146] and thus represents a surrogate marker to monitor tumor response after preoperative therapy.[146]

The utilization of new radionuclides such as ^{18}F-fluoroestradiol is a valid alternative to the common ^{18}F-FDG for low-proliferating luminal A tumors with modest glucose metabolism. Interestingly, tumor avidity to ^{18}F-fluoroestradiol can be considered a pharmacodynamic biomarker that has the potential to predict response to neoadjuvant therapy. In the randomized NEOadjuvant Chemotherapy versus ENdocrine Therapy (NEOCENT) study, postmenopausal women with ER-positive/^{18}F-fluoroestradiol-PET-negative EBC benefit most from neoadjuvant systemic therapy rather than neoadjuvant endocrine therapy.[147]

Overall, the integration of dynamic or functional imaging procedures to the conventional ultrasound or mammography can result in a greater accuracy monitoring neoadjuvant treatment response. Further research is warranted in this context to confirm whether novel radiotracers for PET scans in combination with MRI could offer advantages for therapy response assessment.

Neoadjuvant targeted therapies. Efforts to prevent early recurrences and development of metastases as well as improve survival in patients with hormone receptor-positive/HER2-negative EBC are ongoing. Apart from extending the duration of endocrine treatment or developing new endocrine agents, there is a great deal of interest in generating novel treatment strategies.

According to their design, studies focusing on neoadjuvant endocrine treatment in combination with targeted therapies can be divided into at least five types:

1. Classic neoadjuvant trials with a 3- to 6-month treatment period followed by surgery, such as the IMPACT[148] or RAD001 study.[149] Despite the preliminary assessment of the complete cell cycle arrest (CCCA, in terms of Ki67 levels $<2.7\%$ on tissue biopsy 2 or 3 weeks from treatment start) rate, no therapy modification is introduced.
2. Enrichment-adaptive design trials such as the ALTERNATE,[150] ACOSOG Z1031-B,[151] and WSG-ADAPT HR+/HER2 studies, where patients could either receive endocrine therapy alone until the surgery or switch to alternative therapies depending on the level of Ki67 tumor suppression as determined from the 2- to 3-week tissue biopsy.
3. Multi-arm, lead-in design trials such as the LORELEI[152] or PALbociclib LETrozole (PALLET)[153] studies, analyzing the ability of short-term preoperative endocrine therapy with or without an investigational drug to induce Ki67 reduction in 2 weeks. After the post-induction Ki67 assessment, patients will receive a 4- to 6-month treatment regimen with endocrine therapy plus the investigational drug until the surgery.[152,153]
4. Single-arm trials with multiple tissue biopsies such as the neoMONARCH study[154] assessing the efficacy of CDK4/6 inhibitors in patients irresponsive to aromatase inhibitor regimen.
5. Window of opportunity trial such as the POETIC,[45,46] Mammary ONcology Assessment of LEE011's Efficacy and SAfety (MONALEESA)-1,[155] ARB,[156] and coopERA Breast Cancer[157] studies, where the biomarker analysis during the 2- or 3-week period of preoperative therapy between diagnosis and primary surgery will provide additional data on molecular mechanisms of action of a drug, prove the feasibility of candidate predictive biomarkers, or assess drug efficacy.

The addition of the three currently approved CDK4/6 inhibitors (palbociclib, ribociclib, abemaciclib) to endocrine therapy has been the most

successful strategy in the treatment of hormone receptor-positive/HER2-negative advanced breast cancer by improving PFS and even OS significantly compared with endocrine therapy alone, with a favorable toxicity profile.[158–164] Successes obtained in the metastatic setting have prompted investigation of this therapeutic approach in EBC.

A preplanned interim analysis of the randomized, open-label phase 3 monarchE trial indicated that adding abemaciclib to standard-of-care adjuvant ET reduces the risk of disease recurrence of patients with hormone receptor-positive/HER2-negative high-risk EBC (the estimated 3-year invasive DFS rates were 88.8% for abemaciclib plus endocrine therapy *versus* 83.4% for endocrine therapy alone).[165] The clinically meaningful benefit of abemaciclib even beyond the 2-year treatment period in these patients granted the recent approval by the FDA.

In contrast to monarchE, the randomized phase 3 PALbociclib CoLlaborative Adjuvant Study (PALLAS) trial showed no benefit after 2 years of palbociclib with endocrine therapy in terms of risk reduction for disease recurrence compared with standard-of-care endocrine therapy in patients with hormone receptor-positive/HER2-negative EBC (the estimated 3-year invasive DFS was 88.2% for palbociclib plus endocrine therapy *versus* 88.5% for endocrine therapy alone).[166] Thus, although a longer follow-up from monarchE is required, a new potential standard of care has been established in the adjuvant setting for patients with high-risk luminal EBC.

Moving to the neoadjuvant setting, in the NeoPalAna trial, patients with stage II-III ER-positive/HER2-negative breast cancer were initially treated with anastrozole alone for 4 weeks, followed by anastrozole plus palbociclib for 4 months. The primary endpoint, the CCCA, was significantly higher at cycle 1 day 15 with palbociclib plus anastrozole than at cycle 1 day 1 with anastrozole alone (87% *versus* 26%, respectively).[167]

Subsequent studies demonstrated that adding CDK4/6 inhibitors to neoadjuvant endocrine therapy led to higher cycle arrest rates. Although the clinical response was not significantly different between the treatment groups, the PALLET trial revealed that more patients on palbociclib plus letrozole achieved CCCA than the letrozole groups (90% *versus* 59%, respectively).[153]

In phase 2 neoMONARCH study, women were randomized to receive either abemaciclib plus anastrozole, abemaciclib alone, or anastrozole alone for the first 2 weeks. After undergoing a second biopsy, patients received the abemaciclib plus anastrozole for 14 weeks and more patients in the abemaciclib-containing arms *versus* anastrozole alone achieved CCCA (58% and 68% *versus* 14% in the abemaciclib plus anastrozole, abemaciclib alone, and anastrozole alone, respectively).[154]

The randomized, phase 2 NeoPAL study compared the combination of letrozole plus palbociclib with a systemic regimen of 5-fluorouracil, epirubicin, and cyclophosphamide followed by docetaxel in patients with PAM50-determined luminal B or luminal A and node-positive who were not a candidate for breast-conserving surgery. At surgery, the proportion of patients with a Residual Cancer Burden index of 0–1 was not statistically different between both treatment arms (7.7% in letrozole plus palbociclib *versus* 15.7% in chemotherapy).[168]

The ongoing phase 2 DxCARTES trial (ClinicalTrials.gov identifier: NCT03819010) aims to explore the ability of preoperative letrozole plus palbociclib to induce global molecular downstaging in two cohorts of high-risk patients, luminal B EBC, with pretreatment RS 18–25 or RS 26–100 using the Oncotype DX Breast RS® assay.[169]

Also, ribociclib has been evaluated in the neoadjuvant setting and the CORALLEEN trial postmenopausal women with PAM50-determined luminal B breast cancer were randomized to receive either six cycles of letrozole plus ribociclib or a standard anthracycline- and taxane-based chemotherapy similar to the design of the NeoPAL trial. At surgery, a similar proportion of patients achieved molecular downstaging of their disease in terms of PAM50-determined low risk of relapse in both treatment arms (46.9% in the ribociclib plus letrozole group and 46.1% in the chemotherapy group).[168] Taken together, despite being insufficiently conclusive to adopt this strategy as a standard of care, findings from NeoPAL and CORALLEEN trials suggest that a high proportion of patients achieve molecular downstaging of clinically high-risk breast cancer after a chemotherapy-free strategy, providing evidence for additional future investigations on long-term survival outcomes.

Hormone receptor-positive breast cancers are considered 'cold' tumors due to their lower levels of TILs, higher tumor mutational burden, PD-L1, and human leukocyte antigen expression compared with other breast tumor subtypes.[9,170] A strategy adopted in the I-SPY2 study involved increasing immunotherapy responsiveness within the luminal subtype of breast cancer by combining neoadjuvant chemotherapy with pembrolizumab. In this study, 30% of patients achieved pCR and the 3-year EFS for patients who achieved pCR was 93%, suggesting the activity of this immunotherapy could be similar to what is observed in TNBC.[130]

In the ULTIMATE phase 2 study (ClinicalTrials.gov identifier: NCT02997995), patients will receive a single infusion of the immune attractant tremelimumab plus exemestane followed by a 6-month regimen of durvalumab plus exemestane only for patients with CD8 tumor infiltration greater than 10%. The primary objective is the rate of pCR.

The ongoing Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging And moLecular Analysis (I-SPY TRIAL) (ClinicalTrials.gov identifier: NCT01042379) is analyzing the efficacy and safety of the combination of pembrolizumab with paclitaxel in both hormone receptor-positive/HER2-negative breast cancer and TNBC.

Finally, two ongoing randomized phase 3 trials [the Keynote756[171] (ClinicalTrials.gov identifier: NCT03725059) and Checkmate7FL[172] (ClinicalTrials.gov identifier: NCT04109066)] promise to provide a firm conclusion regarding the benefit of adding immune checkpoint inhibitors pembrolizumab or nivolumab to neoadjuvant chemotherapy in patients with luminal high-risk EBC. Both studies present improved pCR (ypT0/is, ypN0) and EFS as co-primary endpoints.

Conclusion

The neoadjuvant setting has long represented the optimal scenario for testing the clinical activity of new treatments. Efficacy and safety information is usually obtained faster and with fewer patients than those accrued into adjuvant trials. Using EBC as an example, we evaluated the underlying evidence for the surrogate endpoints listed in the FDA's Table of Surrogate Endpoints. Regulatory agencies have accepted pCR as a valid surrogate marker in the neoadjuvant setting to accelerate drug approval for high-risk patients with breast cancer and ensuring that positive results in terms of DFS/OS are ultimately obtained. The prognosis of patients with highly aggressive tumors who achieve pCR is similar to that of patients with less aggressive tumors. Thus, pCR could be considered an adequate intermediate endpoint for studies involving patients with TNBC, HER2-positive, and high-grade hormone receptor-positive breast cancers.

Historically, hormone receptor-positive/HER2-negative breast cancers – especially of the luminal A subtype – pose a considerable therapeutic challenge due to a minimal response to neoadjuvant systemic chemotherapy. In these patients, Ki67 levels and PEPI score represent the most accepted surrogate markers for neoadjuvant treatment benefit. The availability of multigene signatures – such as Oncotype DX® and MammaPrint® that have been validated by prospective, randomized phase 3 trials – is currently paving the way for estimating the ROR and survival after neoadjuvant therapy.

In HER2-positive breast cancer, the main strategical aim over the last decade is to potentiate the efficacy of standard treatment with chemotherapy plus trastuzumab and pertuzumab for high-risk patients and develop chemotherapy de-escalation regimens for patients less likely to relapse.

For TNBC, anthracycline- and taxane-based chemotherapy remains the mainstay of systemic treatment in its early stages. However, PARP inhibitors in patients with impaired homologous recombination repair and immune checkpoint inhibitors targeting the anti-PD1/PD-L1 axis have generated encouraging results for the treatment of early TNBC.

Finding the optimal balance between timely access to new drug therapies and ensuring its efficacy is mandatory to safeguarding patients' health. Thus, regardless of breast cancer subtype, the preliminary results obtained in small neoadjuvant trials on prognosis-related factors or early biomarkers of response/resistance to the treatment deserve further evaluation to validate their efficacy in confirmatory, well-conducted clinical trials. Future strategies to tailor systemic treatments for EBC will depend upon genomic signatures that will be further implemented in the

clinic. Integrative modeling algorithms will consider both clinical and biological tumor characteristics to describe the unique molecular portrait associated with a tumor and to escalate or de-escalate treatment with less chemotherapy, fewer targeted drugs, or shorter duration.

Author contributions

All authors involved in conception and design, contributed to the review of literature, helped in critical revision of the manuscript, and have read and agreed to the published version of the manuscript. A.M. helped in drafting the first version of the manuscript and final editing.

Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Gion reported having a consulting role for Roche and Pfizer. Dr. Pérez-García reported having a consulting role for Roche, Lilly, and Daichii-Sankyo; receiving travel compensation from Roche; to being part-time employee of Medica Scientia Innovation Research (MEDSIR) during the conduct of the study. Dr. Llombart-Cussac reported playing a leadership role at Eisai, Celgene, Lilly, Pfizer, Roche, Novartis, and Merck Sharp & Dohme (MSD); intellectual property for MEDSIR and Initia-Research; a consulting role for Lilly, Roche, Pfizer, Novartis, Pierre-Fabre, Genomic Health, and GlaxoSmithKline (GSK); to be part of the speaker bureau for Lilly, AstraZeneca, and MSD; to receive research funding from Roche, Foundation Medicine, and Pierre-Fabre, and Agendia, and travel compensation from Roche, Lilly, Novartis, Pfizer, and AstraZeneca during the conduct of the study. Mr. Sampayo-Cordero reported receiving honoraria from MEDSIR, Syntax for Science, and Nestlé; research funding from MEDSIR, Syntax for Science, and Roche; travel compensation from MEDSIR, Syntax for Science, and Roche; serving as a consultant to MEDSIR, Syntax for Science, and Nestlé; to being on the speaker bureau for MEDSIR, Syntax for Science, Roche; to being part-time employee of MEDSIR during the conduct of the study. Dr. Cortés reported serving as a consultant to Roche, Celgene, Cellestia, AstraZeneca, Biothera Pharmaceutical, Merus, Seattle Genetics, Daiichi Sankyo, Erytech, Athenex, Polyphor, Lilly, Servier, MSD, GSK, Leuko, Bioasis, and Clovis Oncology; providing intellectual property to MEDSIR; receiving honoraria from Roche, Novartis, Celgene, Eisai, Pfizer, Samsung Bioepis, Lilly, MSD, and Daiichi Sankyo; receiving institutional research funding from Roche, Ariad Pharmaceuticals, AstraZeneca, Baxalta GMBH/Servier Affaires, Bayer Healthcare, Eisai, Hoffman-La Roche, Guardant Health, MSD, Pfizer, Piqur Therapeutics, Puma C, and Queen Mary University of London during the course of the study. Dr. Malfettone is a full-time employee of MEDSIR.

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