Preoperative systemic therapy (PST) has emerged from an infrequently used approach in the management of patients with breast cancer to one with an established role in both inoperable and operable breast cancer. In particular, it now has an established role in drug development and biomarker discovery programs. In this era of molecular-based therapies, the unique resource of paired pre- and post-treatment tissue in PST trials represents a powerful research tool for the \textit{in vivo} study of biologic mechanisms of systemic therapy. It is possible to examine downregulation of signaling pathways that are known to be activated, and off-target effects through the identification of pathways not previously known to be activated. In addition, correlative studies may identify predictive biomarkers of response and resistance, which has the potential to inform the design of larger and more expensive adjuvant trials. The aims of this article are to review key developments in PST trials, particularly in specific breast cancer subtypes, and identify areas in which PST trials can facilitate drug development and rapidly translate its findings into clinical practice.

**Keywords:** breast cancer subtype • midcourse response assessment • pathological complete response • predictive biomarker • surrogate end point

Preoperative (also known as primary or neoadjuvant) systemic therapy (PST), in which systemic therapies are administered between diagnosis and definitive surgery, is now widely used in the management of locally advanced breast cancer and in women with relatively large tumors who are interested in breast conservation. Moreover, PST is used increasingly to evaluate new therapeutic approaches in patients with early-stage breast cancer. It holds great promise as a research tool to study the biologic impact of systemic therapy on breast tumor cells through the availability of pre- and post-treatment tissue in treatment-naive patients. PST represents a fertile setting for tissue-intensive correlative research to identify predictive biomarkers of response and resistance. This approach is in contrast to conventional drug development pathways in which new treatments are initially established in the metastatic setting and subsequently evaluated in the adjuvant setting (Table 1). This conventional process can be both protracted and expensive, and it is both unusual and difficult to obtain tissue in patients with metastatic disease for correlative studies. Few studies in the metastatic setting include mandatory tissue biopsies, and when biopsies are optional in this context, only a small minority of patients has tissue obtained. Increasingly, a drug development paradigm without tissue-based studies is inadequate for rational drug development of agents directed against molecular targets, particularly if the therapy is only active in tumors with a particular targeted molecular alteration or phenotype.

Novel therapies may be integrated with standard treatments in the preoperative setting using various strategies, including the use of targeted therapy prior to standard PST that allows for the performance of correlative studies on treatment-naive tumors (Figure 1). A variation of PST is the window of opportunity study.
Table 1. Paradigms for drug development.

<table>
<thead>
<tr>
<th>Initial testing of novel agents</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic</td>
<td>Shorter duration to survival and progression end points</td>
<td>Patients are often heavily pretreated and effects of novel agents may be masked by previous therapies</td>
</tr>
<tr>
<td></td>
<td>Established path for translation into the adjuvant setting</td>
<td>Limited availability of tissue for correlative studies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biomarkers for predicting response not always well established</td>
</tr>
<tr>
<td>Preoperative</td>
<td>Treatment-naive patients</td>
<td>Potential delay to definitive curative treatment if experimental drugs are ineffective</td>
</tr>
<tr>
<td></td>
<td>Availability of paired pre- and post-treatment tissue for correlative studies, including identification of predictive biomarkers to specific therapies</td>
<td>Treatment decisions based on diagnostic biopsy may result in sampling error in cases of intra-tumoral heterogeneity</td>
</tr>
<tr>
<td></td>
<td>Early introduction of systemic chemotherapy</td>
<td>Long duration to survival and progression end points</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Surrogate end points of survival need to be validated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Effects of long-term toxicity need to be considered</td>
</tr>
</tbody>
</table>

in which short-term treatment with novel therapies is administered during the interval between diagnostic biopsy and standard PST or planned surgery. The goal of this approach is not to downsize the tumor nor improve breast-conserving surgery (BCS) rates, but to utilize the availability of pre- and post-treatment tissue; the primary goal of window of opportunity studies is to identify pharmacodynamic end points and other correlative analyses. Such an approach has been used successfully to identify predictive biomarkers for endocrine and HER2-directed therapies [1,2]. The goal of biomarker discovery in PST trials is to identify surrogate end points of clinical outcomes, such as biomarkers that predict therapeutic response or resistance to specific therapies. Correlative tissue studies in PST can be valuable in the study of mechanisms of primary resistance in nonresponding patients.

By facilitating efficient testing of novel therapies and identification of predictive biomarkers, PST trials can provide preliminary data to guide decisions as to whether to proceed to larger and more expensive adjuvant trials. Increasingly, preoperative and adjuvant trials of novel therapies are planned together. Results from PST trials not only establish the activity of novel therapies, but also help identify predictors of therapeutic benefit that can be used for patient selection and can be evaluated in the context of adjuvant trials. This article aims to identify areas in which PST trials can facilitate drug development and rapidly translate their findings into clinical practice.

Lessons from the development of PST trials in breast cancer

Historically, the goal of PST was to improve the operability of breast cancer in the setting of inoperable locally advanced breast cancer (LABC) and inflammatory breast cancer (IBC). In a subset of these patients, PST resulted in downstaging of the primary tumor and improved operability. PST has also resulted in improved survival rates when compared with historical controls managed with local therapy alone [3–5], and has consequently established itself as the initial management of choice in LABC and IBC [6]. Interest in PST subsequently shifted into the arena of operable breast cancer and was focused primarily on chemotherapy-based regimens, initially due to the relatively shorter duration of treatment required. In more recent years, the interest in PST has extended to endocrine and novel therapies.

There are four distinct periods in PST trials in operable breast cancer to date. The first evaluated established adjuvant anthracycline regimens in the preoperative setting (Table 2) [7–13]. End points assessed included tumor response and BCS rates, as well as long-term outcomes such as overall survival (OS) and disease-free survival (DFS). PST was typically well tolerated and did not result in problems related to the surgery or radiotherapy that followed. Clinical objective responses (OR) were typically good and a small but significant subgroup of patients attained a pathological complete responses (pCR). As a result, there was an increase in the proportion of patients offered BCS instead of traditional adjuvant therapy. In most of these trials, patients with a pCR had improved survival rates with longer-term follow up, regardless of the therapy administered or breast cancer subtype [7,8]. As a result, pCR was established as a valuable end point in assessing new therapies and established its role as a prognostic marker. A recent Cochrane meta-analysis of eight randomized studies of 4620 women, comparing preoperative and adjuvant chemotherapy for operable breast cancer, demonstrated equivalent OS rates with a hazard ratio of 0.98 (0.87–1.09; p = 0.67) [14]. PST was associated with fewer adverse effects and higher rates of BCS (hazard ratio: 0.71, 0.67–0.75; p < 0.001). In addition, patients who achieved a pCR had a better survival than those who had residual disease in the breast and lymph nodes (hazard
ratio: 0.48; 0.33–0.69; p < 0.001). PST was associated with a small increase in the risk of loco-regional recurrence in patients who went on to receive radiotherapy without surgery as local therapy [14,15].

The second phase of PST trials focused on improving tumor response and BCS rates through the optimization of chemotherapy combinations and scheduling in the hope that these changes would translate into improved long-term outcomes. The addition of taxanes to anthracycline-based regimens in the preoperative setting have resulted in improved pCR rates and survival outcomes, independent of hormone receptor (HR) status [7,16,17]. In the National Surgical Breast and Bowel B-27 trial, the addition of docetaxel to doxorubicin and cyclophosphamide (AC) resulted in a higher pCR rate compared with AC alone (26.1 vs 12.8%) [18]. A third treatment arm in this trial consisted of preoperative AC followed by adjuvant docetaxel. Whilst pCR remained a significant predictor of OS and DFS, unexpectedly, the differences in pCR rates did not translate into improvements in long-term outcomes for the three treatment arms, with no statistically significant differences in OS and DFS after 8 years of follow-up [7]. Possible explanations include the potential dampening of chemotherapy benefit with concurrent tamoxifen given to hormone receptor (HR)-positive patients and tumor heterogeneity from a lack of stratification of breast cancer subtypes. Many patients who did not achieve a pCR nonetheless did well, and obtaining a pCR in these patients by administering additional therapy prior to surgery would have no impact on DFS or OS. Alternatively, it may be the ability to achieve pCR rather than necessarily achieving it that is important. If this is the case, then combining therapies with the explicit goal of achieving a pCR may be misguided. Regardless of the explanation, at this time pCR remains a less than adequate surrogate for DFS or OS when one is comparing two different treatment approaches.

The optimal duration of preoperative anthracycline-taxane regimens was assessed in the Austrian Breast Cancer Study Group Trial-14 trial, which evaluated three versus six cycles of epirubicin and docetaxel.
There was a threefold increase in the pCR rates (15.9 vs 4.9%; p = 0.011), and a smaller increase in BCS rates (76 vs 67%; p = 0.01) with longer treatment [19]. As a result of these and other studies, most current guidelines recommend six to eight cycles of PST. Attempts at improving pCR rates through the addition of other chemotherapy agents and intensifying dosing schedules have not resulted in consistent additional benefits in pCR and BCS rates.

Patients who do not achieve a good clinical response after two to four cycles of PST represent a subgroup who are chemotherapy resistant, with a pCR rate of approximately 5% [20–23], and a high risk of recurrence in the long-term. Several groups have used clinical mid-course response assessments during PST as a decision aid to guide subsequent therapy in either the preoperative or adjuvant settings; however, there is still a lack of consistent improvement in outcome with such an approach using conventional chemotherapy [21,24]. Patients with poor interim response to PST may represent an ideal patient cohort to trial novel agents, particularly agents with a different mechanism of action.

The third phase of PST trial development has involved the adoption of different strategies to treat different subtypes of breast cancer. Early PST trials were predominantly conducted in unselected breast cancer subtypes, resulting in heterogeneous responses to different systemic therapies. There was limited ability to determine if these differences in survival were as a result of tumor growth and metastatic potential or differential treatment sensitivity. Critical studies highlighted differences in pCR rates between breast cancer subtypes (Table 3) [25,26], and as a result, subsequent PST trials have centered on assessing novel agents and the identification of predictive biomarkers in different subtypes.

Table 2. Pivotal Phase III trials comparing preoperative and adjuvant systemic therapy in operable early stage breast cancer.

<table>
<thead>
<tr>
<th>Treatments (no. of cycles)</th>
<th>Stage Patients/ follow-up (years)</th>
<th>pCR (%)</th>
<th>Clinical outcomes</th>
<th>pCR vs non-pCR</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSABP B-18: AC (×4)</td>
<td>T1–3 N0–1</td>
<td>1493/16</td>
<td>13</td>
<td>PST vs adjuvant OS: HR = 0.99 DFS: HR = 0.93 BCS: 68 vs 60%†</td>
<td>OS: HR = 0.32†</td>
</tr>
<tr>
<td>EORTC 10902: FE60C (×4)</td>
<td>T1–4 N0–1</td>
<td>698/10</td>
<td>3.7</td>
<td>PST vs adjuvant OS: HR = 1.09 DFS: HR = 1.12 BCS: 35 vs 22%</td>
<td>OS: HR = 0.91</td>
</tr>
<tr>
<td>ECTO: AP (× 4) → CMF (x4)</td>
<td>T1–3 N0–1</td>
<td>892/6.3</td>
<td>20</td>
<td>PST vs adjuvant OS: HR = 1.10 RFS: HR = 1.21 BCS: 65 vs 34%†</td>
<td>RFS: HR = 0.43†</td>
</tr>
<tr>
<td>ABCSG-07‡ CMF (×3)</td>
<td>T1–3 N0–2</td>
<td>423/9</td>
<td>5.9</td>
<td>Adjuvant vs PST OS: HR = 0.8 RFS: HR = 0.7 BCS: 66 vs 60%</td>
<td>NR</td>
</tr>
</tbody>
</table>

†p < 0.05.
‡ Patients received further adjuvant systemic therapy.

A: Doxorubicin; ABCSG: Austrian Breast Cancer Study Group; BCS: Breast cancer survival; C: Cyclophosphamide; DFS: Disease-free survival; E: Epirubicin; ECTO: European Cooperative Trial in Operable Breast Cancer; EORTC: European Organisation for Research and Treatment of Cancer; F: 5-fluorouracil; HR: Hazard ratio; M: Methotrexate; NR: Not reported; NSABP: National Surgical Breast and Bowel Trial; OS: Overall survival; P: Paclitaxel; pCR: Pathological complete response; PST: Preoperative systemic therapy; RFS: Relapse-free survival.

Table 3. Comparison of pathological complete response and clinical response rates with preoperative anthracycline and taxane chemotherapy in stage II and III breast cancer by cancer subtype.

<table>
<thead>
<tr>
<th>End point</th>
<th>Basal/TNBC (%)</th>
<th>Luminal/HR:HER2 (%)</th>
<th>HER2-amplified/HER2:HR (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCR</td>
<td>24.2–45</td>
<td>1.8–7</td>
<td>7.7–45</td>
<td>[26]†</td>
</tr>
<tr>
<td>Clinical OR</td>
<td>85</td>
<td>15</td>
<td>70</td>
<td>[25,27]†</td>
</tr>
</tbody>
</table>

†mRNA used to define molecular subtypes.
‡ Retrospective series.
The fourth and current phase of PST development is characterized by the integration or coupling of preoperative and adjuvant trials with the aim to efficiently translate knowledge of preliminary data gleaned from PST trials to more comprehensive and more expensive adjuvant trials. Proof of principle of this approach was obtained retrospectively from the Immediate Preoperative Anastrozole, Tamoxifen, or Combined With Tamoxifen (IMPACT) trial, a Phase III PST trial comparing anastrozole, tamoxifen and a combination of the two in postmenopausal women with HR-positive tumors [28]. The same treatment arms were used in an earlier adjuvant Arimidex, Tamoxifen Alone or in Combination (ATAC) study [29], and the investigators had set out to determine if the PST results would predict for the long-term outcome in the adjuvant setting. Although the clinical OR rate, which was the primary end point of this study, did not predict for long-term outcome in the ATAC trial, reduction in Ki67 after 2 and 12 weeks of treatment was significantly higher in the anastrozole compared with the tamoxifen treatment arms [1]. This result mirrors the DFS results seen in the adjuvant ATAC study, suggesting that early and late changes in proliferation after short-term PST may be predictive of outcome in the adjuvant setting. Another example is the concurrently run Neo-tAnGo and tAnGo trials, which sought to determine the benefit of adding gemcitabine to epirubicin, cyclophosphamide and paclitaxel in the treatment of high-risk early breast cancer in the preoperative and adjuvant settings [30,31]. The PST Neo-tAnGo trial included correlative studies with molecular profiling, proteomics and candidate gene analysis. The Neo-tAnGo results confirm those of the adjuvant tAnGo trial with no improvement of DFS and OS with the addition of gemcitabine. A similar approach is used in the neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (Neo-ALTTO) trial [101] and the Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (ALTTO) trial [102]. The results of these two trials have yet to be reported, and it is anticipated that this parallel strategy would lead to the identification of biomarkers that would allow for the early detection of benefit from trastuzumab and lapatinib and optimize patient selection in the adjuvant setting.

**Lessons from PST trials in breast cancer subtypes**

The identification of molecular subtypes of breast cancer using gene profiling studies has been a major advance in understanding the heterogeneity of treatment response and survival [32–34]. At a minimum, breast cancer is now divided into three major subtypes based on the pattern of expression of hormone receptors, estrogen receptor (ER) and progesterone receptor (PR), and HER2; comprising HR-positive, HER2-amplified and triple-negative breast cancer (TNBC) subtypes. The basal/TNBC and HER2-amplified subtypes have the highest pCR rates to preoperative chemotherapy (22–62% and 36–45%, respectively) compared with the luminal/HR-positive subtype (0.5–7%) (Table 4) [22,26,35–38]. Pathologic CR has therefore been used most commonly as an end point in PST trials of HER2-amplified and TNBC subtypes [25,26]. Despite higher pCR rates in basal/TNBC tumors, poorer outcomes persist due to a higher relapse rate in the non-pCR subgroup. A recent retrospective analysis of patients receiving PST at the MD Anderson Cancer Center from 1985 to 2004 identified 255 out of 1118 patients with TNBC [39]. TNBC was associated with higher pCR rates compared with non-TNBC (22 vs 11%; p<0.05), and patients with residual disease after PST had significantly decreased OS compared with patients with non-TNBC and residual disease (hazard ratio: 1.5; p < 0.0001). The highest pCR rates in TNBC were observed for patients treated with taxane followed by anthracycline regimens.

**BRCA1**-associated tumors have characteristic DNA-repair defects that confer sensitivity to cisplatin and poly [ADP-ribose] polymerase inhibitors [40,41]. As the gene signatures of the basal/TNBC subtype co-localize with **BRCA1** mutation-associated tumors, these novel therapeutic approaches may be relevant to TNBC [42], and are now being assessed in the preoperative setting. Recent preoperative trials have demonstrated encouraging anti-tumor activity with platinum chemotherapy although the numbers of patients analyzed were small [36–38]. A recent large retrospective analysis found that unlike the basal/TNBC subtype, **BRCA1** mutation-associated tumors have a low pCR rate following combination chemotherapy with doxorubicin–docetaxel and oral cyclophosphamide/methotrexate/fluorouracil (CMF; 8 and 7%, respectively) and a high response rate to cisplatin (83%) [40], suggesting that the chemotherapy response profile may differ between the two groups. The basal/TNBC subtype is heterogeneous in its therapeutic response, and PST trials would be particularly valuable in identifying surrogate biomarkers that can predict for therapeutic response.

Progress in the PST of HER2-amplified breast cancer has mirrored the advances made in the metastatic and adjuvant setting. The addition of trastuzumab to chemotherapy has consistently demonstrated significant improvements in pCR rates in operable breast cancer (Table 4) [43–45], and long-term outcomes in patients with LABC and IBC [44,46]. Trastuzumab has demonstrated significant single-agent anti-tumor activity in treatment-naive HER2-amplified breast tumors after only 3 weeks of treatment [2], and pCR rates were doubled when combined with taxanes and anthracyclines compared with chemotherapy alone [44,45,47–50]. Interestingly, there were few short-term cardiac side effects with the
concurrent use of trastuzumab and anthracyclines \cite{43,44,51}. Trastuzumab-based regimens now form the backbone of PST trials in HER2-amplified tumors. Important insights into the mechanisms of trastuzumab resistance have been obtained from correlative studies in PST trials, with preliminary evidence suggesting that HER2-amplified tumors with a basal-like phenotype, or expression of IGF-1 receptor and other proteins involved in growth factor pathways, are predictors of resistance to the combination of trastuzumab and vinorelbine \cite{52}. Another important observation is that approximately 15–30% of patients with residual disease following trastuzumab regimens have a change in HER2 expression (from positive to negative) or lose HER2 amplification \cite{49,50,53}. In one study, loss of amplification was associated with a poorer relapse-free survival (RFS) compared with patients who retained HER amplification (3-year RFS 50 vs 87.5%; \(p < 0.05\)) \cite{49}. This change in HER2 status needs to be validated in larger studies and, if the finding is consistent, the underlying mechanism will need to be investigated further. One possible explanation is sampling error arising from intrinsic heterogeneity of HER2 expression or the elimination of HER2-amplified clones in the initial treatment of these tumors. This finding could have important implications on the treatment of HER2-amplified tumors in the metastatic setting, and provides some evidence to suggest that rebiopsy at progression may be prudent in some patients. Current PST trials with other HER2-directed therapies, such as lapatinib, trastuzumab-DM1 and pertuzumab, and novel agents targeting the phosphotidylinositol 3-kinase (PI3K) pathway, will hopefully identify predictive markers of response and mechanisms of resistance to these agents.

| Table 4. Pathological complete response and response rates following preoperative systemic therapy in triple-negative breast cancer and HER2\textsuperscript{2} breast cancer subtypes. |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment (no. of cycles) | Pts | Stage | pCR (%) | CR (%) | Notes | Ref. |
|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| TNBC subtype             |                 |                 |                 |                 |                 |                 |                 |
| Cisplatin E\textsubscript{2}F \(\times4\) \(\rightarrow\) P \(\times4\) | 30 | T\(_2,3\) N\(_0,3\) | 40 | 86 | [38] |
| Cisplatin E\textsubscript{2}P \(\times8\) weeks + GCSF | 74 | T\(_2,3\) N\(_0,3\) | 62 | 100 | pCR vs non-pCR 5-year DFS: 90 vs 56% [36] |
| Cisplatin \(\times4\) | 28 | T\(_1,4\) N\(_0,3\) | 22 | 64 | 2/28 pts were BRCA1 mutation carriers, and both patients had a pCR [37] |
| HER2-amplified subtype   |                 |                 |                 |                 |                 |                 |                 |
| VH \(\times4\) | 48 | T\(_1,4\) N\(_0,3\) | 20 | 88 | [52] |
| PH \(\times4\) | 40 | T\(_1,4\) N\(_0,2\) | 18 | 75 | 17.5% with residual tumors had reduced HER2 expression [50] |
| DH + carboplatin \(\times6\) | 70 | T\(_2,3\) N\(_0,2\) | 39 | 95 | [54] |
| DH + cisplatin \(\times4\) | 48 | T\(_3,4\) N\(_0,3\) | 17 | 100 | pCR vs non-pCR 4-year OS: 100 vs 86% 4-year DFS: 100 vs 81% [53] |
| PH \(\times4\) \(\rightarrow\) FE\textsubscript{75}C \(+\) H \(\times4\) | 142 | T\(_1,4\) N\(_0,3\) | 51 | NR | 32% with residual tumors were HER2 negative; residual tumors with HER2 amplification vs HER2 nonamplified 3-year RFS: 87.5 vs 50% [49] |
| EC \(\times4\) \(\rightarrow\) D \(+\) X \(\times4\) (sequential vs concurrent) \(\rightarrow\) DH + X \(\times4\) (sequential vs concurrent) | 1495 | T\(_1,4\) N\(_0,3\) | 16 vs 32 | 76 vs 81 | [45] |
| P \(\times4\) \(\rightarrow\) FE\textsubscript{75}C \(\times4\) vs P \(\times4\) \(\rightarrow\) FE\textsubscript{75}C \(+\) H \(\times4\) | 42 | T\(_1,4\) N\(_0,2\) | 25 vs 67\textsuperscript{'} | 84 vs 96 | [43] |
| AP \(\times3\) \(\rightarrow\) P \(\times3\) \(\rightarrow\) CMF \(\times3\) vs AP + H \(\times3\) \(\rightarrow\) P + H \(\times3\) \(\rightarrow\) CMF + H \(\times3\) | 235 | T\(_{IBC}\) N\(_0,2\) | 19 vs 38\textsuperscript{'} | 74 vs 87 | With trastuzumab vs without 3-year DFS: 56 vs 71% [44] |
| A: Doxorubicin; C: Cyclophosphamide; CR: Complete response; D: Docetaxel; DFS: Disease-free survival; E: Epirubicin; F: 5-fluourouracil; H: Trastuzumab; M: Methotrexate; NR: Not reported; OS: Overall survival; P: Paclitaxel; pCR: Pathological complete response; RFS: Relapse-free survival; V: Vinorelbine; X: Capecitabine. |
do obtain a reduction in breast tumor volume with chemotherapy, translating to a clinically meaningful improvement in BCS rate (Table 5) [25,26,35,55]. The low pCR rate is perhaps not surprising given the biology of HR-positive disease and its relative lack of chemosensitivity in the metastatic and adjuvant setting compared with other subtypes. Preoperative endocrine therapy has been effective in many women, although its use has been largely limited to postmenopausal patients. Although it is very rare to see a pCR with endocrine therapy, tumor shrinkage and downstaging does occur, and some patients who are not initially thought to be candidates for conservative surgery can avoid a mastectomy as a result of preoperative endocrine therapy [55–63]. In general, aromatase inhibitors have resulted in higher response rates than tamoxifen. A recent randomized Phase II trial comparing 4 months of preoperative letrozole, anastrazole and exemestane for stage II/III breast cancer with high Allred scores reported impressive response rates of 69–79% [62]. Clinical OR rates correlated with duration of preoperative endocrine therapy, particularly in the subset of patients who were deemed endocrine sensitive when assessed at an earlier time point during PST [63,64] and with the degree of ER expression. The largest reductions in tumor volumes typically occur in the subsets of patients with the highest ER Allred score [62,65–67]. It is important to note that this subset is also the least likely to obtain benefit from preoperative chemotherapy [68]. Higher pCR rates have been obtained with combination chemo–endocrine PST in premenopausal women; however, it has not translated to a difference in long-term outcomes between adjuvant and preoperative approaches [69]. Alternative intermediate end points are required, particularly in HR-positive tumors, as the majority of these tumors will not attain a pCR with PST.

The heterogeneity of tumors within the HR-positive subtype is likely to account for the variability in biological behavior and treatment response. HR-positive breast cancer is comprised of at least two molecular subtypes, luminal A and B, and the latter subtype is characterized by a higher expression of Ki67 and/or HER2 amplification. Relative to luminal A tumors, luminal B tumors have poorer clinical outcome to adjuvant endocrine therapy alone, and conversely derive a greater benefit from adjuvant chemotherapy [70,71]. The challenge, therefore, in PST trials is to identify subgroups more likely to respond to chemotherapy and to endocrine therapy. Knowledge about predictive markers of therapy such as Ki67 assessments may be validated prospectively in a similar manner to the 21-gene recurrence score [72]. As most of the endocrine-based trials have been in postmenopausal women, the generalizability of these findings to premenopausal patients is uncertain. Important considerations include the need for concurrent leutenizing hormone-releasing hormone agonists with aromatase inhibitor therapy, and differences in the distribution of endocrine- and chemotheraphy-responsive HR-positive tumors compared with postmenopausal women. The choice to combine a novel agent with either an endocrine- or chemotherapy-based preoperative regimen in the HR-positive subtype is not straightforward and measures of high cell proliferation, such as Ki67 labeling index, and the 21-gene recurrence score assay may be considered to assist in the identification of patients who might benefit from preoperative chemotherapy [73,74].

**Table 5. Response to preoperative endocrine therapy in postmenopausal patients with HR-positive breast cancer.**

<table>
<thead>
<tr>
<th>Phase: treatment (duration [months])</th>
<th>Pts</th>
<th>Stage</th>
<th>Clinical OR (%)</th>
<th>pCR (%)</th>
<th>Improvement in BCS (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase III: letrozole (4) vs tamoxifen (4)</td>
<td>324</td>
<td>T2-4 N0-2</td>
<td>35 vs 25</td>
<td>0.6 vs 0.6</td>
<td>45 vs 35</td>
<td>[56]</td>
</tr>
<tr>
<td>Phase III: anastrozole (3) vs tamoxifen (3)</td>
<td>251</td>
<td>T2-4 N0-2</td>
<td>40 vs 35</td>
<td>NR</td>
<td>43 vs 31</td>
<td>[58]</td>
</tr>
<tr>
<td>Phase III: anastrozole (3) vs tamoxifen (3) vs combination (3)</td>
<td>330</td>
<td>T1-4 N0-1</td>
<td>24 vs 20 vs 28</td>
<td>NR</td>
<td>46 vs 22 vs 26</td>
<td>[28]</td>
</tr>
<tr>
<td>Phase II: exemestane (4) vs letrozole (4) vs anastrozole (4)</td>
<td>374</td>
<td>T2-4 N0-1</td>
<td>69 vs 79 vs 77</td>
<td>&lt;1</td>
<td>68 vs 58 vs 74</td>
<td>[62]</td>
</tr>
<tr>
<td>Phase II: exemestane (3) vs doxorubicin + paclitaxel (x4 cycles)</td>
<td>239</td>
<td>T2-4 N0-2</td>
<td>40 vs 46</td>
<td>3 vs 6</td>
<td>33 vs 24</td>
<td>[55]</td>
</tr>
<tr>
<td>Phase II: exemestane (4)</td>
<td>80</td>
<td>T2-4 N0-2</td>
<td>39</td>
<td>3</td>
<td>NR</td>
<td>[61]</td>
</tr>
</tbody>
</table>

* p < 0.05.

Clinical OR determined with ultrasound measurements; OR determined by ultrasound.

BCS: Breast cancer survival; NR: Not reported; OR: Overall response; pCR: Pathological complete response.
platforms for biomarker research include gene expression profiling, functional imaging modalities, such as fluorodeoxyglucose positron emission tomography (FDG-PET) and MRI, and circulating tumor cells (Table 6). Important considerations in biomarker studies include the variability in cellular composition and the confounding effects of tumor stroma in research core biopsies [75]. These factors mandate careful processing of biopsy material and evaluation of amount of tumor tissue within the biopsy. In addition, the standardization of biomarker assessment methodology and definitions are vital in PST trials to facilitate comparability across trials. Finally, pharmacogenomic and pharmacodynamic studies may be used to study difference in drug metabolism and may involve use of surrogate tissues, such as hair and skin.

Pathologic CR is the most studied intermediate end point in PST trials. pCR following PST has been shown to correlate with long-term outcome in most studies, with patients achieving pCR demonstrating better OS and DFS compared with patients with residual tumor following PST, regardless of breast cancer subtype [7,9,12,25,53,76–78]. For this reason, pCR has been adopted as a study end point in most PST trials. Underlying some of the variation in pCR rates across trials is the different definitions used for this end point, thereby highlighting the importance of standardization of assessment for comparison of results across studies. Importantly, if one compares treatment arms within a particular study, improvements in pCR rates do not consistently translate into clinically or statistically significant improvements in clinical outcomes [7]. The correlation between pathologic response and subsequent outcome is further confounded if patients receive active adjuvant therapy, such as adjuvant endocrine therapy. There is little question that we need more sensitive surrogate markers that can allow investigators to move beyond pCR. Although pCR is unable to fulfill the strictest definition of a surrogate end point for survival and cannot be used solely for the registration of novel therapies, it does have a vital role in identifying early positive signals from novel therapies. Pathologic response may also aid in the decision to proceed with larger adjuvant studies.

Despite its widespread use in PST studies, there are a number of valid criticisms of the use of pCR as a biomarker of treatment response. One of the limitations of pCR is that it is a binary end point and therefore it does not adequately discriminate between patients with no response to therapy and those with a major response that may fall just short of a pCR. Alternative methods of assessing PST response include the residual cancer burden (RCB), which is calculated as a continuous variable [79]. RCB measurements take into account tumor response based on primary tumor dimensions, and axillary nodal burden. It allows for the quantification of minimal residual disease and the prognostication of patients who did not achieve pCR, and was correlated with a doubling of risk of relapse for each unit of increase in the RCB index in a cohort of patients treated with an anthracycline regimen.

Alternative predictive biomarkers include indices of cellular proliferation and apoptosis, which are also biological determinants of tumor regression and progression and may be more appropriate surrogates in some settings. For example, prolonged therapy with a novel agent that results in cell cycle arrest or the downregulation of cell proliferation alone may not lead to pCR, but may increase the proportion of cells undergoing apoptosis. Of the proliferation markers, Ki67 has been the most extensively studied in the PST setting, particularly in HR-positive tumors. It is a proliferation

<table>
<thead>
<tr>
<th>Table 6. Intermediate and proposed surrogate end points in preoperative systemic therapy trials.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomarker</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>pCR</td>
</tr>
<tr>
<td>Residual tumor burden</td>
</tr>
<tr>
<td>DNA damage response score</td>
</tr>
<tr>
<td>Cleaved caspase-3</td>
</tr>
<tr>
<td>Ki67 reduction</td>
</tr>
<tr>
<td>PEPI score</td>
</tr>
<tr>
<td>FDG-PET</td>
</tr>
<tr>
<td>MRI</td>
</tr>
</tbody>
</table>

DFS: Disease-free survival; FDG-PET: Fluorodeoxyglucose PET; NA: Not applicable; OR: Overall response; OS: Overall survival; pCR: Pathological complete response; PEPI: Preoperative endocrine prognostic index; PST: Preoperative systemic therapy; RFS: Relapse-free survival; TNBC: Triple-negative breast cancer.
antigen that identifies cells in the G1/S and M phases of the cell cycle. Ki67 has been shown to be a marker of poor prognosis and a predictor of clinical response to chemotherapy [80]. Whilst HR-positive breast cancer generally has low pCR rates (0–7%), an early decrease in Ki67 with endocrine therapy has been shown to predict for endocrine responsiveness and survival outcomes in patients not achieving pCR [81–83]. A preoperative endocrine prognostic index used to predict for RFS was developed from the data generated in a Phase III PST trial of letrozole versus tamoxifen, and incorporated the Ki67 natural log intervals with pathological tumor size, pathological node status and ER Allred score [74]. This was subsequently validated in a similar large trial of anastrozole versus tamoxifen [28], and has the utility of predicting in HR-positive tumors which patient subgroup was more likely to have poor outcomes and therefore require additional adjuvant treatment following surgery.

Other immunohistochemistry-based biomarkers that have been evaluated in the preoperative setting include cleaved caspase-3, a marker of apoptosis, and measurements of DNA damage repair. Chemotherapy has been shown to induce DNA damage and apoptosis in a small percentage of cells within 24 h after exposure [84]. In a preoperative trial of trastuzumab in HER2-amplified breast cancer, there was a significant clinical OR rate after 3 weeks of therapy. The anti-tumor response correlated with the apoptotic index based on cleaved caspase-3 measurements, with a median increase of 35% above baseline [2]. Interestingly, cell cycle and proliferation markers were not increased after 1 and 3 weeks of treatment in this study, suggesting that apoptosis markers may be a more predictive biomarker of treatment response in this tumor subtype. In another preoperative trial, paired biopsies were obtained before and at 18–24 h after the initiation of epirubicin and cyclophosphamide in order to measure a DNA damage repair (DDR) score comprising DNA damage repair proteins such as conjugated ubiquitin, BRCA1-, gH2AX- and Rad5-foci, as a predictive biomarker for DNA damage-inducing chemotherapy [85]. The DDR score was found to be a potentially useful predictive biomarker of therapy as it inversely correlated with tumor response to chemotherapy whilst the clinico-pathological factors analyzed did not.

FDG-PET is an imaging modality that shows promise as a potential biomarker to predict treatment response in PST by measuring changes in tumor metabolism. Most studies have demonstrated a correlation between early changes in the maximum standardized uptake values (SUVmax) and pCR rates after one to three cycles of PST [86–89], however there is consistent heterogeneity of results across studies, partly explained by differences in Δ-SUV thresholds to define treatment responsiveness, timing of early FDG-PET evaluations and patient metabolic factors. As such, FDG-PET assessments of tumor response during PST will still need to be standardized and prospectively validated. FDG-PET remains investigational in the preoperative setting and is not recommended outside a clinical trial. Another imaging modality currently being assessed as a predictive biomarker of therapy is contrast-enhanced breast MRI [90]. The I-SPY 1 and 2 studies are ambitious ongoing projects in the preoperative setting for women with locally advanced breast cancer focusing on the clinical development of paired oncologic therapies and biomarkers [103]. These studies integrate imaging with diverse genetic profiling expression and comparative genome hybridization data throughout the breast cancer treatment cycle, with the overarching goal of effectively identifying surrogate markers for early response and therefore more effective therapies for breast cancer patients.

Intensive correlative research is also focusing on the use of gene expression profiling in the context of PST trials. By comparing transcriptional profiles between pre- and post-treatment samples, and tumor samples from responders and nonresponders, differentially expressed genes can be used to derive treatment-specific gene signatures that may predict response in new cases [75,91]. Using a functional genomics approach to identify a paclitaxel-specific predictor of pCR in TNBC derived from an RNA interference (RNAi) screen in breast cancer cell lines, a paclitaxel-response metagene was found to be significantly associated with pCR in paclitaxel-treated patients (OR: 19.9; p < 0.01) and validated retrospectively in PST trials [92]. Another group have identified a stroma-related gene signature expressed in reactive stroma obtained from micro-dissected breast tumors, and it was found to predict for anthracycline-resistance in HR-negative breast tumors [93]. This signature did not predict for survival in patients who did not receive chemotherapy, therefore suggesting that the stroma metagene was not a prognostic marker. These results support the hypothesis that stromal factors may contribute to chemotherapy resistance, and that mammary epithelial cells may have acquired this stromal metagene signature through a process of epithelial-to-mesenchymal transition [94].

In summary, tissue-intensive correlative PST studies represent a vital area for the identification of surrogate markers of long-term outcomes, as well as treatment-specific predictive biomarkers, which may shed light on the underlying mechanisms of response and resistance. Predictive biomarkers identified in this way have the potential to be used for patient selection in subsequent larger trials in the adjuvant setting. Biomarkers are required.
to identify patients that are likely to relapse, whereby further therapy may be warranted either in the preoperative or adjuvant setting, representing a potential niche setting in which to trial novel therapies. Standardization of the optimal timing and method of measuring these intermediate end points, and correlation with long-term survival end points are essential prerequisites before their routine use in the clinical setting.

Integration of new therapies in the PST setting
Novel therapies have been trialled either alone or in combination with established PST treatments. Correlative studies are especially valuable in elucidating the mechanisms of action through evidence of downregulation of signaling pathways that are known to be activated, and off-target effects through the identification of pathways not previously known to be activated. In a window of opportunity study, 6–10 days of erlotinib prior to surgery inhibited cell proliferation (Ki67) and other post-receptor signaling proteins only in HR-positive/HER2-negative tumors, but not in HER2-amplified or TNBC [95]. These results have potential implications if one were to design an adjuvant trial with erlotinib. In this case, additional pilot testing would clearly be needed before an adjuvant trial could be launched. In a second example, everolimus, an inhibitor of mammalian target of rapamycin, given in combination with letrozole in HR-positive breast cancer resulted in an improved clinical OR rate compared with letrozole alone. Correlative studies demonstrated that combination treatment resulted in downregulation of downstream effectors of the PI3K pathway, and a larger reduction in Ki67 compared with letrozole treatment alone. PIK3CA exon nine mutations were identified as a predictive factor for response to combination therapy, supporting an association between PI3K pathway alteration and endocrine insensitivity in HR-positive tumors. Together with preclinical studies, this finding has contributed to the patient selection criteria of forthcoming adjuvant trials of PI3K pathway inhibitors.

Conclusions & future perspective
Preoperative systemic therapy is no longer a novelty, but a mainstream treatment approach. In the research setting, it has a number of clear advantages including an ability to move drug development and biomarker discovery programs forward. Differences in biology between breast cancer subtypes have added another layer of complexity to its integration into routine clinical practice. Whilst having a pivotal role in treating LABC and IBC and in the trials of novel therapies in defined tumor subtypes, it should be considered as an option for patients with operable breast therapy only in the setting of a well-coordinated multidisciplinary team. The change in sequencing of local and systemic therapies has necessitated closer monitoring of the treatment response of the primary tumor and lymph nodes, and careful surgical monitoring to determine optimal margins and pathological assessment for residual disease. PST requires flexibility, as patients who fail to respond or progress on treatment may require earlier surgery and additional treatment in the adjuvant setting.

To ensure comparability of results across trials, it is vital that the definitions of biomarkers be standardized to make sense of the results across studies. As with breast cancer treatment in the adjuvant and metastatic setting, PST trials should, at a minimum, report the results separately for the subgroups that are defined by ER and PR expression and by the presence or absence of HER2 amplification. Similarly, as IBC is characterized by an unusual biological behavior and genetic profile [96], it should also be reported separately in PST trials. PST should be tailored according to the breast cancer subtype, with the inclusion of endocrine and HER2-directed therapies into the PST of HR-positive and HER2-amplified tumors, respectively.

In addition to the type, combination and duration of treatment, consideration should also be given to the choice of biomarkers and intermediate end points to be measured. As best responses have been achieved with anthracycline and taxane regimens for women with high-risk breast cancer, it should form the backbone of therapy outside a clinical trial. In addition, one should consider a relatively longer duration of preoperative endocrine treatment for women with HR-positive breast cancer, particularly in postmenopausal women who are more likely to have slow-tempo disease. In the setting of HER2-amplified tumors, HER2-directed therapy should generally be given in combination with chemotherapy. PST is not recommended in patients who are managed outside a multidisciplinary setting, when close monitoring of the treatment response of the primary tumor and lymph nodes is not feasible, and when systemic therapy is not indicated. Surgery remains the initial treatment modality of choice in these scenarios.

Testing novel therapies during the preapproval process has been shown to be feasible in the preoperative setting. The utility of clinical mid-course response assessments during PST as a decision aid to guide subsequent systemic therapy in either the preoperative or adjuvant setting may also have an impact on PST trial design for the testing of novel therapeutics, whereby nonresponders may be considered for additional novel therapies. It is important to consider that the biological behavior of the tumor in the metastatic setting may differ from the preoperative setting and therefore results from one setting may not always translate to the other and therefore the preoperative evaluation of novel therapies must always
be validated in larger adjuvant studies. The integration or coupling of PST and adjuvant trials will allow the efficient translation of knowledge gleaned from preliminary data in PST trials, such as early treatment response and predictors of therapeutic response for patient selection, to be applied in the adjuvant setting. Whilst it offers a potentially quicker and cost-saving alternative for testing therapeutics by requiring a smaller patient study cohort compared with the adjuvant setting, reliable and validated intermediate and long-term end points are still required in the long run. In the era of molecularly based therapeutics, correlative tissue-based studies are critical if we are to learn how to individualize these treatments. The full potential of PST will not be realized simply by bringing forward drugs currently used in the adjuvant and metastatic setting, but rather through parallel tissue-intensive biomarker discovery and correlative mechanistic studies. Priorities include the validation of surrogate end points with long-term outcomes and identifying predictive biomarkers of therapeutic response in order to prospectively identify subgroups of patients most likely to receive the greatest benefit and those at high risk of recurrence following therapy. These findings may subsequently be used to determine eligibility in adjuvant trials, thereby reducing the dilution of clinical efficacy in unselected patient cohorts.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary
- Preoperative systemic therapy (PST) trials should be tailored to specific breast cancer subtypes, with the inclusion of endocrine and HER2-directed therapies in HR-positive and HER2-amplified tumors, respectively.
- Appropriate surrogate end points in PST trials vary with breast cancer subtype and treatment.
- Paired pre- and post-treatment tissue allows for the identification of surrogate end points of long-term outcomes, and predictive biomarkers of therapeutic response to prospectively identify patient subgroups most likely to receive the greatest benefit and those at high risk of recurrence following therapy.
- Patients who do not respond to PST represent a group with poor prognosis, in whom additional therapy should be considered.
- Preoperative testing of novel therapies during the pre-approval process is feasible. It may be used to provide proof of principle for novel therapies, determining if these treatments merit further evaluation and determining patient selection criteria in a larger resource-intensive adjuvant setting.

Bibliography
Papers of special note have been highlighted as:

therapeutic perspective


[26] With [26], highlights differences in pathological complete response rates in different subtypes of breast cancer. These studies contribute significantly to the growing understanding of the different biological behavior of breast cancer subtypes, and reinforces the relationship between pathological response rates and survival outcomes.


[27] With [25], highlights differences in pathological complete response rates in different subtypes of breast cancer. These studies contribute significantly to the growing understanding of the different biological behavior of breast cancer subtypes, and reinforces the relationship between pathological response rates and survival outcomes.


[33,34] With [33,34], these are seminal papers that first described the molecular subtypes of breast cancer using DNA microarrays and their distinct clinical behavior, strongly supporting the idea that breast tumor subtypes represent biologically distinct disease entities.


[35] With [32,34], these are seminal papers that first described the molecular subtypes of breast cancer using DNA microarrays and their distinct clinical behavior, strongly supporting the idea that breast tumor subtypes represent biologically distinct disease entities.
Will preoperative trials change future clinical practice? Therapeutic Perspective


With [32,33], these are seminal papers that first described the molecular subtypes of breast cancer using DNA microarrays and their distinct clinical behavior, strongly supporting the idea that breast tumor subtypes represent biologically distinct disease entities.


Therapeutic Perspective


68 Colleoni M, Viale G, Goldhirsch A. Lessons on responsiveness to adjuvant systemic therapies learned from the neoadjuvant setting. Breast 18(Suppl. 3), S137–S140 (2009).


Will preoperative trials change future clinical practice?

### Therapeutic Perspective


### Websites

