

Translating neoadjuvant therapy into survival benefits: one size does not fit all

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Abstract | Neoadjuvant therapy has been established as an effective therapeutic approach for patients with locally advanced breast cancer. Similar outcomes between neoadjuvant and adjuvant chemotherapy have been demonstrated in several trials. Nevertheless, neoadjuvant therapy has some advantages over adjuvant therapy, including tumour downstaging, *in vivo* assessment of therapeutic efficacy, reduced treatment durations, and the need to enrol fewer patients for clinical trials to reach their preplanned objectives. The number of neoadjuvant trials in patients with breast cancer has increased substantially in the past 5 years, particularly in the context of HER2-positive disease. Substantial improvements in the pathological complete response rate to anti-HER2 therapy, a proposed surrogate end point for long-term clinical benefit, have been observed with neoadjuvant dual-agent HER2 blockade. Thus, it was hypothesized that this approach would provide additional survival benefits over standard-of-care therapy with the anti-HER2 antibody trastuzumab in the adjuvant setting. Emerging data, however, are calling this notion into question. We discuss potential reasons why results of neoadjuvant trials of targeted therapies have not been mirrored in the adjuvant setting, and other than inherent differences in clinical-trial designs and statistical power, we consider how the biology of the disease, patient characteristics, and drug administration and schedule might influence the results.

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Preoperative, or 'neoadjuvant', therapy is a treatment option for patients with early stage breast cancer and is the standard of care for patients with locally advanced breast cancer¹. Neoadjuvant therapy has advantages over adjuvant therapy, given that preoperative therapy often results in downstaging of both the primary tumour and axillary disease, enables *in vivo* assessment of tumour biology, and represents the ideal scenario for studying predictive biomarkers and intermediate end points that might predict long-term clinical outcomes^{2,3}. In addition, the neoadjuvant approach offers opportunities for response-guided therapeutic strategies, whereby therapeutic regimens can be adjusted when tumour tissue is available for response monitoring⁴.

Neoadjuvant chemotherapy, compared to conventional adjuvant therapy, does not seem to improve the overall survival of patients with breast cancer^{5,6}. Indeed, randomized controlled trials have demonstrated similar outcomes, in terms of disease-free survival (DFS) and overall survival, between neoadjuvant and adjuvant systemic chemotherapy or endocrine therapy in patients with breast cancer⁵⁻⁷. Nevertheless, the increasing rates of pathological complete response (pCR) to neoadjuvant therapy have had a marked effect on locoregional-treatment considerations, as neoadjuvant

chemotherapy can provide increased opportunities to perform breast-conserving surgery in patients with locally advanced breast cancer⁸. Patient preferences, surgeons' recommendations, and the possible failure to achieve tumour control through breast-conserving surgery (in circumstances such as a predicted insufficient response to chemotherapy, or a patient not being a suitable candidate for breast-conserving surgery) contribute to the choice of the timing for systemic therapy^{9,10}.

The number of trials investigating neoadjuvant therapy for breast cancer increased substantially over the past decade, particularly in the context of HER2-positive disease, a subtype that is associated with a poor prognosis if not treated with anti-HER2 agents^{11,12}. HER2-positive breast cancers account for 15–20% of all invasive breast cancers¹³. The state-of-the-art treatment for HER2-positive breast cancer consists of trastuzumab-based therapy, which has been shown to improve the DFS and overall survival of patients with early stage and metastatic HER2-positive breast cancer, compared with chemotherapy alone^{12,14-16}. In the neoadjuvant setting, the addition of trastuzumab to standard chemotherapy results in an increase in the pCR rate compared with neoadjuvant chemotherapy alone^{17,18}. Likewise, the use of trastuzumab, in addition to

Key points

- Neoadjuvant therapy is the standard of care for patients with locally advanced breast cancer and can improve operability of breast cancer
- For patients with HER2-positive breast cancer, the long-term outcomes of neoadjuvant treatment with the anti-HER2 agent trastuzumab should be considered equivalent to those of adjuvant therapy with this drug
- Despite the success of some neoadjuvant trials of dual-agent HER2 therapy for HER2-positive breast cancer, additional overall survival benefits of this approach over single-agent trastuzumab have not been documented in adjuvant trials
- Differences in clinical-trial designs, patient characteristics, breast-cancer biology, and the sequence and schedule of drug administration might have influenced the results of neoadjuvant trials and the contrasting results seen in adjuvant trials
- Prospective neoadjuvant trials with treatment informed by biomarkers and administration of matched experimental drug regimens should address the biological heterogeneity observed within breast-cancer subtypes

standard chemotherapy, is associated with confirmed long-term improvements in DFS and overall survival in large adjuvant studies^{15,16,19}.

Other novel anti-HER2 therapeutic strategies have been approved or are under investigation for the treatment of patients with HER2-positive breast cancer^{20–23}. Notably, a pertuzumab-based neoadjuvant treatment regimen, also comprising trastuzumab and standard chemotherapy, was approved by the FDA for patients with locally advanced, inflammatory, or early stage HER2-positive breast cancers greater than 2 cm in diameter and/or with axillary-lymph-node involvement^{24,25}.

The benefit of neoadjuvant dual-agent HER2 blockade in patients with HER2-positive breast cancer has been supported by both preclinical and clinical studies. For instance, in preclinical studies, compelling evidence indicates that dual-agent HER2 blockade with trastuzumab and the small-molecule HER1/2-tyrosine-kinase inhibitor lapatinib has better efficacy than trastuzumab alone because of nonoverlapping mechanisms of action and synergic interaction between these agents^{26,27}. In the setting of HER2-positive breast cancer, four phase III neoadjuvant trials testing trastuzumab plus another anti-HER2 agent versus trastuzumab alone have shown dramatic increases in pCR rates, a proposed surrogate end point for long-term clinical benefit: NeoSPHERE (Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation)²⁴, NeoALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation)²⁸, CHER-LOB (Chemotherapy, Herceptin and Lapatinib in Operable Breast Cancer)²⁹, and LPT 109096 (preoperative lapatinib and trastuzumab, separately and combined before neoadjuvant chemotherapy)³⁰. Two of these neoadjuvant trials, NeoSPHERE²⁴, and NeoALTTO²⁸, have adjuvant counterparts: APHINITY (Adjuvant Pertuzumab and Herceptin in Initial Therapy; NCT01358877)³¹ and ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation; NCT00490139)³², respectively.

On the basis of the statistically significant improvements in pCR rates observed with neoadjuvant dual-agent HER2 blockade, and the association between pCR and long-term outcomes of other neoadjuvant treatments, it was hypothesized that this approach would also provide additional survival benefits over standard-of-care

therapy (comprising single-agent HER2 blockade with trastuzumab) in the adjuvant setting; however, data emerging from the ALTTO trial since 2014 have called this notion into question^{28,32}. A similar situation has also been observed with the incorporation of the anti-VEGF antibody bevacizumab into adjuvant therapy (chemotherapy with an anthracycline or taxane, or both) for triple-negative breast cancer (TNBC) in the BEATRICE trial³³, and in unselected patients with HER2-negative disease in the E5103 study³⁴ (with anthracycline and taxane chemotherapy). This treatment approach had previously been associated with an increase rate of pCR in the neoadjuvant setting, although this effect has not been translated into improved survival outcomes^{35–37}.

In this Review, we address the potential reasons why changes in pCR rates following neoadjuvant therapy might not equate with changes in DFS of the same magnitude when the same treatment is applied in the adjuvant setting, or even improvements in long-term outcomes in the neoadjuvant setting. We discuss the hypothesis that, in addition to inherent differences in the designs, statistical power, and use of surrogate markers between neoadjuvant and adjuvant trials, the biology of the disease and the characteristics of the drugs might also influence the translation of increased pCR rates into improvements in DFS and overall survival in the adjuvant setting. Furthermore, the ongoing studies that could shed additional light on this topic are reviewed, and we suggest additional approaches that might help realize the potential use of pCR rates after neoadjuvant therapy to infer the actual effect of systemic therapies on the survival of patients with breast cancer.

Comparing NeoALTTO with ALTTO**Treatment efficacy**

In NeoALTTO and ALTTO^{28,32}, investigators compared dual-agent HER2 blockade consisting of trastuzumab plus lapatinib to either lapatinib or trastuzumab alone, in the neoadjuvant and adjuvant settings, respectively, with the aim of improving the outcomes of patients with HER2-positive breast cancer. In NeoALTTO, the trastuzumab plus lapatinib cohort were treated with both drugs concomitantly, whereas in the ALTTO trial, these drugs were used either concomitantly or sequentially (that is, trastuzumab together with or followed by lapatinib) in different patient subgroups (FIG. 1). Of note, the lapatinib alone arm of the ALTTO trial was closed in 2011 on the recommendation of an Independent Data Monitoring Committee (IDMC), after the first planned interim analysis. The IDMC reported that the comparison of lapatinib alone versus trastuzumab alone crossed the futility boundary, indicating that the lapatinib alone arm was unlikely to meet the prespecified criteria to demonstrate non-inferiority to trastuzumab alone, with respect to DFS^{28,32}.

Despite being performed in patients with the same disease subtype, the NeoALTTO and ALTTO trials had fundamentally different results. The primary objective of the NeoALTTO trial was met, with a substantial increase in the pCR rate with dual-agent HER2 blockade compared with trastuzumab alone (51.3% versus 29.5%, $P=0.0001$)²⁸. By contrast, the primary end point of the

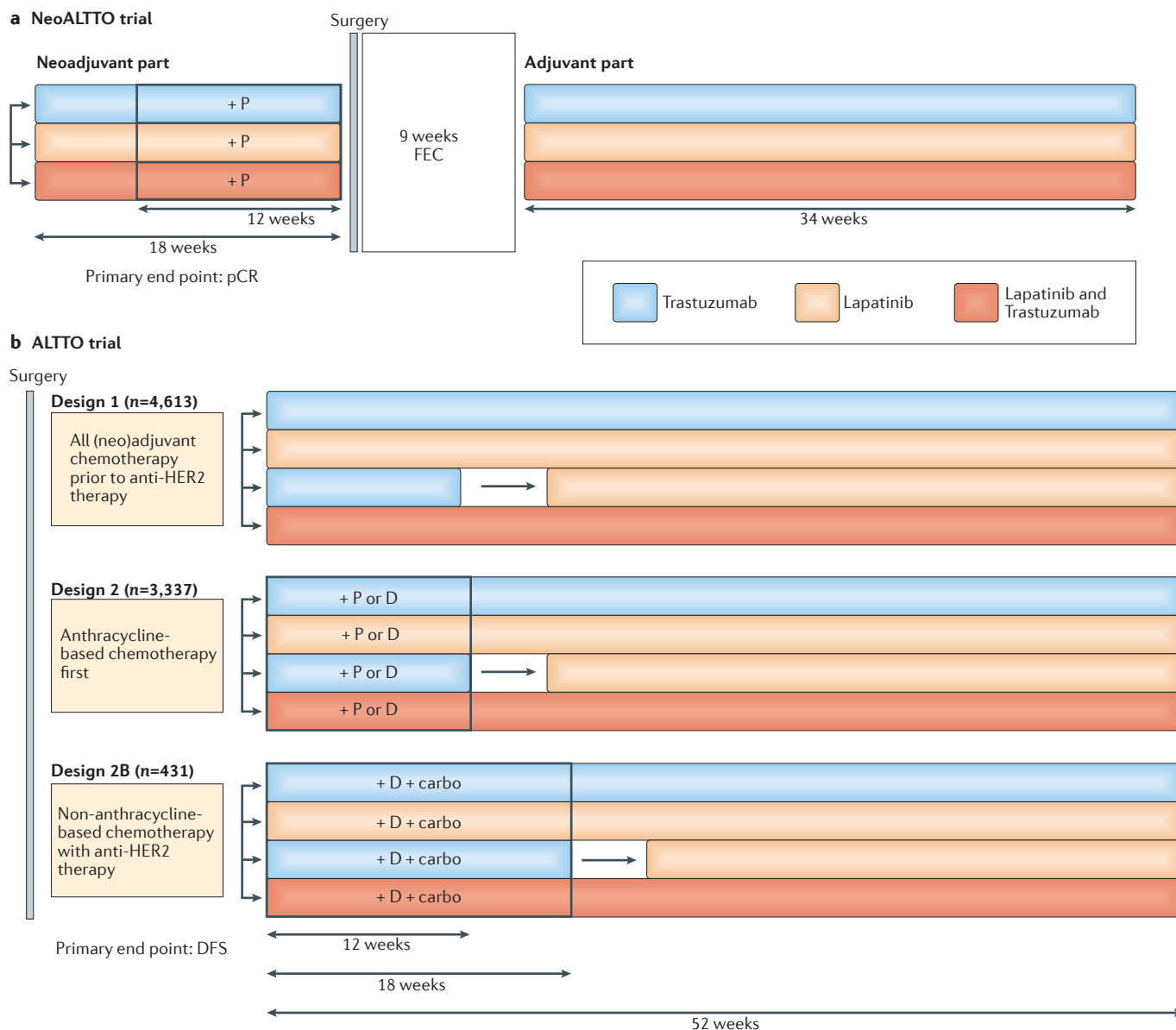


Figure 1 | Design of the ALTT0 and NeoALTT0 clinical trials. The efficacy of lapatinib monotherapy, standard-of-care trastuzumab monotherapy, and dual HER2 blockade with lapatinib and trastuzumab for patients with HER2-positive breast cancer was compared in the adjuvant and neoadjuvant settings in the ALTT0 and NeoALTT0, respectively. In both trials, patients also received chemotherapy according to varying regimens, as indicated. **a** | In the NeoALTT0 trial, patients continued to be treated with the same anti-HER2 agent(s) that they received during the neoadjuvant period. **b** | The ALTT0 trial included an extra treatment arm to determine the efficacy of trastuzumab (for 12 weeks in design 2 and for 18 weeks in design 2B), followed by lapatinib (for 34 and 28 weeks, respectively) after a 6-week ‘wash out’ period. Anti-HER2 therapy was initiated after completing all chemotherapy (design 1), concurrently with a taxane after chemotherapy with an anthracycline (design 2), or concomitant with platinum-containing, non-anthracycline chemotherapy (design 2B). The lapatinib-only arm of the ALTT0 trial was closed in August 2011 for futility. Carbo, carboplatin (AUC 6); D, docetaxel (75–100 mg/m² every 3 weeks); FEC, 5-fluorouracil (500 mg/m²), epirubicin (100 mg/m²), and cyclophosphamide (500 mg/m²) given on the same day every 3 weeks; P, paclitaxel (80 mg/m² weekly).

ALTT0 trial, was not met; that is, no statistically significant DFS benefit for adjuvant dual-agent HER2 blockade was observed at a median follow up for 4.5 years (HR 0.84 for the comparison of concomitant trastuzumab and lapatinib versus trastuzumab alone, $P=0.048$ — the protocol modification required a P value of ≤ 0.025 for significance)³².

Of note, however, survival outcomes were not superior for the dual-agent HER2 blockade group versus the trastuzumab alone cohort overall in the NeoALTT0 trial (event-free survival (EFS): HR 0.78, 95% CI 0.47–1.28, $P=0.33$; overall survival: HR 0.62, 95% CI 0.30–1.25, $P=0.19$)³⁸. In a landmark analysis (an approach used to compare time-to-event outcomes between groups,

determined during study follow up, which includes controls for guarantee time bias³⁹) performed at 30 weeks after randomization, the NeoALTTO investigators demonstrated that 3-year EFS (HR 0.38, 95% CI 0.22–0.63, $P=0.0003$) and 3-year overall survival (HR 0.35, 95% CI 0.15–0.70, $P=0.005$) were significantly improved in women who achieved a pCR compared with those who did not³⁸. These improvements were observed for all of the patients with a pCR, as a whole (that is, when the treatment administered was not considered), as well as for the hormone-receptor-negative group, overall (3-year EFS: HR 0.34, 95% CI 0.17–0.63, $P=0.001$; 3-year overall survival: HR 0.29, 95% CI 0.11–0.62, $P=0.003$)³⁸. Caution should be exercised when interpreting these data, however, because patients who were hormone-receptor negative were more likely to be included in the landmark analysis than those who were hormone-receptor positive³⁸; moreover, the hormone-receptor-negative patients were also more likely to achieve a pCR and to have an disease-progression event during follow up³⁸. In addition, although achievement of a pCR was associated with improved 3-year EFS and overall survival in the hormone-receptor negative group, and not in hormone-receptor positive group, the test for interaction was not significant ($P_{\text{interaction}}=0.34$)³⁸.

Disparities between the trials

A number of potential factors might explain the disparities between the results, with regard to the primary end points, of the NeoALTTO and ALTTO clinical trials. These factors are discussed the following sections.

Patient/disease characteristics. Differences in the characteristics of the patients with HER2-positive breast cancer enrolled in the NeoALTTO and ALTTO trials can be recognized (TABLE 1). Notably, in the ALTTO clinical

trial, patients had low risk of recurrence, on the basis that at least ~85% of patients had tumours ≤5 cm in diameter (compared with a maximum of <65% of the NeoALTTO cohorts), and 40% had axillary-lymph-node-negative disease^{28,32}. These discrepancies in clinicopathological data (that is, smaller tumour sizes and less axillary-lymph-node involvement) suggest that patients in the ALTTO trial had less-aggressive disease than those in NeoALTTO trial. These characteristics might explain the low frequency of DFS events reported and, therefore, might underlie the failure to demonstrate a statistically significant difference in this end point between the treatment groups³².

Pathological features that include tumour grade, and the expression levels of oestrogen receptors (ERs), progesterone receptors (PR), and markers of cell proliferation (that is, the Ki67 index) seem to be associated with different responses to neoadjuvant therapies: patients harbouring tumours with a high proliferative index and/or of a high grade have a higher likelihood of achieving a pCR^{40,41}. For example, in neoadjuvant trials of anti-HER2 agents, patients with ER-negative and HER2-positive (ER-/HER2+) breast cancer have been found to have remarkably different pathological outcomes compared with those with ER+/HER2+ disease^{24,28,42}. Indeed, in the NeoALTTO trial²⁸, the pCR rate was higher in patients with ER-/HER2+ tumours, compared with those with ER+/HER2+ disease, in all treatment groups (dual-agent HER2 blockade, 61.3% versus 41.6%; single-agent HER2 blockade with trastuzumab, 36.5% versus 22.7%). In the landmark analysis performed by the NeoALTTO trial investigators, the hazard ratios for EFS in the patients who achieved a pCR versus those without a pCR were significant in the hormone-receptor-negative cohort (HR 0.34, 95% CI 0.17–0.63, $P=0.001$), but not in the hormone-receptor-positive cohort (HR 0.50, 0.18–1.13, $P=0.13$). These findings support the notion that these subgroups comprise different disease entities within the HER2-positive breast-cancer subtype. This information could potentially be useful in the stratification of patients who are likely to derive additional benefit from the administration of dual-agent HER2 blockade versus trastuzumab monotherapy in future clinical trials. In the NeoALTTO trial, however, no statistically significant difference between the treatment groups was demonstrated when EFS and overall survival were analysed by hormone-receptor status. Notably, no statistically significant differences between the effects of therapy on the outcomes of patients with these disease subtypes have been demonstrated in the postoperative setting, including the ALTTO trial^{15,16,32}. Nevertheless, NeoALTTO comprised a higher proportion of patients with ER-negative disease than ALTTO (>48% versus <43%), which could potentially have contributed to the differences in the outcomes of these trials, with regard to the primary end points.

Treatment regimens and schedules. Treatment-related issues might be relevant to understanding the possible reasons for the disparity between the results of both trials. In particular, the timing of anthracycline administration in the NeoALTTO and ALTTO, and the administration

Table 1 | Clinicopathological characteristics of the NeoALTTO and ALTTO trials

Characteristic	NeoALTTO (n=455)	ALTTO (n=8,381)
Primary end point	Breast pCR	DFS
Approach to dual-agent HER2 blockade	Lapatinib plus trastuzumab	Lapatinib plus trastuzumab, or trastuzumab then lapatinib
Menopausal status	NA	<ul style="list-style-type: none"> • Postmenopausal or male: 56–57% (0–0.25% male) • Premenopausal: 43–44%
Tumour size*	<ul style="list-style-type: none"> • ≤2cm: 1.3–2.6% • >2cm and ≤5: 44.3–61.8% • >5 cm: 35.5–53% • Missing or NA: 0–1.3% 	<ul style="list-style-type: none"> • ≤2cm: 41% • >2cm and ≤5: 44–45% • >5 cm: 5–6% • Missing or NA: 9–10%
Axillary-lymph-node status*	<ul style="list-style-type: none"> • N0–1: 83.8–84.6% • ≥N2, Nx, or missing: 15.4–16.2% 	<ul style="list-style-type: none"> • N0: 40% • N1–3: 29–30% • ≥N4: 22% • Missing or NA: 8–9%
ER status*	<ul style="list-style-type: none"> • ER negative: 48.1–49.7% • ER positive: 50.3–51.9% 	<ul style="list-style-type: none"> • ER negative: 42–43% • ER positive: 57–58%

DFS, disease-free survival; ER, oestrogen receptor; N, regional lymph-nodes stage; NA, not available; Nx, regional lymph nodes could not be assessed (for example, previously removed); pCR, pathological complete response. *Ranges represent the different values across treatment arms.

schedule of lapatinib deserve to be mentioned. In the NeoALTTO trial²⁸, anthracycline-based chemotherapy was administered after surgery — not as part of the pre-operative treatment. In the ALTTO trial³², anthracycline-based chemotherapy was incorporated as part of design number 1 (anti-HER2 therapy after all chemotherapy) and design 2 (concomitant anti-HER2 therapy and taxane chemotherapy after anthracycline chemotherapy), although design 2B (simultaneous anti-HER2 and platinum–docetaxel doublet chemotherapy) was planned not to include anthracycline administration. Whether achievement of a pCR would have translated into a 3-year EFS and overall survival benefit in the NeoALTTO trial³⁸ if no anthracycline-based chemotherapy had been given after surgery remains an open question.

With regard to the lapatinib schedule, preclinical and early clinical evidence indicate that intermittent administration of lapatinib is associated with better efficacy than standard continuous dosing^{43,44}. Thus, the use of the standard schedule of lapatinib in the ALTTO and NeoALTTO trials, in addition to the consistent reports of increased adverse event rates and discontinuation or interruption of treatment occurring in the lapatinib-containing arms³⁸, could potentially indicate that the efficacy of lapatinib might not have been optimal in these studies.

Primary end points. The designs and primary end points of both clinical trials were different. The primary end point of the NeoALTTO trial²⁸ — a significant increase in the rate of pCR in the breast (not including the axilla) between the dual-agent HER2 blockade and trastuzumab monotherapy cohorts — was met. In the ALTTO trial³², however, the primary end point was DFS, which was not met as the treatment cohorts shared similar DFS rates at a median follow up of 4.5 years: 86% to 88%.

Relevant concerns over the discrepancies between the neoadjuvant and adjuvant trials of dual-agent HER2 blockade, with regard to achievement of the primary end points, relate to statistical power. The investigators of the ALTTO trial planned to perform the primary statistical analysis when either 850 DFS events required for the comparison of lapatinib plus trastuzumab versus trastuzumab had occurred (for 80% power to detect a HR of 0.80 using a two-sided alpha error of 0.0167), or a median follow up of 4.5 years was reached^{28,32}. Although the ALTTO trial involved more than 8,300 patients, the first analysis was based on 555 DFS events — that is, 30% less than the planned total of 850 events required for 80% power — at a median follow up of 4.49 years (range: 1 day to 6.4 years)^{28,32}. Notably, the time-driven analysis was used in favour of the event-driven analysis to obtain earlier results. The fact that this trial did not demonstrate the superiority of dual-agent anti-HER2 therapy was probably because of the time-driven primary end point used. Indeed, the high DFS rates at the planned median follow-up point suggest that a longer period of time would be needed to observe any statistically significant effect of dual-agent HER2 blockade. Thus, a more-mature analysis (after a greater number of events) should be explored and might provide different results.

As described, in the ALTTO trial^{28,32}, the DFS HR for the combination of anti-HER2 agents versus trastuzumab alone was 0.84 (97.5% CI 0.70–1.02), with a two-sided *P* value of 0.048. This result was not significant owing to the splitting of the type I error rate in the original design between the comparisons with the sequential and concurrent approaches to dual-agent HER2 blockade^{28,32}; however, the *P*-value of ≤ 0.025 required for significance was rather restrictive. In other words, the investigators added another comparison, to establish the non-inferiority of trastuzumab followed by lapatinib versus trastuzumab alone⁴⁵. Thus, if the ALTTO trial had randomly assigned patients to receive anti-HER2 therapy with only trastuzumab or concomitant trastuzumab plus lapatinib, the *P* value obtained for this comparison would have been considered statistically significant and the trial positive for its primary end point.

Another important point to highlight is that the overall-survival data for patients in the ALTTO trial was not inferior to that of women in the HERA trial (also known as the Herceptin Adjuvant trial), in which investigators compared treatment with trastuzumab for 1 and 2 years with observation after standard neoadjuvant chemotherapy or adjuvant chemotherapy, or both^{15,46}. Performing an accurate head-to-head comparison between these trials is not feasible; bearing in mind the caveats of indirect comparison, however, the HRs for overall survival reported for dual-agent HER2 blockade versus trastuzumab arms in the ALTTO trial are similar to those for the comparison between trastuzumab and observation (after standard neoadjuvant and/or adjuvant chemotherapy) in the HERA trial: HRs of 0.80 and 0.91 for trastuzumab plus lapatinib and trastuzumab followed by lapatinib, respectively, versus trastuzumab in the ALTTO trial³²; HR 0.76 for trastuzumab-based therapy versus observation in the HERA trial^{15,46}.

The absence of a requirement for a lymph-node complete response in the definition of pCR used in the NeoALTTO trial is another potential reason why the primary outcome of the NeoALTTO was met, whereas that of the ALTTO trial was not (see ‘*Insights from other neoadjuvant trials*’ section), and further exploratory analysis should shed some light on this possibility. According to the FDA criteria, absence of disease at both the primary-tumour site and axillary lymph nodes seems to be the optimal definition of a pCR when considering accelerated approval of a treatment. Indeed, evidence suggests that a response in both primary breast tumour and axillary lymph nodes is associated with prolonged survival; hence, definitions of pCR that include response in only the primary breast tumour, as used in the NeoALTTO trial, might not correlate as closely with long-term outcomes.

Of note, in the NeoALTTO trial, the secondary EFS end point was more analogous to the primary end point of the ALTTO trial, DFS, and this did not differ for the dual-agent versus the monotherapy HER2-blockade cohorts overall. Of note, the small sample size limited the power to reveal differences in EFS in the neoadjuvant setting, and the fact that the effects of the adjuvant parts of NeoALTTO treatment schedules could not be

considered in the pCR assessment, but are relevant to EFS, is an important concern. For example, patients without a pCR might benefit from subsequent anthracycline treatment, whereas patients who already have pCR might not. Fundamentally, therefore, the differing results between NeoALTTO and ALTTO might be a consequence of being able to stratify those patients with a pCR in the neoadjuvant setting, and report significantly improved long-term outcomes for these patients, despite no overall improvement in the outcomes of the entire treatment cohort. In the adjuvant setting, stratifying patients on the basis of an end point associated with residual disease is not possible at present. If such an end point could be assessed in the adjuvant setting (for example, using assays for detecting minimal residual disease, such as circulating tumour DNA (ctDNA) or circulating tumour cells), the findings of ALTTO and NeoALTTO might have been similar — that is, if patients who had no residual tumour cells after adjuvant therapy could be identified, these patients would probably have better long-term outcomes (that is, DFS and/or overall survival) than those with residual cells. Thus, stating that those patients with a pCR in the NeoALTTO trial have better long-term outcomes is important, but this ignores the patients who do not achieve a pCR and the fact that the magnitude of the effect of dual-agent HER2 blockade was insufficient to make this approach superior for the overall population. Currently, one cannot predict the patients who will have a pCR to neoadjuvant therapy, and, therefore, the long-term outcomes of the entire cohorts are critically important for clinical decision-making. Hence, from this perspective, the situation seems to be analogous in both the neoadjuvant and adjuvant trials (that is, NeoALTTO and ALTTO): dual-agent HER2 blockade has no significant effect on overall survival, for the overall ‘intention-to-treat’ population. Only with regard to the primary end points is NeoALTTO considered a success while ALTTO is not.

pCR as a surrogate for survival

Varying definitions and correlations

The achievement of a pCR, albeit strongly correlated with EFS and overall survival after neoadjuvant chemotherapy, is known to be an imperfect surrogate for prediction of the survival of patients with breast cancer. Furthermore, the pCR end point can also vary in its definition^{35,47,48}. Indeed, a universally accepted definition for pCR is not available at present, and differences in the definitions of pCR used in neoadjuvant trials have rendered the reporting and interpretation of data challenging. For the purpose of designing trials, the FDA⁴⁸ recognizes the following two definitions of a pCR to neoadjuvant systemic therapy, based on haematoxylin and eosin (H&E) evaluation of the complete resected breast specimen and all sampled regional lymph nodes: either the absence of residual invasive cancer (classified as ypT0/Tis ypN0 according to the current American Joint Committee on Cancer (AJCC) staging system); or, the absence of residual invasive and *in situ* cancer (AJCC stage ypT0 ypN0).

In addition, previous studies have addressed clinical and biological factors associated with a pCR to neoadjuvant chemotherapy in patients with breast cancer. In these studies, investigators demonstrated that a high tumour grade and/or ER-negative disease, as well as combined trastuzumab treatment and chemotherapy for HER2-positive disease, as factors associated with a greater likelihood of achieving a pCR^{17,24,28,40,49–52}. In fact, the frequency and prognostic value of achieving a pCR was shown to vary among breast cancer intrinsic subtypes^{47,53}. Specifically, for tumour subtypes characterized by slow proliferation (such as, luminal-A-like breast cancer), pCR is not associated with prognosis, whereas for subtypes comprising highly proliferating tumours (such as TNBC), pCR can be used to accurately discriminate between patients with a good or a poor prognosis⁵⁴. The findings of NeoALTTO²⁸, as well as those of other trials^{29,55} that used dual-agent HER2 blockade in the context of HER2-positive breast cancer, indicate that ER-positive disease and ER-negative disease discriminate two different subgroups of patients with HER2-positive breast cancer. As patients with ER-negative tumours had higher pCR rates than those with ER-positive disease, the new generation of neoadjuvant trials should study these two patient populations independently.

Indeed, the validity of using pCR as a surrogate end point for survival in patients with breast cancer is an issue that has been raised in various discussions of the NeoALTTO and ALTTO trials^{28,32,47,56,57}. An FDA-led meta-analysis⁵⁸, in which data from 12 international trials and a total of 11,955 patients were assessed, has often been cited in this context. This study demonstrated unequivocal associations between achievement of a pCR and long-term survival benefits. For example, in patients with HER2-positive tumours, achievement of a pCR was strongly associated with EFS (HR 0.39, 95% CI 0.31–0.50) and overall survival (HR 0.34, 95% CI 0.24–0.47)⁵⁸. The study found no clear relationship, however, between increases in the pCR rate and the HRs for EFS and overall survival at the level of the entire trial cohorts, and thus failed to establish the pCR rate as a surrogate end point for improved EFS and overall survival⁵⁸. The Prentice criterion⁵⁹ for surrogate end points essentially requires the surrogate variable to capture any relationship between the treatment and the true end point. To meet this criterion, statistical hypothesis testing is required to establish that the treatment has no residual effect on the true end point after adjusting for the surrogate measure^{59–64}; pCR rate did not satisfy this criterion in the context of the FDA meta-analysis⁵⁸. The evidence that a pCR to treatment is predictive of a good outcome in patients with breast cancer is, nevertheless, unequivocal. Thus, the key question is, in a neoadjuvant trial, what magnitude of difference in pCR rates between two treatment arms and across different breast cancer subtypes would translate into statistically significant improvements in survival in the overall trial population?

As discussed, pCR rates for patients with the different clinical subtypes of breast cancer vary, and are lower in those with ER+/HER2– breast cancer than in

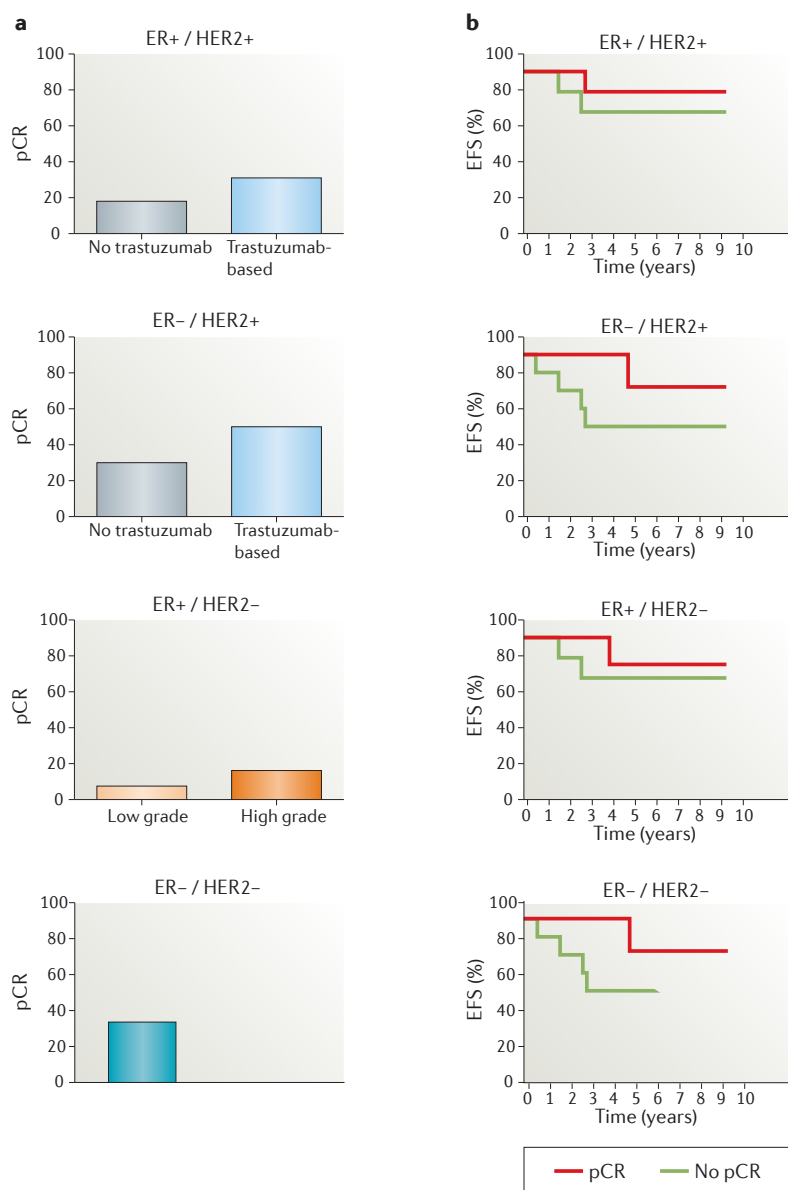


Figure 2 | Pathological complete response (pCR) and outcomes. a | The graphs demonstrate the proportion of patients who achieve a pCR to neoadjuvant therapy with systemic treatments relevant to each of the major breast-cancer subtypes, based on the findings of a pooled analysis of clinical-trial data by Cortazar *et al.*⁵⁸ For patients with HER2-positive primary tumours (both oestrogen receptor (ER)-positive and ER-negative), pCR data for the trastuzumab-based and no trastuzumab treatment arms are shown. In the ER+/HER2- group, pCR is shown for both low-grade and high-grade tumours. In the ER-/HER2- group, pCR data is shown without dichotomization. **b** | Illustrative Kaplan–Meier curves showing the association between pCR and event-free survival (EFS), stratified according to breast-cancer subtype.

women with HER2-positive or ER-/HER2- disease subtypes (FIG. 2a). Furthermore, the strongest associations between pCR and long-term outcome have been reported for aggressive breast cancer subtypes (that is, ER-/HER2- and ER-/HER2+; FIG. 2b)⁵⁸. In the FDA-led meta-analysis⁵⁸, most of the studies examined enrolled patients with biologically heterogeneous breast-cancer subtypes; an exception to this pattern was the disease-specific NOAH trial⁵¹, in which trastuzumab plus chemotherapy or chemotherapy alone were

administered only to patients with HER2-positive breast cancer (64% ER negative and 36% ER positive for both treatment arms). In the NOAH trial⁵¹, EFS was strongly associated with pCR, particularly in patients given trastuzumab: the hazard ratio for EFS between the patients with pCR who received trastuzumab versus those with a pCR who did not receive this agent was 0.29 (95% CI 0.11–0.78, $P=0.0135$). This finding suggests that the relationship between pCR and long-term outcomes might also depend on the specific therapy used.

Likewise, the updated data from the NeoALTTO trial³⁸ indicate that, although no statistically significant differences in the secondary end points — EFS and overall survival — were observed between the treatment arms, patients who experienced a pCR had significantly better outcomes than those patients without a pCR. This relationship between achievement of a pCR and survival outcomes held true for EFS and overall survival in the entire cohort of patients and the subset of patients with hormone-receptor-negative disease, and pCR was also independently associated with EFS, but not overall survival, in the patients treated with dual-agent HER2 therapy³⁸; in the other treatment groups, pCR was not associated with EFS or overall survival³⁸. It should be emphasized, however, that this trial was not powered to detect statistically significant differences in EFS or overall survival. Thus, evidence suggests that if a new therapeutic option produces an increase in the frequency of pCR compared with standard therapy alone, the novel treatment is likely to result in long-term survival benefits (EFS and overall survival), particularly if treatment is focused on well-defined breast-cancer subtypes that are known respond better to neoadjuvant therapy (such as ER-/HER2- and ER-/HER2+ disease). Nevertheless, adequately powered trials are required to confirm this holds true for dual-agent HER2 blockade.

Insights from other neoadjuvant trials

In addition to NeoALTTO³⁸, other neoadjuvant studies have investigated the addition of lapatinib to trastuzumab for the treatment of patients with HER2-positive breast cancer^{42,55,65}. The Cancer and Leukaemia Group B 40601 trial (CALGB 40601)⁴² quantified the pCR rates of patients who received weekly paclitaxel and either trastuzumab plus lapatinib or trastuzumab alone; the pCR rate was modestly higher with combined dual-agent HER2 blockade, but this difference did not reach statistical significance: 51% (95% CI 42–60) versus 40% (95% CI 32–49), $P=0.11$. The authors also analysed the results according to gene-expression-based intrinsic molecular subtypes, and gene-sequence and copy-number abnormalities in primary tumours and residual disease; these studies revealed that pCR rates were higher among the patients with a specific molecular disease subtype defined as ‘HER2-enriched’ (REFS 42,66). In addition, the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-41 trial⁵⁵ and European Organisation for Research and Treatment of Cancer (EORTC) 10054 trial⁶⁵ randomly assigned patients to receive neoadjuvant chemotherapy with either trastuzumab, lapatinib, or the combination of both anti-HER2 agents. The dual-agent

HER2-targeted therapy was associated with numerically higher, but not statistically significant, pCR rates than single-agent HER2-directed therapy in both trials^{55,65}.

Thus, unlike the NeoALTTO findings²⁸, the results of the CALGB 40601, NSABP B-41, and the EORTC 10054 trials did not demonstrate a statistically significant increase in pCR rates for the trastuzumab plus lapatinib combination^{42,55,65}. Of note, the NSABP B-41 treatment regimen was more similar to that used in the ALTTO study than the NeoALTTO treatment approach^{28,32}; however, the difference in the characteristics of the study populations might also, at least partially, explain the discordance between the results of these trials, as well as the NeoSPHERE and NeoALTTO trials^{24,28,55,65}. In particular, the negative results of both the CALGB 40601 and the NSABP B-41 trials might also be at least partially explained by the inclusion of a higher proportions of women with ER-positive disease (with a generally lower frequency of pCR than patients with ER-negative tumours) than in the NeoALTTO and NeoSPHERE trials^{24,28,55,65}. Of note, in the CALGB 40601 trial⁴², patients with ER-negative tumours had higher pCR rates after dual-agent versus single-agent HER2 blockade (77% versus 55%), but the differences were modest in those with ER-positive tumours (42% versus 39%); however, the improvement with the addition of lapatinib to trastuzumab was not statistically significant in either group.

In the CALGB 40601, NSABP-B41, EORTC 10054 trials^{42,55,65}, pCR was defined as breast pCR only. Similarly, the primary end point of the NeoALTTO trial, as reported in 2012 (REF. 28), was the rate of pCRs defined as the absence of only invasive tumour cells in the breast at the time of surgery (ypT0). Additionally, however, the rate of locoregional total pCR, as defined by no invasive cancer in the breast and no pathological involvement of the axillary lymph nodes (ypT0/Tis ypN0), was a secondary end point of NeoALTTO. This secondary end point was included in NeoALTTO protocol amendment 3 (published on 17th May 2013), and was introduced to achieve consistency with recommendations from the FDA³⁸ — the FDA suggests that either ypT0/Tis ypN0 or ypT0 ypN0 should be used as the primary definition of pCR in neoadjuvant trials⁵⁸. The absence of a requirement of lymph-node complete response in the NeoALTTO trial might constitute another reason why differences in the primary end points were observed between the NeoALTTO and ALTTO trials. Further exploratory analysis of these factors is warranted.

By contrast, the lack of efficacy with lapatinib monotherapy has been consistently demonstrated in the neoadjuvant trials that compared anti-HER2 monotherapy with dual-agent HER2 blockade, as well as in the ALTTO trial in the adjuvant setting^{28,32,42,55,65,67}. Moreover, akin to the NeoALTTO trial, the results of the Geparquinto trial⁶⁷ indicated a significantly lower pCR rate with lapatinib versus trastuzumab monotherapy, when added to a 24-week chemotherapy regimen comprising an anthracycline and taxane. The CALGB 40601 trial⁴² data also showed a trend towards a lower pCR rate with 16 weeks of lapatinib versus trastuzumab

therapy, as did data from the EORTC 10054 study⁶⁵. In the NSABP B-41 trial⁵⁵, investigators also demonstrated no difference in pCR rates between the lapatinib and trastuzumab monotherapy arms, but a higher pCR rate for dual-agent HER2 blockade.

Using pCR to identify new biomarkers

At present, we cannot limit dual-agent HER2 treatment to only those patients who are likely to benefit (over standard therapy) in either the neoadjuvant or the adjuvant setting. Thus, when treating the entire population of HER2-positive patients (assuming we cannot select those who will achieve a pCR beforehand) only a small fraction (20%) might gain additional benefit from dual-agent therapy, compared with the standard of care, but the larger fraction of HER2-positive patients will not and will be exposed to the excess biological toxicity and costs of dual-agent HER2 blockade. Further combined analysis of the pCR data and tumour molecular characteristics might enable those patients who will benefit from dual-agent (over the single-agent) HER2-targeted therapy to be identified, so that future trials can be stratified by predictive biomarkers — ultimately making the harm-to-benefit ratio of the dual-agent approach more favourable. Deciphering which patients are likely to achieve a pCR is, therefore, a thought-provoking topic; theoretically, the same features that make patients 'responders' in the neoadjuvant setting might also have the same implications in the adjuvant setting.

Studies have been conducted to discover biomarkers indicative of response to dual-agent therapy using NeoALTTO tumour-tissue biopsy samples^{68,69}. For example, baseline biopsy samples were screened for mutations in the gene encoding the phosphatidylinositol 3-kinase (PI3K) catalytic subunit (*PIK3CA*)⁶⁸. Patients treated with a dual-agent HER2 therapy who had a wild-type *PIK3CA* status had a total pCR rate of 53.1% versus 28.6% in patients with tumours that harboured *PIK3CA* mutations ($P=0.012$). This finding has already enabled stratification of patients for treatment to be incorporated into the design of some clinical trials. For instance, in the NeoPHOEBE trial (NCT01816594)⁷⁰, patients with breast cancer have been molecularly stratified to determine whether any benefit of the addition of a PI3K inhibitor to neoadjuvant trastuzumab and paclitaxel is dependent on *PIK3CA*-mutation status.

High levels of tumour-infiltrating lymphocytes (TILs) have been consistently linked to a more-favourable prognosis in patients with early stage triple-negative and HER2-positive breast cancers^{71–73}. Thus, investigators have also analysed whether the presence of TILs at diagnosis was associated with achievement of a pCR and/or EFS in patients treated in the NeoALTTO trial⁶⁹. They found that the presence of TILs at diagnosis was an independent prognostic marker for a pCR — TILs comprising >5% of the tumour were associated with higher pCR rates, independent of treatment group, (adjusted odds ratio 2.60, 95% CI 1.26–5.39, $P=0.01$)⁶⁹. EFS was also related to TIL levels: every 1% increase in the proportion of TILs was associated with a 3% decrease in the event rate (HR 0.97, 95% CI 0.95–0.99, $P=0.002$)

across all treatment groups⁶⁹. These results suggest that enhancing antitumour immune responses is important to improving survival outcomes.

Other challenges in (neo)adjuvant trials

The design of neoadjuvant and adjuvant clinical trials in patients with early stage breast cancer can influence the tumour responses and thus patients' prognoses. Neoadjuvant and adjuvant clinical trials, however, have important differences (TABLE 2): compared with adjuvant clinical trials, neoadjuvant trials have smaller sample sizes; the patients enrolled sometimes have shorter durations of drug exposure and follow up, resulting in less long-term safety information being obtained; and the primary end point is usually pCR, rather than outcomes related directly to disease progression and/or survival. In addition, some of the greatest benefits of using the neoadjuvant approach are possibility of tumour downstaging (local control), and the ability to assess the clinical and radiological responses of the primary breast tumour and axillary lymph nodes *in vivo*³ — which can vary from mild responses (or even progression) to complete responses (that is, pCRs). Neoadjuvant trials are important, however, not only for local control of disease, but also to accelerate drug development⁵⁷.

Adjuvant trials have become less sustainable because they take a long time to complete, are costly, and require enrolment of a large number of patients⁵⁷. Neoadjuvant trials could potentially overcome many of these challenges if the short-term results (pCR) are able to predict long-term outcomes, such as EFS and overall survival. At present, the literature includes evidence that cancer biology, tumour heterogeneity, and mechanisms of

resistance are other variables, in addition to the inherent differences in clinical-trial designs and statistical power, that might have an important role in explaining the discrepancies between neoadjuvant and adjuvant trials in breast cancer; however, no definitive answers are available, and preclinical and clinical validation is required. Furthermore, comparing the results of neoadjuvant trials that used the imperfect primary end point of pCR (and indeed, demonstrated no effect on EFS or overall survival) and adjuvant trials with traditional survival end points has obvious caveats. We nevertheless discuss the potential biological reasons to explain the differences in the results of neoadjuvant and adjuvant studies that use essentially the same therapeutic regimens.

Capturing the dynamics of resistance

Given the often shorter treatment durations and higher tumour burdens involved in neoadjuvant trials versus adjuvant trials of the same drugs, capturing the dynamics of resistance might be useful in predicting therapeutic resistance and the dynamics of tumour repopulation by a resistant subclone. Treatment resistance due to resistant clones that either pre-existed before treatment or were acquired over the course of therapy is the key contributor to the lack of long-term survival improvement in both neoadjuvant and adjuvant settings. Potentially, the presence of a higher tumour burden at the time of systemic treatment in the neoadjuvant setting versus the adjuvant setting could increase the probability that therapy will select for more-aggressive clones that, if left behind after surgery, repopulate the tumour more quickly. Thus, one could hypothesize that patients without a pCR might have worse outcomes with neoadjuvant versus adjuvant

Table 2 | Comparison of main characteristics of neoadjuvant and adjuvant trials

Parameter	Neoadjuvant trials	Adjuvant trials
Definition	Treatment given before surgery	Treatment given after surgery
Sample size	Smaller	Larger
End points	Response rates and duration (pCR, EFS)	Relapse and survival (DFS, overall survival)
Time taken to complete	Months	Years
Costs	Lower	Higher
Advantages	<ul style="list-style-type: none"> • Tumour downstaging—that is, converting a previously unresectable, locally advanced breast cancer to an operable tumour • Enables early assessment of response to treatment • Response-guided therapeutic planning is possible • Enables comparison of the characteristics of pretreatment and post-treatment tumour samples (in those without a pCR), which might facilitate biomarker discovery, prognostication, patient stratification, and assessment of responses to therapy 	<ul style="list-style-type: none"> • Designed to increase the chance of long-term survival • Quality-of-life questionnaires can be used to assess the long-term effects of treatment in multiple domains
Disadvantages	<ul style="list-style-type: none"> • Local tumour control might be delayed • Complicates pathological assessments and staging • In some circumstances, duration of drug exposure is shorter, which might reduce its effectiveness • Less long-term safety information is available • Potential for selection of resistant clones owing to relatively high disease burden at the time of systemic treatment 	<ul style="list-style-type: none"> • Risk of exposing a large number of patients to treatment that is more toxic and/or no more (or even less) effective than the current standard of care • Precludes assessment of response to systemic treatment and, thus, does not enable response-guided treatment planning

DFS, disease-free survival; EFS, event-free survival; pCR, pathological complete response.

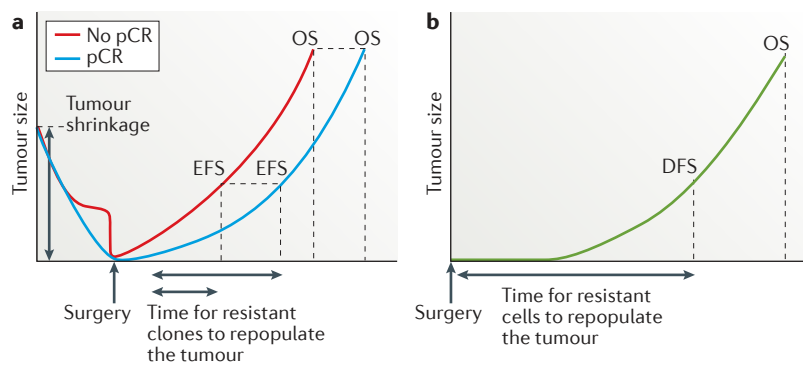


Figure 3 | The influence of therapeutic response on survival. a | In the neoadjuvant setting, achievement of a pathological complete response (pCR) is hypothesized to result in prolonged event-free survival (EFS) and overall survival (OS), compared with an incomplete response, because fewer drug-resistant clones are present and, therefore, even fewer are likely to be left behind after completion of surgery and systemic treatments; thus repopulation of the tumour and/or the development of clinically detectable metastases is delayed or might never happen, and OS is prolonged in the population of patients with a pCR. The early benefit on EFS associated with achieving a pCR to neoadjuvant therapy, compared with an incomplete response, might be diminished in the long-term, however, as resistant clones repopulate the tumour and eventually lead to clinical disease recurrence and, in some cases, death; that is, attaining a pCR might improve OS, but the proportion of patients who are cured might not be significantly different. **b** | In the adjuvant setting, the time taken for any residual resistant cells to repopulate the tumour is reflected in the duration of disease-free survival (DFS) and OS. In all scenarios, the probability of pre-existing resistance, and thus the chance of residual cells being left after all treatment is completed, increases with tumour size at diagnosis and might be at least partially related to the breast cancer subtype. It should be noted, for example, that triple-negative breast cancers tend to be larger and to have more-rapid growth than other breast cancer subtypes¹¹⁶; therefore, the risk of relapse might be higher, and the time to relapse shorter, for this subtype compared with others. Ultimately, the benefit of complete tumour shrinkage in patients with a pCR, in comparison with the outcomes of patients who receive only adjuvant therapy, might be minimal — assuming that almost all tumour cells are removed when R0 resection is achieved and that, in the adjuvant setting, the small number of residual cells are less likely to be treatment resistant.

treatment because, presumably, the level of intrinsic resistance is already higher in these individuals. This possibility might also provide an explanation for the lack of EFS and overall survival benefit observed when considering the entire dual-agent HER2 treatment cohort, despite the improved pCR rate — that is, the patients without a pCR might have had worse outcomes after neoadjuvant therapy (rapid disease progression owing to selection of resistant clones), thus diluting the pCR benefit in the overall treatment cohort.

Conceivably, the early benefit of novel therapies in patients who achieve a pCR versus those who do not achieve a pCR, as observed in neoadjuvant trials, might be diminished in the long term as resistant clones repopulate the tumour and eventually lead to disease recurrence in selected cases (FIG. 3). The Luria-Delbrück models⁷⁴ of bacterial resistance have been adapted to study pre-existing drug resistance in several cancer types, including chronic myeloid leukaemia (CML)^{75,76}, lung cancer⁷⁷, and colorectal cancer⁷⁸. The theoretical predictions imply that the probability of pre-existing resistance increases with tumour size at diagnosis and the mutation rate^{79–81}. Diaz *et al.*⁷⁸ performed mathematical calculations based on the dynamics of *KRAS* mutations in ctDNA over the course of anti-EGFR therapy in 28 patients with colorectal

cancer. Their results suggest that resistance mutations in *KRAS* and other genes were highly likely to be present in subclonal tumour-cell populations before treatment initiation⁷⁸. This hypothesis is in line with existing experimental evidence for other targeted agents^{76,82–84}. The authors concluded that resistance is a *fait accompli*, and the time to recurrence is thus the interval required for the resistance clones to repopulate the tumour⁷⁸.

One can also posit, therefore, that if the effectiveness of analogous neoadjuvant and adjuvant therapy are the same, the extent of tumour-cell killing/removal would predominantly determine recurrence and survival outcomes. Thus, the degree and characteristics of any micrometastatic residual disease that remains after neoadjuvant or adjuvant therapy might ultimately negate any overall benefit expected based on increased pCR rates or DFS rates with novel therapies, respectively. In this context, identifying micrometastatic residual disease after neoadjuvant and also after adjuvant therapies might indicate targetable biomarkers (which can be different from those present in the primary breast tumour) that are associated with therapeutic resistance and create opportunities for therapeutic interventions before the development of clinical metastasis⁸⁵. The non-invasive monitoring of micrometastatic residual disease through liquid biopsies (that is, ctDNA) might be used as a surrogate or complementary tool for response-guided therapy, and could lead to the optimization of response assessment in the neoadjuvant and adjuvant settings^{85–87}. In patients with nonmetastatic breast cancer, liquid biopsies offer the potential to capture and monitor minimal residual disease following curative resection, preceding the development of clinical or radiological recurrence, and represent a tool to facilitate assessments of tumour dormancy^{85–92}.

Tumour heterogeneity and drug resistance

The development of massively parallel sequencing technologies and digital genomic analyses has provided preliminary evidence of breast-cancer heterogeneity. Tumour heterogeneity has been documented to be both spatial^{93–96} and temporal^{93,97,98}, and might explain why not all patients who receive a given therapeutic approach benefit from it, or why some patients have only transient responses. How tumour heterogeneity affects clinical outcomes is a subject for ongoing research^{86,96,99–102}.

Using laser-capture microdissection and *in situ* single-cell analysis, investigators have demonstrated that *HER2* amplification and *PIK3CA* mutations are distributed heterogeneously in breast tumours^{96,103}. This spatio-temporal intratumour heterogeneity could interfere with responses to neoadjuvant and adjuvant treatments. For instance, when tumour samples collected from patients with *HER2*-positive breast cancer before and after neoadjuvant therapy were compared, the frequency of cells containing *HER2* amplification was reduced and samples were enriched for cells containing *PIK3CA* mutations⁹⁶. This observation might go some way to explaining the suboptimal efficacy of anti-*HER2* therapy in the post-surgery period after neoadjuvant therapy (that is, using EFS and overall survival as end points), as proportionally fewer cells containing *HER2* amplification might

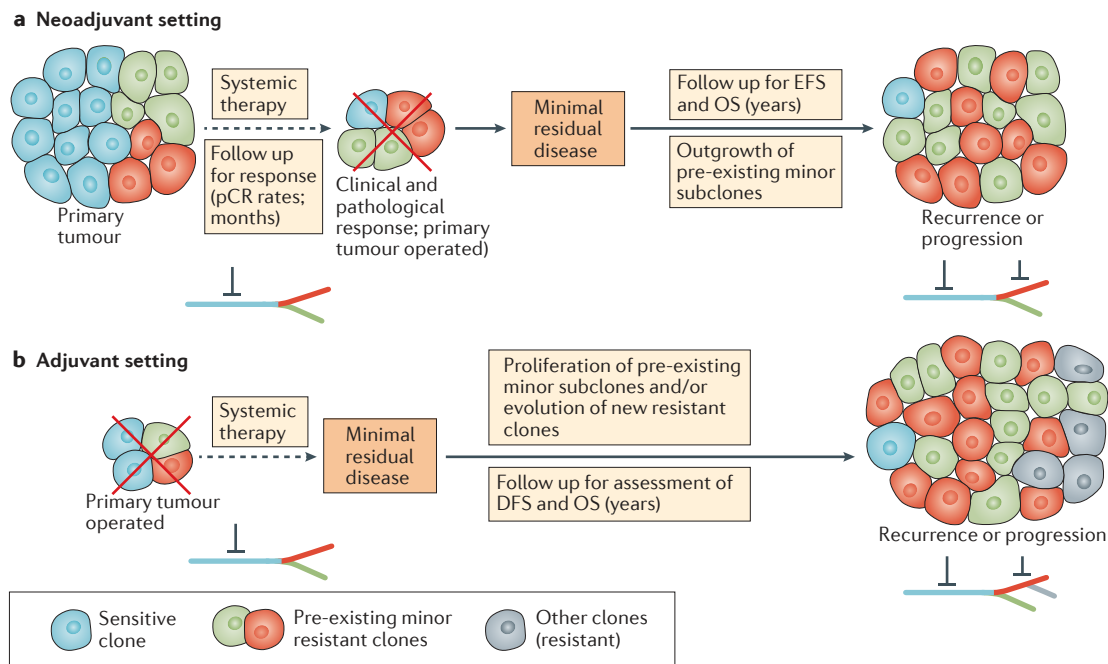


Figure 4 | Tumour heterogeneity and drug resistance in the neoadjuvant and adjuvant breast-cancer settings.
a | The efficacy of neoadjuvant treatment targeting ‘trunk’ (clonal) mutations or aberrations that are expressed by most or all of the tumour cells might nevertheless be limited by the overgrowth of spatially distinct distributed subclones with intrinsic resistance mechanisms (green and red cells), leading to primary drug resistance. This outgrowth of resistant subclones might underlie incomplete responses, or eventual relapse in those with a complete response. **b** | In the course of adjuvant therapy, the treatment duration and follow-up time are longer, creating a niche for the development of secondary (acquired) resistance to therapy, and/or outgrowth of pre-existing minor resistant subclones that were left behind after surgery and could persist despite systemic therapy. Contemporary data suggest, however, that the latter process is likely to predominate — most resistant clones that underlie recurrence are detectable before treatment and, therefore, eventual acquired resistance is probably a consequence of low-level intrinsic resistance. The trunk–branch model for tumour heterogeneity is shown: the trunk of the tree bears ubiquitous genomic alterations; acquired subclonal mutations that are present only in a subset of the tumour cells compose the branches. Inhibitory symbols represent pharmacological blockade. DFS, disease-free survival; EFS, event-free survival; OS, overall survival; pCR, pathological complete response

be present in some patients after surgery. By contrast, the enrichment of *PIK3CA*-mutant cells by neoadjuvant therapy could potentially increase the chance of these resistant cells being left behind after surgery, compared with the use of adjuvant systemic therapy only, thus negatively affecting the outcomes of neoadjuvant therapy. Of note, however, in some breast cancers with a heterogeneous *HER2* amplification pattern (that is, *HER2*-amplified and *HER2*-non amplified components in the same tumour), the *HER2*-amplification might not be truncal in the tumour (that is, is not present in all cancer cells of a tumour), and other genetic alterations could be driving the *HER2*-negative cells of those tumours¹⁰³. Cancer seems to follow Darwinian rules of evolution^{93,104,105}. The trunk–branch model for tumour heterogeneity suggests that the trunk (clonal events, present in all tumour cells, at all tumour regions or sites) and branch (subclonal events, present in a limited number of cells, potentially at distinct sites of a tumour) phylogeny of primary tumours could provide hints for deciphering therapeutic resistance (FIG. 4). Ideally, targeting actionable truncal genetic alterations, which are less susceptible to sampling bias and are present ubiquitously, should represent the best therapeutic strategy¹⁰⁶. If a tumour has multiple branches, however, monotherapy could

represent a specific selective pressure that promotes the outgrowth of resistant clones over time. The branches should also be targeted because subclones that harbour a resistant event can be present. Thus, in addition to targeting truncal events, targeting subclones (the branches) and their inter-relationships should be another strategy to prevent clonal outgrowth and resistance. Neoadjuvant therapy seems to interfere with the genetic diversity of the tumour-cell population and, therefore, poses challenges for the clinical management of patients and for the development of systemic therapeutics — as the molecular characteristics of tumours might change under the selective pressure of treatment¹⁰⁷. Evidence indicates that post-treatment tumours have different biological characteristics compared with primary tumours^{96,99–102}. Indeed, residual tumours after neoadjuvant treatment are likely to have a different, and frequently less-favourable, characteristics and composition than those of the diagnostic sample^{108,109}. At present, however, no data exist regarding the influence of tumour evolution on the achievement of a pCR and the subsequent translation of such responses into overall survival benefits. Such data might shed some light on the biological heterogeneity of breast tumours and support the need for retesting the *HER2* status of the surgical sample

after neoadjuvant therapy, in order to accurately determine the appropriate use of further targeted therapy, in suitable patients.

At present, firm evidence on the levels to which heterogeneity affects the clinical management of patients with breast cancer is lacking, and to what extent this issue might explain the discrepancies between the results of neoadjuvant and adjuvant trials in breast cancer remains unknown. Potential reasons for why pCR rates increased with dual-agent HER2 blockade, whereas DFS and overall survival in the adjuvant trials (as well as EFS and overall survival in the neoadjuvant trials) did not, might be manifold. First, if the higher tumour burden present during neoadjuvant therapy increases the risk that treatment will result in selection of resistant clones, the outcomes with dual-agent HER2 blockade should have been better in the adjuvant setting — wherein most of the tumour is removed, reducing the probability of resistant clones remaining and also the tumour heterogeneity); however, evidence from NeoALTTO and ALTTO have shown the opposite in terms of primary end points (but not long-term outcomes). Second, the properties of any minimal residual disease in the post-surgery setting could contravene the potential benefits of a therapy that is known to result in deeper responses (increase pCR rates) in the neoadjuvant setting.

The use of adaptive trial designs, in which genetic information for the tumours of patients with breast cancer is used to guide therapeutic approaches, should be envisioned. Trials incorporating this approach should be adapted to encompass low-frequency somatic events that are associated with therapeutic resistance and further morbidity.

Conclusions

Neoadjuvant studies have provided insights into the association between pCR and long-term outcomes in patients with breast cancer, particularly for those with HER2-positive disease. The neoadjuvant setting seems to be a different scenario compared with the adjuvant setting. Indeed, therapeutic benefits detected in the neoadjuvant setting — as defined by increases in pCR — have not been translated into significant survival increases when the same modern targeted therapies are used in the adjuvant setting. In general, however, neoadjuvant therapies do not seem to improve the overall survival of unselected patients with breast cancers, when compared with conventional postoperative adjuvant therapy^{5,6}. The increasing rates of pCR following neoadjuvant therapy have, nevertheless, had a clinically significant effect on locoregional treatment considerations.

At present, the data describing long-term outcomes of patients after anti-HER2 based neoadjuvant therapy remain sparse¹⁰. In the neoadjuvant clinical trials, empirical definitions of pCR have been used, with the purpose of utilizing pCR as a surrogate for long-term outcomes. Nevertheless, the burden of residual disease after neoadjuvant chemotherapy has been associated with long-term prognosis^{111,112}. For example, data from the NeoALTTO trial suggested better outcomes in those with a pCR, independent of treatment, but in the overall cohorts, the

increased response rates with dual-agent HER2 blockade did not correlate with EFS or overall survival²⁸. Indeed, evidence regarding the association between pCR and EFS for new therapies in breast cancer remains limited^{57,58}.

The design of future clinical trials in the neoadjuvant setting should incorporate several features to overcome the lack of evidence as to whether increased pCR rates translate into better long-term outcomes: standardization of the definition of pCR across clinical trials; biomarker-based stratification of patients who are most likely to benefit from a specific regimen; and investigation of whether agents brought from the metastatic setting to the neoadjuvant setting would be active and induce pCRs, and the time span of these events (that is, do they occur within the duration of typical neoadjuvant therapy or require a longer period).

In fact, neoadjuvant trials to test specific agents based on biomarker signature of HER2-positive breast cancers have already been initiated^{113,114}. For example, the I-SPY 2 trial¹¹⁴ is a neoadjuvant dynamic adaptive-design phase II trial that is enrolling patients with high-risk breast cancer. In this trial, tumour biomarker signatures are being determined in order to match experimental drug regimens to the patients who would most benefit from them. The results of this study will likely shed some light on the predictive and prognostic values of pCR, while also providing information on tumour biology and the simultaneous administration of novel therapeutic agents. A phase III registration trial (I-SPY 3) of neratinib (a small-molecule inhibitor of HER2) plus standard neoadjuvant therapy is planned, based on the identification of HER2-positive hormone-receptor-negative group in the phase II study who might benefit from this therapy¹¹⁴.

In addition to lapatinib plus trastuzumab, dual-agent HER blockade with pertuzumab and trastuzumab is being explored in the adjuvant APHINITY trial³¹, and whether the positive results for this approach observed in the NeoSPHERE trial (which reported doubling of the pCR rate for dual-agent HER2 blockade as compared to single-agent HER2 inhibition with trastuzumab) will be translated to the adjuvant setting (or even into an EFS and overall survival benefit in the neoadjuvant setting) is unknown. In addition, whether a subgroup of patients with HER2-positive breast cancers who would be sufficiently treated with trastuzumab as the only anti-HER2 therapy (as opposed to dual-agent HER2 blockade) can be identified remains to be determined.

The new wave of neoadjuvant targeted therapy trials has resulted in a paradigm shift in the treatment of breast cancer; however, the limitations of the current approaches, as outlined herein, should be recognized. Intratumour heterogeneity and mathematical modelling might have thought-provoking roles in addressing such challenges. In the neoadjuvant and adjuvant settings, biomarkers that predict response and resistance, and that can capture the molecular characteristics of micrometastatic disease are urgently needed¹¹⁵. Further developments of massively parallel sequencing technologies should provide more opportunities to characterize the genetics of micrometastatic disease and help realize the potential of precision medicine.

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Author contributions

All authors contributed to researching the data for the article, discussions of content, and the writing, review and editing of the manuscript.

Competing interests statement

The authors declare no competing interests.

Review criteria

A review of the biomedical English-language literature was conducted using the PubMed and MEDLINE databases. Articles published before October 2015 were included. The search terms used included “breast cancer”, “neoadjuvant therapy”, “adjuvant therapy”, “targeted therapy”, “NeoALTTO”, and “ALTO”. Relevant abstracts of studies presented at the ASCO and ESMO annual meetings, and San Antonio Breast Cancer Symposium were also included.