Combined Peri-operative Lapatinib and Trastuzumab in Early HER2-Positive Breast Cancer Identifies Early Responders: Randomised UK EPHOS-B Trial Long term results

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Translational relevance:

- In a randomised trial of 257 HER2-positive breast cancer patients, lapatinib (alone 66% or in combination 74%) for 11 days produced higher Ki67 response rates than trastuzumab alone (37-45%) or control (5-7%).
- Combination treatment achieved a pCR or RCB1 in 26% cancers.
- After 6 years median follow-up, perioperative falls in Ki67% of 50% or more were associated with a lower relapse rate than smaller or no decrease in Ki67.
- Early response to therapy identifies cancers dependent on the HER2 pathway, allowing individualization of treatment.

ABSTRACT (254 words)

Background

EPHOS-B aimed to determine whether peri-operative anti-HER2 therapy inhibited proliferation and/or increased apoptosis in HER2-positive breast cancer.

Patients and methods

This randomised phase 2, 2-part, multi-centre trial included newly diagnosed women with HER2-positive invasive breast cancer due to undergo surgery. Patients were randomised to: Part-1(1:2:2), no treatment (control), trastuzumab or lapatinib; Part-2(1:1:2) control, trastuzumab, or lapatinib and trastuzumab combination. Treatment was given for 11 days pre-surgery. Co-primary endpoints were change in Ki67 and apoptosis between baseline and surgery tumour samples (biological response: ≥30% change). Central pathology review scored Residual Cancer Burden (RCB). Relapse-free survival (RFS) explored long-term effects.

Results

Between November 2010 and September 2015, 257 patients were randomised (Part-1: control 22, trastuzumab 57, lapatinib 51; Part-2: control 29, trastuzumab 32, combination 66). Ki67 response was evaluable for 223 patients: in Part-1 Ki67 response occurred in 29/44 (66%) lapatinib vs 18/49 (37%) trastuzumab (p=0.007) and 1/22 (5%) control (p<0.0001); in Part-2 in 36/49 (74%) combination vs 14/31 (45%) trastuzumab (p=0.02) and 2/28 (7%) control (p<0.0001). No significant increase in apoptosis after 11 days was seen in treatment groups. Six patients achieved complete pathological response (pCR, RCB0) and 13 RCB1, all but two in the combination group. After 6-years median follow-up, 28 (11%) had recurrence, and 19 (7%) died. No recurrences or deaths were observed amongst patients who had a pCR. Ki67% falls ≥50% associated with fewer recurrences (p=0.002).

Conclusions

Early response after short duration anti-HER2 dual therapy identifies cancers dependent on the HER2 pathway providing a strategy for exploring risk-adapted individualised treatment de-escalation.

Introduction

The human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor amplified or over-expressed in 15-20% of breast cancers. HER2 lacks a specific ligand, and signalling occurs after the formation of heterodimers with HER-1 and HER-3.[1] Targeting this pathway improves outcomes for patients with HER2-positive breast cancer. Trastuzumab interacts with the extra-cellular domain of the HER2 protein to inhibit its function,[1,2] but the mechanism of action is incompletely understood. Lapatinib blocks the HER1/2 internal tyrosine kinase domain and inhibits proliferation of HER2-positive cancers[3] as shown in a small preoperative trial.[4]

Changes in proliferation biomarkers, including Ki67, predict clinical response and long-term outcome after two weeks of endocrine therapy in oestrogen receptor (ER)-positive breast cancer.[5-7] Incompletely excised breast cancers requiring re-excision within 48 days of surgery showed a significant increase in proliferation if they were HER2-positive, but not if they were HER2-negative.[8] Preventing these early changes provides a rationale for window-of-opportunity studies investigating response to short-term treatment, enhancing prospects for personalising medicine by identifying tumours sensitive to anti-HER2 therapy (without added chemotherapy).

The Effect of Peri-operative Anti-HER2 therapy on Early Breast Cancer Study – Biological phase (EPHOS-B) was designed to assess whether either single agent lapatinib or trastuzumab given as peri-operative treatment had effects on Ki67 and/or apoptosis compared to no anti-HER2 therapy prior to surgery (Part-1). Emerging evidence from the NeoSphere trial[9] on the safety and efficacy of combination anti-HER2 therapy led to a protocol amendment, enabling patient allocation between control, trastuzumab alone or the combination of lapatinib and trastuzumab (Part-2). Whilst the primary biological endpoint reported here is a short-term biomarker, its presentation is accompanied by analyses illustrating impact on 5-year disease outcomes and exploratory analysis associating response with stromal tumour infiltrating lymphocytes (TILs).

Methods

Design and patients

EPHOS-B (NCT01104571) was a phase II, open-label, randomised, UK multi-centre trial conducted in 2 parts, in newly diagnosed women with HER2-positive invasive breast cancer due to undergo surgery within 28 days. Patients had to be willing to undergo adjuvant chemotherapy and trastuzumab post-surgery as per standard-of-care and provide written informed consent for participation and donation of tissue and blood samples. Patients with significant cardiac abnormalities were ineligible. Baseline left ventricular ejection fraction (LVEF) ≥55% was required for trial entry. Full selection criteria are to be found in Appendix 1.

Procedures

In Part-1, patients were randomised (1:2:2) to receive no peri-operative treatment (control), trastuzumab alone or lapatinib alone. In Part-2, patients were allocated (1:1:2) to control, trastuzumab, or lapatinib and trastuzumab combined. Treatment commencement date was agreed prior to randomisation as 11 days (+2/-1) before the scheduled surgery. Treatment allocation was open-label, computer-generated and centrally performed via telephone to the trials unit. In Part-1, permuted blocks (up to size 12) stratified by centre were used; in Part-2, minimisation with a random element and centre as balancing factor was adopted to avoid imbalance given the smaller than expected number of patients randomised per centre.

Trastuzumab (alone or in combination) was given intravenously before surgery on days 1 and 8 at an accelerated loading schedule dose of 6mg/kg[10] (to achieve faster steady state levels of therapeutic efficacy) and one dose of 2mg/kg was given after surgery between days 15-19. In Part-1, lapatinib was given at a dose of 1500 mg/day orally continuously for 28 days including the day of surgery. In Part-2, when combined with trastuzumab, the lapatinib dose was 1000mg/day orally for 28 days.

Definitive surgery was according to local practice and patient choice. If nodal involvement was identified preoperatively, axillary clearance was the standard treatment. Adjuvant treatment was as per local policy and not influenced by EPHOS-B allocated treatment (see

Appendix 1). Patients were followed-up for cardiac toxicities and disease outcome every 6 months for 2 years after randomisation, then annually.

Assessment of biomarkers

Formalin-fixed, paraffin embedded (FFPE) tumour blocks from diagnostic core biopsy and surgical specimens were centrally assessed for quality and tumour content, and analysed for Ki67 and activated caspase 3 (apoptosis) by immunohistochemistry (IHC) using methods previously described.[4,5,11] Hormone receptor status (ER and, when available, progesterone receptor [PgR]) was locally evaluated by IHC: Allred (or Quickscore), percentage tumour cells or H-score were recorded if available. The cut-off for positivity was ≥1% tumour cells, or Allred/Quickscore ≥3. HER2 was evaluated locally and judged positive by IHC 3+ score or fluorescence *in situ* hybridization (FISH) amplification.[12,13] FISH assessment was retrospectively repeated centrally. Central scoring of stromal tumour infiltrating lymphocytes (TILs) was conducted by specialist breast pathologists following international recommendations[14] on scanned H&E baseline and surgery slides.

Unexpectedly, a proportion of patients had insufficient tumour tissue in the surgical specimen for biomarker analysis. A review of pathology reports blinded to allocated treatment was undertaken to identify cases with evidence of potential tumour regression, and their pathology centrally assessed. Tumour bed sections at surgery were reviewed to confirm pathological complete response (pCR) or, if tumour still present, to assess the two largest measurable spans of tumour, cellularity of the tumour within the tumour bed and ductal carcinoma *in situ* (DCIS) within the tumour. Lymph node stage was recorded from pathology reports, and the size of the largest metastatic deposit measured. If there was detectable evidence of tumour response and nodal status was known, Residual Cancer Burden score (RCB) and class (RCB0 [pCR]; RCB1 [minimal residual disease]; RCB2/3 [moderate or extensive residual disease]) were calculated.[15] The remaining cases not selected for central pathology review were considered RCB2/3.

Outcomes

The co-primary endpoints were change in Ki67 and apoptosis, with biological response defined as a relative decrease in Ki67 of >30%, or increase in apoptosis of >30% between baseline and surgery.[16] Secondary endpoints included relapse-free survival (RFS, time from

randomisation to local, regional, distant tumour recurrence or death from any cause, with second primary cancers censored), overall survival and safety. Exploratory endpoints included disease response at surgery, HER2 amplification by FISH, and changes in TILs during the peri-operative period.

Statistical Analysis

The planned sample size (N=250) assumed that biological response in Ki67 or apoptosis would be ≤5% in the control group compared with >30% in the treatment groups, and powered to detect 30% differences in response rate between treatment groups. With 2.5% one-sided type I error, 85% power for the treatment-control comparisons and >80% for between-treatment comparisons, 40 lapatinib (L, Part-1), 55 control (C, Part-1: 20, Part-2: 35), 75 trastuzumab (T, Part-1: 40, Part-2:35) and 80 combination (T+L, Part-2) patients were required. Between-group comparisons were restricted to concurrently randomised patients: L vs C (Part 1), L vs T (Part 1), T+L vs T (Part 2), T+L vs T (Part 2) and T vs C (Part1&Part2).

Peri-operative change endpoints were analysed on all patients who had paired biological data; patients who were found ineligible before starting any treatment were excluded. Surgical Ki67 and apoptosis scores in patients with breast pCR (irrespective of nodal status) were excluded from the analysis. We conducted a sensitivity analysis on Ki67% assuming such patients had 0% score at surgery. Percentage changes by randomised treatment group were compared by Mann–Whitney tests. The proportion of patients responding in Ki67 and/or apoptosis analyses were compared using Fisher's exact test. An alternative threshold of >50% reduction (as used in the MAPLE trial[4]) was also explored. The proportion of patients with pCR or RCB1 was described for each treatment group.

All randomised patients were included in the analysis of time-to-event endpoints, summarised by Kaplan-Meier estimates, and groups compared with log-rank tests. Association of peri-operative biological changes with RFS was considered exploratory in nature; Part-1 and Part-2 were combined for this, and log-rank tests stratified by treatment group. Peri-operative %Ki67 decrease was categorised into decrease of 50% or more, 10% to 50% decrease; or no relevant decrease (<10% decrease or no decrease). Absolute Ki67 values were categorised following on from work in endocrine sensitive breast cancer[7]:

baseline and surgery Ki67 were high if ≥10% or low if <10%, and combined into "high-high", "high-low", "low-high" or "low-low" categories. Patients with pCR or 0% breast cellularity were imputed a value of 0% at surgery and included in these analyses.

TILs were measured as a percentage (occupation of TILs in the tumour stromal surface area), and categorised into low (≤20%) or high TILs (>20%).[17] Analysis of changes in TILS were restricted to patients with paired baseline and surgery data and no evidence of tumour regression at surgery (i.e. RCB2/3), to account for the lack of samples to perform analyses in pCR and RCB1 patients. TILS were associated with trial outcomes, for which Part-1 and Part-2 data were combined.

A 5% significance level was considered for treatment comparisons of primary and secondary endpoints; 1% for all other exploratory analyses. Stata (v13.0 or later) statistical software was used. Data cut-off for biomarker analyses was July 14, 2017; updated for 5-year outcomes on December 20, 2020.

Further details of the methodology are available in the Appendix 1.

Data availability

Formal requests for sharing the data generated in this study will be considered with due regard given to funder and sponsor guidelines. Requests involving collaboration with the EPHOS-B Trial Management Group (TMG) are strongly encouraged. Requests are reviewed by the TMG and will be considered dependent on scientific merit, ethical considerations including patient consent, funding, resources and alignment with the trial objectives. Data sharing is further approved by the Trial Steering Committee.

Results

Patient disposition and baseline characteristics

257 patients were recruited from 21 UK centres; 130 entered Part-1 between November 15, 2010, and July 29, 2013, and 127 entered Part-2 between August 6, 2013 and September 10, 2015. 2 (1%) were found ineligible before starting treatment and excluded from the analysis of peri-operative endpoints (Figure 1). Overall, 172 patients (67%) were ER-positive, with a

median tumour size of 2.2cm (Table 1). Details of adjuvant treatment following surgery are provided in Appendix 2; with no differences between randomised groups in adjuvant treatment received.

Disease Response

40/255 patients showed evidence of potential tumour regression in the central review of pathology reports, and underwent central RCB scoring; the remainder were considered RCB2/3. Although this analysis was originally unplanned, it became an essential component of the main trial analysis in order to inform impact of disease regression on the primary biomarker endpoints (informative censoring).

In Part-1, 1/56 (2%) pCR was observed in the trastuzumab group. In Part-2, 1/32 (3%) pCR occurred in the trastuzumab group, while in the combination group 4/65 (6%) pCR and 13/65 (20%) RCB1 were identified, including two node positive patients who were node negative at surgery (Appendix 3). Two further combination treated patients who had scored RCB1 and RCB2/3 (due to nodal involvement) showed no residual disease in the breast (0% breast cellularity). Amongst the 19 pCR or RCB1 patients, 14 (74%) were ER-positive (Appendix 3). All but one (patient choice) received adjuvant chemotherapy as per local practice.

Before therapy, median (min–max) radiological tumour size was 2cm (0.9–2.8) for pCR patients, 1.4cm (0.5–4.5) for RCB1 patients and 1.9cm (0.1–10) for RCB2/3 patients. In a multivariable analysis in the combination group, only size of tumour was associated with observing pCR/RCB1 response (Appendix 3).

Ki67

Waterfall plots illustrating the range of percentage change in Ki67 observed in individual patients are presented in Figures 2A&2B.

In Part-1, 29/44 (66%) lapatinib patients had Ki67 response (≥30% reduction) compared with 18/49 (37%) trastuzumab patients (P_{LvT}=0.007) and 1/22 (5%) control patients (P_{LvC}<0.0001). Median percentage change in Ki67 was -43% (IQR -68% to -21%) with lapatinib, -14% (IQR -

51% to +6%) with trastuzumab and +2% (IQR -9% to +15%) with control (Table 2, Appendix 4.1).

In Part-2, 36/49 (74%) combination patients had Ki67 response compared with 14/31 (45%) trastuzumab (P_{T+LvT}=0.02) and 2/28 (7%) control (P_{T+LvC}<0.0001) patients. Median percentage change in Ki67 was -49% (IQR -78% to -25%) with combination, -26% (IQR -46% to -6%) with trastuzumab and -2% (IQR -20% to +7%) with control (Table 2, Appendix 4.1).

When combining Part1 & &Part2, 32/80 (40%) trastuzumab patients had Ki67 response compared with 3/50 (6%) control patients (P_{TvC}<0.001). Median percentage change in Ki67 was -20% (IQR -50% to +2%) with trastuzumab and 0% (IQR -13% to +11%) with control.

In sensitivity analyses where 0% Ki67 at surgery was imputed in those patients with breast pCR, similar results were obtained (Appendix 4.2). Treatment differences remained after adjusting for known prognostic factors (Appendix 4.3). In exploratory multivariable analyses of the pooled dataset, no other factors (including ER and PgR status) were found to be associated with Ki67 decrease (Appendix 4.4).

HER2 gene amplification ratio (HER2/CEP17 ratio) by FISH (centrally assessed) correlated with change in Ki67 in the trastuzumab group, both in Part-2 (P=0.008), and Part-1&Part-2 combined (P=0.04); no association was found between amplification ratio and other trial outcomes (Appendix 5).

Apoptosis

In Part-1, 2/37 (5%) lapatinib patients had apoptosis response (>30% increase) compared with 7/38 (18%) trastuzumab patients (P_{LvT}=0.15) and 7/19 (37%) control patients (P_{LvC}=0.01). Median percentage change in apoptosis was -25% (IQR -42% to +1%) with lapatinib, -5% (IQR -18% to +21%) with trastuzumab, and +24% (IQR -10% to +57%) with control (Table 2).

In Part-2, 8/41 (20%) combination patients had apoptosis response compared with 11/30 (37%) trastuzumab (P_{T+LvT}=0.17) and 10/28 (36%) control (P_{T+LvC}=0.17). Median percentage

change in apoptosis was -34% (IQR -56% to +10%) with combination, +4% (IQR -32% to +48%) with trastuzumab, and -2% (IQR -15% to +63%) with control (Table 2).

When combining Parts 1& 2, 18/68 (26%) trastuzumab patients had an apoptosis response compared to 17/47 (36%) control patients (P_{TvC}=0.31). Median percentage change in apoptosis was +2% (IQR -28% to +36%) with trastuzumab and +6% (IQR -12% to +62%) with control.

Changes from baseline in apoptosis correlated positively with changes in proliferation only in the combination group (P= 0.034) (Appendix 6).

5-year time-to-event outcomes

After median follow-up of six years (IQR 5.2 – 7.4), 28 women (11%) had breast cancer recurrence and 19 patients died, with all but one due to breast cancer following recurrence (Appendix 7). The proportion free from breast cancer recurrence at five-years (5yr-RFS) (95% CI) was, in Part-1, trastuzumab 88% (76-94), lapatinib 90% (77-96) and control 95% (72-99); in Part-2, trastuzumab 87% (69-95), combination 92% (83-97), and control 90% (71-97) (Figure 2B). When combining Part-1 and Part-2, 5yr-RFS were trastuzumab 87% (79-93) and control 92% (80-97). There were no significant differences between randomised groups (Figure 2C), even when adjusting by known prognostic factors (Appendix 7.1), although the study was not powered for such comparisons. Overall survival is shown in Figure 2D. None of the patients with pCR recurred or died; only one RCB1 patient had a RFS event (local recurrence).

For analysis of peri-operative Ki67 changes and RFS, 231/257 patients were included. When categorizing Ki67 change (Figure 3A), 2/72 (2.8%) RFS events (local only recurrences, one of these followed by distant recurrence) were observed in the group with Ki67 reductions ≥50%, while 17/77 (22%) RFS events (15 distant recurrences, 2 local only) occurred in the group with reductions between 10% and 50%, and 7/82 (8.5%) RFS events (six distant, one local only) were observed in the group with no relevant reduction; RFS was significantly different between the three groups (P=0.002). Such difference remain in multivariable analysis with other prognostic factors (Appendix 7.2). When categorizing absolute Ki67

values, 189 patients (82%) remained with Ki67 high after 11 days of peri-operative treatment ("high-high"), 38 patients (16%) reduced to low ("high-low") and 4 (1.7%) remained low ("low-low"). No patient increased Ki67 from low to high after 11 days peri-operative treatment. Of the 26 RFS events observed, all but one (the local recurrence in a patient with RCB1 response) occurred in the "high-high" group (Figure 3B).

Exploratory analyses on Stromal Tumour Infiltrating Lymphocytes (TILs)

Baseline TILs (bTILs) could be scored for 230/255 patients (90%); 50 carcinomas (22%) showed high bTILs (>20%); no significant differences were found in bTILs between randomised groups (Appendix 8). We did not observe an association between bTILs and disease response (P=0.58). When associated with RFS (Figure 3C), 2/50 (4%) high bTILs experienced an RFS event, vs 23/180 (13%) amongst patients with low bTILs (P=0.06).

Ki67 change was -33% (IQR -62 to -8) for carcinomas with high bTILs, and -23% (IQR -56 to 2) for low bTILs (P=0.19). In the trastuzumab group Ki67 responses were observed in 8/13 (62%) high bTILs vs 23/62 (37%) low bTILs (P=0.10, Appendix 8).

Change from baseline TILs at surgery was calculated in 191/236 (81%) RCB2/3 patients (Appendix 8). ≥20% TILs increase was observed in 38/69 (20%) trastuzumab, 16/43 (23%) lapatinib, 12/33 (36%) combination and 1/46 (2%) control patients (p=0.002). Ki67 response was observed in 21/35 (60%) patients with ≥20% TILs increase, and in 56/152 (37%) patients without (P=0.012). Having high TILs at surgery seemed to explain improved PFS (P=0.02, Figure 3D) rather than having a ≥20% TILs increase between baseline and surgery (P=0.16).

Safety

Sixteen serious adverse events were reported in 14/257 (5%) patients. Six were unrelated to treatment (four allocated trastuzumab alone (Part-1 & 2), and two allocated combination). Ten were classed as expected serious adverse reactions, occurring in two patients allocated to trastuzumab (Part-1 & 2), five allocated to lapatinib (Part-1) and three allocated to combination treatment.

An additional cardiac assessment after treatment but before adjuvant chemotherapy was introduced as of April 2014, affecting 90/127 Part-2 patients. The assessment was done on 70/90 Part-2 patients and one trastuzumab patient showed an abnormal LVEF of 35%, leading to treatment delay.

Further details on safety can be found in Appendix 9.

Discussion

The EPHOS-B Trial met one of its primary objectives, that 11 days of anti-HER2 therapy, between diagnosis and surgery, without chemotherapy, reduced proliferation, which was seen in all active treatment groups but particularly with the dual agent combination where a Ki67 decrease greater than 30% was seen in 74% of cancers. Furthermore, some tumours became too small to be analysed at the time of surgery, and exploratory analysis revealed dual blockade with lapatinib and trastuzumab resulted in 4/65 (6%) of cases having no residual invasive disease in the breast or nodes (pCR) and a further 13/65 (20%) cases with only minimal residual disease (RCB1).

EPHOS-B trial did not meet its second primary objective of showing an increase in apoptosis in treatment groups, in contrast to a small clinical study which reported increases in apoptosis after 7 days after start of treatment[16], so potentially, since the on-treatment assessment was performed after 11 days treatment, in some patients any increase in apoptosis may have been missed. Furthermore, high proliferation values at baseline correlated with higher apoptosis, and, in treatment groups, the fall in proliferation led to a fall in apoptosis, as observed elsewhere[6,18], so few treated patients had a 30% increase in apoptosis. Whereas the baseline core biopsies had high numbers of Ki67 positive cells to count, low values of apoptosis rose in control patients due to the greater accuracy of assessment on the surgical excision specimens, but fell with the antiproliferative effect of treatments.[19]

Both trastuzumab and lapatinib have been previously shown to inhibit HER2-positive breast cancer proliferation when given before surgery.[1,2,4] Changes in proliferation biomarkers, including Ki67, predict clinical response and long-term outcome after two weeks of

endocrine therapy in ER-positive breast cancer.[5-7] In our study several safeguards were in place to enable exploratory associations of biomarkers with long-term outcomes: adjuvant therapy was to be given as per local protocols; Ki67 results were not fed back to investigators; and centralised review of RCB status was done retrospectively. Although adjuvant treatment may be a confounding factor for long-term outcomes, we did not observe differences in adjuvant treatment received between ki67 change groups (see Appendix 2), nor that RCB0 or RCB1 responders received any more or less treatment (see Appendix 3). In these exploratory analyses, patients with Ki67 reductions ≥50% at 11 days had 5-year RFS 99%..

Reporting eradication of primary tumours after only 11 days' dual anti-HER2 blockade therapy is unprecedented. In the NEO-SPHERE trial,[9] 18/107 (17%) patients had pCR after 4 months' treatment with pertuzumab and trastuzumab and no chemotherapy; for ER and/or PgR-positive tumours, only 3/51 (6%) had pCR. In the TBCRC006 study,[1] patients received lapatinib and trastuzumab, with ER-positive patients (62%) also receiving letrozole. After 12 weeks' therapy, 17/64 (27%) patients had pCR: 8/39 (21%) amongst ER-positive, 9/25 (36%) amongst ER-negative. In the WSG-ADAPT study,[20] 12-week treatment of HER2-positive/ER-negative cancers with trastuzumab and pertuzumab (without chemotherapy) led to 74% exhibiting Ki67 reductions ≥30%,and 36% pCR. Ki67 non-responders had an 8% pCR rate. It is worth noting that for neo-adjuvant HER2 trials tumours were typically over 2cm in size at trial entry, while this was not a requirement in EPHOS-B, and the chance of achieving pCR in the combination group was lower for larger tumours.

In EPHOS-B, 26% combination patients whose cancers regressed (pCR or RCB1) was consistent with 30% pCR seen in the PAMELA study[21] and 27% pCR in TBCRC006[1] after 12 weeks of neoadjuvant dual agent therapy. EPHOS-B utilised a trastuzumab accelerated loading dose (6mg/kg)[22] combined with lapatinib 1000mg to ensure maximal HER2 blockade by 11 days, which may partly account for the earlier responses, as previous neoadjuvant studies used a lower initial doses of trastuzumab.[9,21,23]

Imaging sub-studies assessing ^{18}F-FDG PET/CT at 15 days in the Neo-ALTTO and TBCRC026 trials showed greater SUVmax reductions predicted pCR in response to dual anti-HER2

therapy.[24,25] However PET/CT studies are not widely available for clinical practice, whereas Ki67 and tumour response at 11 days are practical for wider implementation in the neoadjuvant setting to predict response on dual antiHER2 therapy potentially reducing toxicity.

ER and HER2-positive tumours are less likely than ER-negative tumours to have pCR in response to several months of anti-HER2 therapy.[9,23,26-28] Observing 6% pCR and 20% RCB1 in a population with two thirds having ER-positive tumours, after 11 days' therapy, was a surprising finding, as there was no evidence that the ER status of the cancer influenced pCR. pCR incidence in ER-positive/HER2-positive cancers is usually lower than in ER-negative/HER2-positive cancers.[29] The observed effects on both Ki67 and tumour response in the ER and HER2-positive cancers may relate to the second anti-HER2 therapy used. Tyrosine kinase inhibitors such as lapatinib interfere with the intercellular tyrosine kinase signalling, which is known to interact with ER signalling. Recent pCR reports in 27–44% HER2-positive cancers after 12–16 weeks combination therapy[30,31] imply that there is a group of HER2 patients whose primary cancers are highly dependent on HER2 signalling, and their early identification would (if validated in further studies) allow testing of the omission of chemotherapy without detrimental effects on oncological outcomes.

The WSG-ADAPT[20] trial tested a de-escalation approach following identification of early responders: in largely stage 1 HER2-positive HR-negative breast cancers, after Ki67 assessment at 3 weeks of pertuzumab and trastuzumab, patients were randomised to continued dual agent therapy up to 12 weeks, or combined it with weekly paclitaxel. Non-response at 3 weeks predicted lack of pCR at 12 weeks, but addition of paclitaxel in early responders produced 79% pCR, superior to 45% pCR observed when no paclitaxel was added.

The PerELISA[32] neoadjuvant study enrolled mainly stage 2/3 HR-negative HER2-positive cancers that were treated for 2 weeks with an aromatase inhibitor and re-biopsied. Reductions in Ki67 ≥50% allowed treatment with dual agent pertuzumab and trastuzumab, whereas non-responders additionally received paclitaxel. After 13 weeks of treatment, pCR and RCB1 occurred in 52% of early responders. If dual antibody therapy can eradicate some

HER2-positive breast cancers in less than two weeks, a similar approach using a letrozole and dual anti-HER2 therapy from initial biopsy may improve selection of patients who can avoid chemotherapy. These studies imply that a Ki67 reduction >50% to anti-HER2 therapy after 2 weeks of treatment predicts outcome, and that approaches to de-escalation will likely differ according to ER status. Our data, taken with these studies, suggest there may be HER2-positive breast cancers that can be eradicated without chemotherapy. Our data adds evidence that reductions in Ki67 or pCR/RCB1 (by image-guided biopsy or surgery) after 11 days of treatment potentially allows clinicians to select patients who could receive less chemotherapy, a strategy that needs validation in further studies.

TILs affect RFS and response to therapy, but the effect seems driven by trastuzumab (alone or in combination): amongst RCB2/3 trastuzumab patients, higher Ki67 response was observed when a relevant increase in TILs occurred (62%) compared to those without increase in TILs (43%, Appendix 7). Moreover, the phenotype of TILs may also alter from suppressor to effector TILs, but we could not assess that on the samples available. The alterations in TILs>20% with the large reduction in tumour proliferation may have produced the tumour shrinkage seen by 11 days.

Early data with trastuzumab, when given concurrently with or after adjuvant chemotherapy, led to concerns about cardiotoxicity,[33] but there were no effects on LVEF in the combination group and no operations were rescheduled because of cardiac issues in our study. All three neoadjuvant trials using dual agent HER2 blockade with chemotherapy have not found increased short-term cardiotoxicity.[2,9,23] A significant part of the cardiac toxicity reported with anti-HER2 therapy may relate to its use with anthracycline chemotherapy.

The next generation of studies of anti-HER2 therapy in early breast cancer need to address both the potential to reduce chemotherapy in some patients, and additional approaches in others, as defined by their demonstrated sensitivity or not to short duration anti-HER2 therapies. The data we report on the early disappearance of tumours 11 days after treatment commencement may identify a patient group highly sensitive to the HER2-pathway who can potentially avoid chemotherapy altogether.

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Table 1: Patient demographics and tumour characteristics at baseline and at surgery, by randomised treatment group

	PART 1						PART 2						Total N=257	
	Trastuzumab N=57		Lapatinib N=51		Control N=22		Trastuzumab N=32		Combination N=66		Control N=29			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Patient demographics														
Age (years)														
Median (IQR)	50 (47 to 62)		51 (48 to 60)		53 (50 to 62)		52 (48 to 55)		53 (47 to 63)		58 (49to 66)		53 (48 to 62)	
Menopausal status[1]														
Pre-menopausal	24	42	21	41	4	18	11	34	25	38	9	31	94	37
Peri/post-menopausal	33	58	30	59	18	82	21	66	41	62	20	69	163	63
Tumour characteristics from the diagnostic core														
Grade														
Grade 1	3	5	0	0	0	0	0	0	2	3	0	0	5	2
Grade 2	20	35	21	41	9	41	14	44	26	39	13	45	103	40
Grade 3	28	49	24	47	13	59	17	53	36	55	14	48	132	51
Unknown[2]	6	11	6	12	0	0	1	3	2	3	2	7	17	7
Histology type														
Infiltrating ductal (no special type)	54	95	45	88	22	100	29	91	59	89	27	93	236	92
Infiltrating lobular	0	0	3	6	0	0	2	6	4	6	1	3	10	4
Mixed	0	0	2	4	0	0	1	3	0	0	1	3	4	2
Mucinous	1	2	1	2	0	0	0	0	1	2	0	0	3	1
Infiltrating micropapillary	0	0	0	0	0	0	0	0	1	2	0	0	1	0
Not known	2	4	0	0	0	0	0	0	1	2	0	0	3	1
Tumour size (cm)[3]														
<2	26	46	21	41	11	50	19	59	39	59	18	62	134	52
2-5	25	44	27	53	10	46	13	41	26	39	11	38	112	44
≥5	6	11	3	6	1	5	0	0	1	2	0	0	11	4
HER2 locally assessed by														
IHC (IHC 3+)	53	93	46	90	17	77	30	94	61	92	28	97	235	91
FISH (IHC 2+ confirmed by FISH)	4	7	5	10	5	23	2	6	5	8	1	3	22	9
HER2 centrally assessed (FISH)[4]														
HER2 amplified	52	91	46	90	21	95	29	91	58	88	29	100	235	91
HER2 not amplified	4	7	4	8	1	5	3	9	1	2	0	0	13	5

	PART 1						PART 2						Total N=257	
	Trastuzumab N=57		Lapatinib N=51		Control N=22		Trastuzumab N=32		Combination N=66		Control N=29			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
FISH data not available	1	2	1	2	0	0	0	0	7	10	0	0	9	4
ER (local assessment)														
Negative	20	35	20	39	7	32	11	34	15	23	12	41	85	33
Positive	37	65	31	61	15	68	21	66	51	77	17	59	172	67
PgR (local assessment)														
Negative	20	35	23	45	8	36	16	50	28	42	17	59	112	44
Positive	21	37	17	33	6	27	8	25	18	27	5	17	75	29
Missing	16	28	11	22	8	36	8	25	20	30	7	24	70	27
<u>Details of surgery</u>														
Definitive breast surgery														
Conservative surgery	26	46	22	43	14	64	20	63	45	68	18	62	145	56
Mastectomy	31	54	29	57	8	36	12	37	21	32	11	38	112	44
Definitive axillary surgery[5]														
Yes	57	100	51	100	22	100	32	100	66	100	28	97	256	99.6
Axillary node clearance	23	41	25	49	6	27	10	31	16	24	9	31	89	34.6
Level 1 sampling	3	5	1	2	0	0	1	3	5	8	2	7	12	4.7
Sentinel lymph node biopsy	31	54	25	49	16	73	21	66	45	68	17	59	155	60.3
No	0	0	0	0	0	0	0	0	0	0	1	3	1	0
<u>Tumour features at surgery</u>														
No. of lymph nodes involved														
0	35	61.4	25	49.0	16	72.7	19	59.4	48	72.7	19	65.5	162	63.0
1-3	14	24.6	17	33.4	4	18.2	8	25.0	12	18.2	7	24.1	62	24.1
4-9	6	10.5	7	13.7	2	9.1	1	3.1	5	7.6	2	6.9	23	9.0
10+	2	3.5	2	3.9	0	0.0	4	12.5	1	1.5	1	3.5	10	3.9
No. of lymph nodes examined														
1-3	29	50.9	23	45.1	12	54.6	19	59.4	45	68.2	14	48.3	142	55.2
4-9	10	17.5	6	11.8	5	22.7	4	12.5	8	12.1	6	20.7	39	15.2
≥10	18	31.6	22	43.1	5	22.7	9	28.1	13	19.7	9	31.0	76	29.6
Grade														
Grade 1	2	3.5	3	5.9	1	4.5	1	3.1	2	3.0	0	0.0	9	3.5
Grade 2	15	26.3	21	41.1	2	9.1	8	25.0	23	34.9	5	17.2	74	28.8
Grade 3	39	68.4	26	51.0	19	86.4	22	68.8	28	42.4	24	82.8	158	61.5

	PART 1						PART 2						Total N=257	
	Trastuzumab N=57		Lapatinib N=51		Control N=22		Trastuzumab N=32		Combination N=66		Control N=29			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
GX	0	0.0	1	2.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.4
Not known	1	1.8	0	0.0	0	0.0	1	3.1	13	19.7	0	0.0	15	5.8
Tumour size (cm)														
<2	26	45.6	18	35.3	9	40.9	18	56.2	43	65.2	14	48.3	128	49.8
2-5	26	45.6	30	58.8	12	54.6	11	34.4	22	33.3	15	51.7	116	45.1
≥5	5	8.8	3	5.9	1	4.5	3	9.4	0	0.0	0	0.0	12	4.7
Missing	0	0.0	0	0.0	0	0.0	0	0.0	1	1.5	0	0.0	1	0.4

IQR = interquartile range; IHC= immunohistochemistry; FISH= fluorescence in situ hybridization; HER2= human epidermal growth factor receptor 2; ER= oestrogen receptor; PgR= progesterone receptor.

(1) Includes 9 patients (2 trastuzumab Part 2; 5 combination Part 2; 2 Control Part 2) with missing menopausal status data who have been classified based on their age (<50 = pre-menopausal, ≥50 =peri/post-menopausal)

(2) Some UK hospitals do not routinely report grade on the diagnostic core.

(3) Pre-surgery, this measurement is either by ultrasound or clinical examination.

(3) For patients with local FISH testing, scores extracted from pathology reports.

(4) Percentage of mastectomy (p=0.017) and axillary clearance (p=0.047) were found to be lower in Part-2 than Part-1.

Table 2: Analysis of Ki67 and apoptosis, by randomised treatment group

IQR = interquartile range; p= p-value. Table summarises Ki67 and apoptosis at baseline (pre-treatment), at surgery and the changes baseline-surgery. Change is summarised by percentage change = ((surgery score+0.1) - (baseline score+0.1))*100/(baseline+0.1) and log-fold change = log((surgery score+0.1)/(baseline score+0.1)). Negative values indicate decrease from baseline; positive values indicate increase from baseline.

	PART 1			PART 2		
	Trastuzumab	**Lapatinib**	**Control**	**Trastuzumab**	**Combination**	**Control**
Ki67						
N with paired data	49	44	22	31	49	28
Baseline Ki67 - median (IQR)	35 (28 to 45)	34 (27 to 42)	37 (24 to 49)	45 (37 to 57)	40 (29 to 54)	42 (31 to 54)
Surgery Ki67 - median (IQR)	31 (18 to 40)	20 (10 to 30)	35 (25 to 54)	30 (22 to 47)	20 (9 to 34)	39 (29 to 48)
Percentage change - median (IQR)	-14 (-51 to 6)	-43 (-68 to -21)	2 (-9 to 15)	-26 (-46 to -6)	-49 (-78 to -25)	-2 (-20 to 7)
Log-fold change - median (IQR)	-0.2 (-0.7 to 0.1)	-0.6 (-1.2 to -0.2)	0.0 (-0.1 to 0.1)	-0.3 (-0.6 to -0.1)	-0.7 (-1.5 to -0.3)	0.0 (-0.2 to 0.1)
Mann-Whitney test for % change – control vs lapatinib / combination		p<0.0001			p<0.0001	
Mann-Whitney test for % change – trastuzumab vs lapatinib / combination	p=0.0034			p=0.0054		
Response (Ki67 decrease >30%) – N (%)	18 (36.7%)	29 (65.9%)	1 (4.5%)	14 (45.2%)	36 (73.5%)	2 (7.1%)
95% confidence interval	[23.4% to 51.7%]	[50.1% to 79.5%]	[0.1% to 22.8%]	[27.3% to 64.0%]	[58.9% to 85.1%]	[0.9% to 23.5%]
Fisher's exact test – control vs lapatinib / combination		p<0.0001			p<0.0001	
Fisher's exact test – trastuzumab vs lapatinib / combination	p=0.007			p=0.02		
Apoptosis						
N with paired data	38	37	19	30	41	28
Baseline apoptosis - median (IQR)	7 (5 to 9)	7 (5 to 9)	7 (6 to 9)	5 (3 to 8)	7 (5 to 9)	7 (4 to 10)
Surgery apoptosis - median (IQR)	8 (4 to 10)	4 (3 to 7)	8 (7 to 13)	6 (3 to 8)	3 (2 to 10)	7 (5 to 12)
Percentage change - median (IQR)	-5 (-18 to 21)	-25 (-42 to 1)	24 (-10 to 57)	4 (-32 to 48)	-34 (-56 to 10)	-2 (-15 to 63)
Log-fold change - median (IQR)	-0.1 (-0.2 to 0.2)	-0.3 (-0.5 to 0.0)	0.2 (-0.1 to 0.5)	0.0 (-0.4 to 0.4)	-0.4 (-0.8 to 0.1)	0.0 (-0.2 to 0.5)
Mann-Whitney test for % change – control vs lapatinib / combination		p=0.0002			p=0.004	
Mann-Whitney test for % change – trastuzumab vs lapatinib / combination	p=0.01			p=0.03		
Response (apoptosis increase >30%) – N(%)	7 (18.4%)	2 (5.4%)	7 (36.8%)	11 (36.7%)	8 (19.5%)	10 (35.7%)
95% confidence interval	[7.7% to 34.3%]	[0.7% to 18.2%]	[16.3% to 61.6%]	[19.9% to 56.1%]	[8.8% to 34.9%]	[18.6% to 55.9%]
Fisher's exact test – control vs lapatinib / combination		p=0.01			p=0.17	
Fisher's exact test – trastuzumab vs lapatinib / combination	p=0.15			p=0.17		

Figure 1: CONSORT diagram

Figure summarises patients recruited into each part of the trial, patients randomised, patients eligible to start treatment, patients who started treatment and those who completed peri-operative treatment as per protocol. In Part-1, 22 patients were allocated to control, 57 trastuzumab, and 51 to lapatinib; in Part-2, 29 were allocated to control, 32 to trastuzumab, and 66 to the combination. Overall, 255 (99%) patients were considered eligible to start treatment and included in the analysis of peri-operative endpoints. Of the 204 patients in the treatment groups, 201 patients (99%) received some peri-operative treatment, with 190/201 (95%) completing the 11 days of peri-operative treatment. Figure also describes how many patients available for analysis of co-primary endpoints Ki67 and apoptosis. Only patients with both paired samples and enough tumour tissue for biomarker analysis were included in the analysis: 223 patients (88%) had paired Ki67 data and 193 (76%) had paired apoptosis data available for analysis. Patients with pathological complete response or 0% breast cellularity were excluded from main analysis of Ki67 and apoptosis.

Figure 2 – Percentage change in Ki67 between pre-treatment (baseline) and surgery for Part 1 (A) and Part 2 (B); Kaplan Meier estimates by treatment group for relapse free survival (C) and overall survival (D)

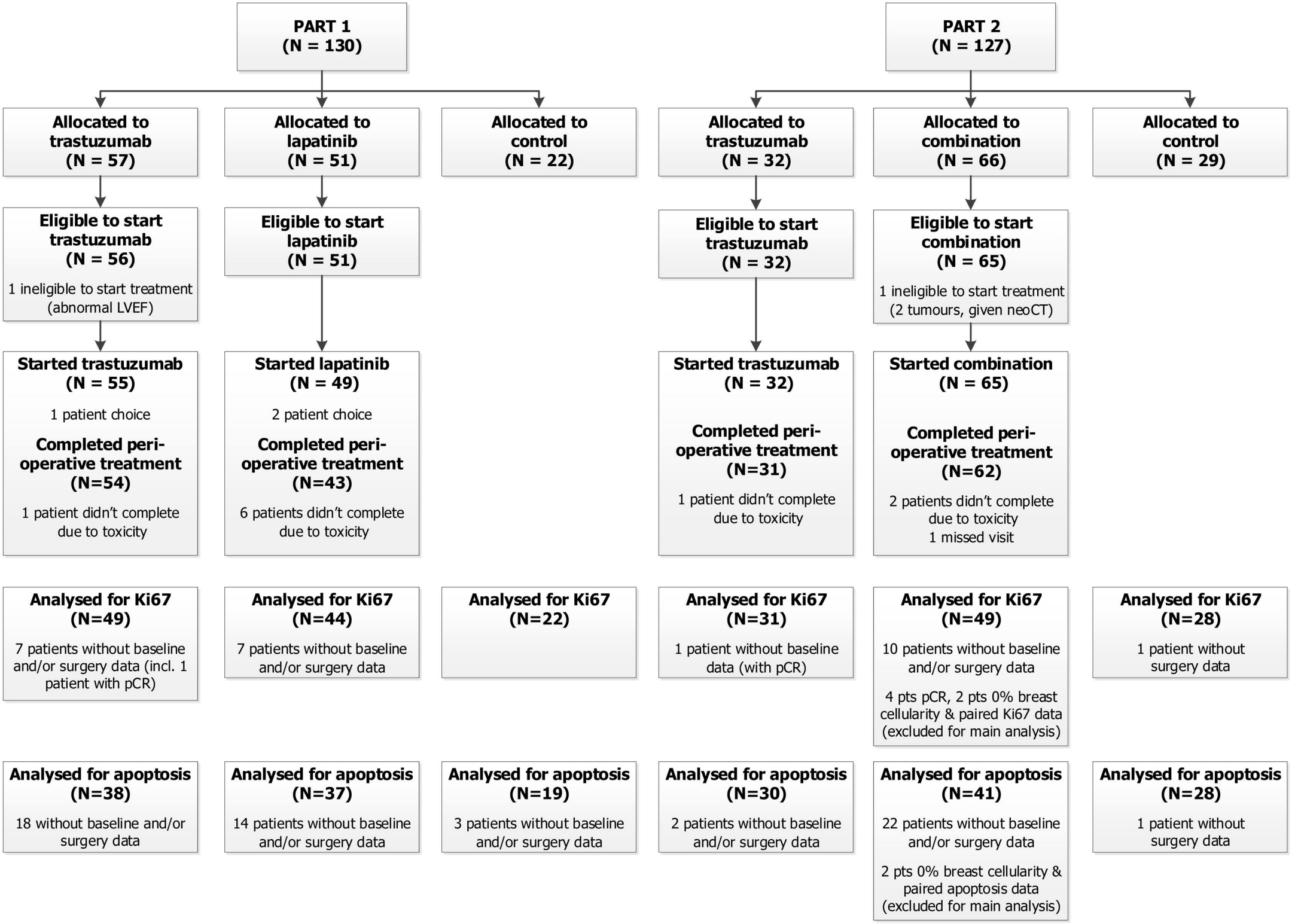
(A) Waterfall plots for Part 1 and Part 2 : for each patient, bar height represents percentage change at surgery from baseline. Percentage change was calculated as [((surgery score+0.1) - (pre-treatment score+0.1))/(pre-treatment score+0.1)]*100. The constant of 0.1 was added to accommodate cases with a value of 0%. Negative values represent decrease from baseline, positive values represent increase from baseline. *pCR in breast:* patients with pCR (no disease in ether breast or nodes) plus 2 additional patients with 0% breast cellularity but nodal involvement are represented as bars of height -120% at the left of the figures and noted “pCR in breast”; any existing Ki67 values for these patients have been excluded of the main analysis; in a sensitivity analysis, we imputed a value of -100% change for these patients (Appendix 2). Small triangles indicate patients with RCB1. Disease recurrences are also indicated at the top of each figure with circles and crosses.

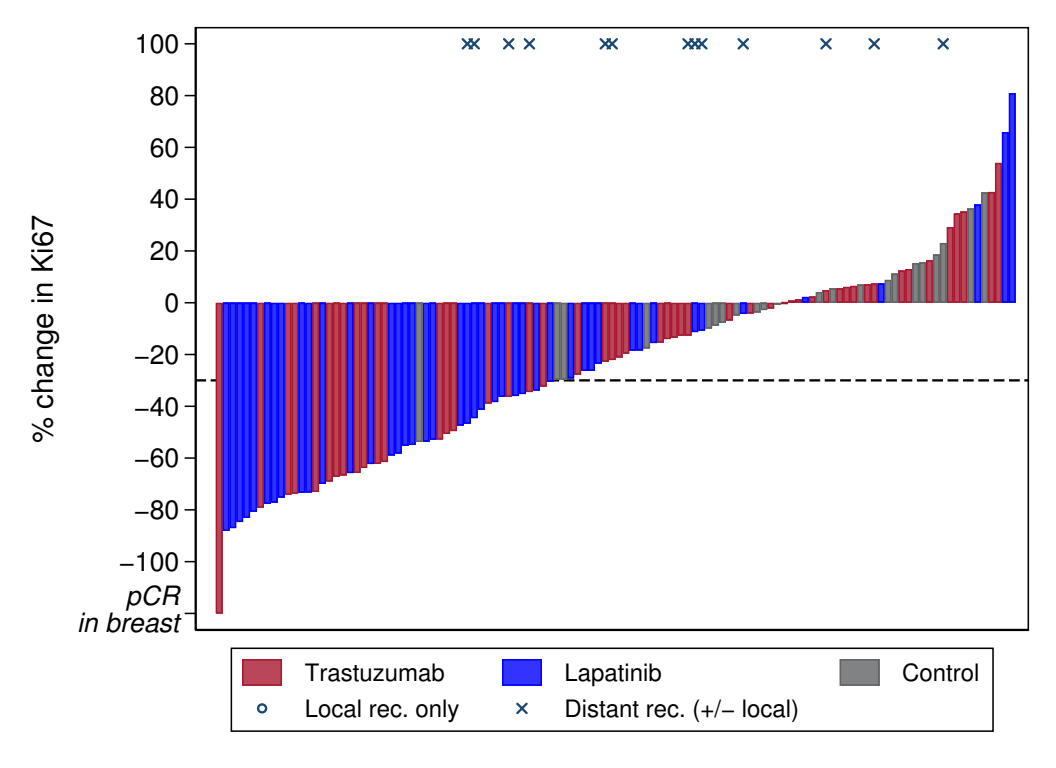
(B) Relapse Free Survival (RFS) is represented in the time interval of up to 6 years after randomisation, as no RFS event occurred later. Overall survival is represented in the fully observed range of values. Log-rank test comparing concurrently randomised treatment groups are reported in the figures. In the figure, trastuzumab and control Part-1 and Part-2 groups are combined to improve readability.

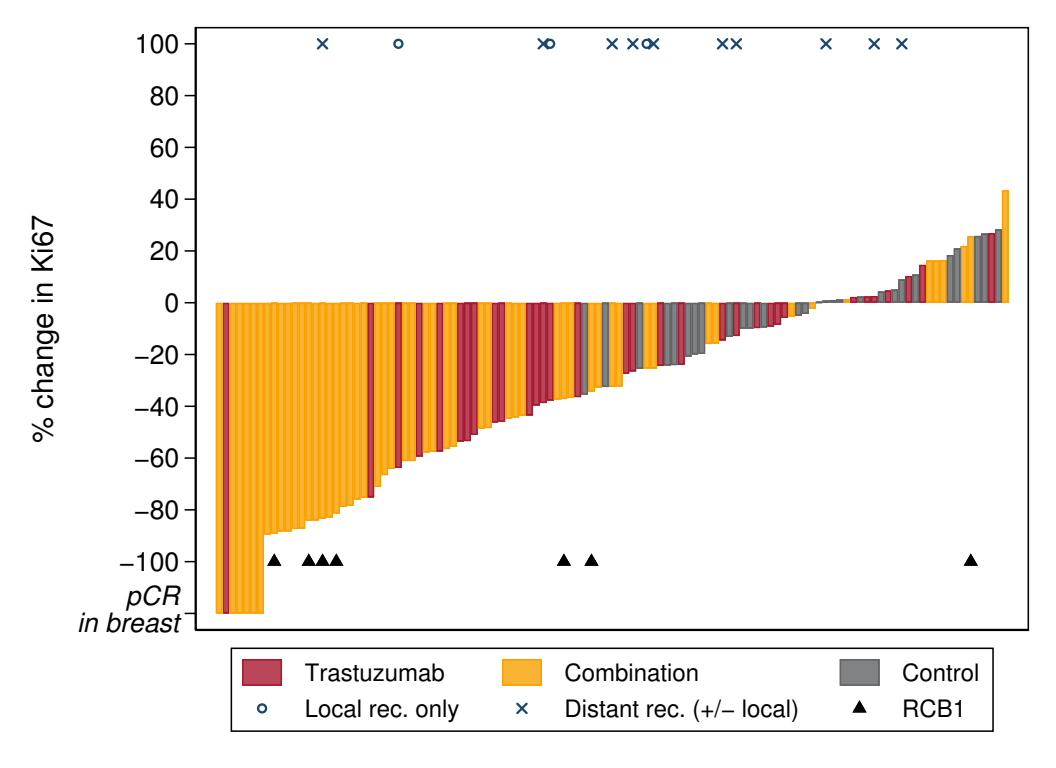
L: lapatinib, C: control, T: trastuzumab, T+L: combination, P1: Part-1, P2: Part-2; all: P1&P2, p=p-value.

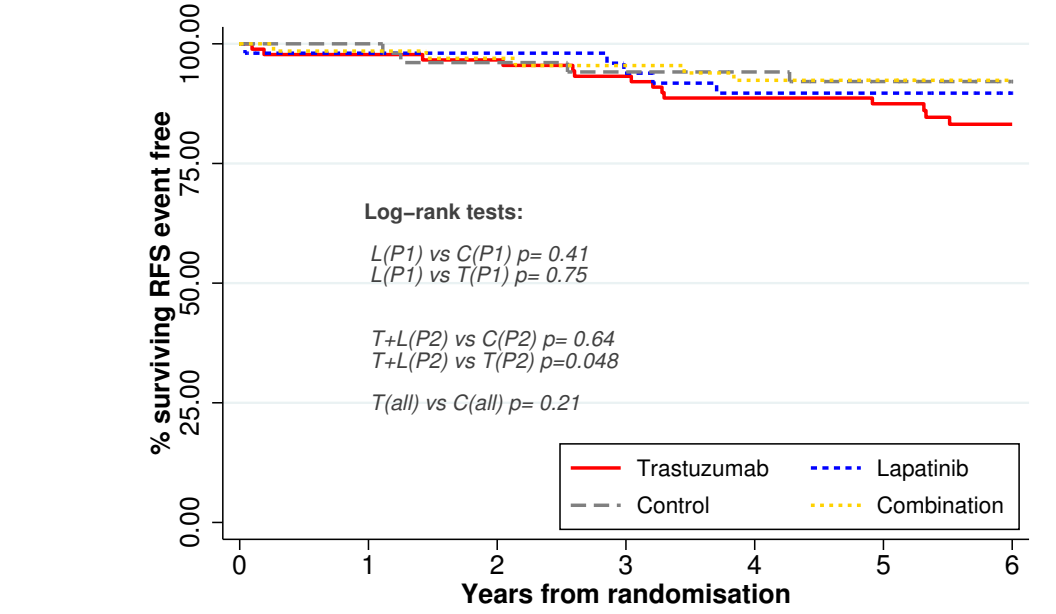
Figure 3: Association of peri-operative changes in biological markers with relapse free survival (A) by categories of Ki67 relative change, (B) by categories of Ki67 absolute change, (C) by baseline TILs, (D) by surgery TILs

Relapse free survival (RFS) is represented in the time interval 0-6 years, as no RFS events occurred beyond 6 years from randomisation. All treatment groups are combined; log-rank tests are stratified by treatment group (p=p-value). For (A) & (B), a value of -100% Ki67 change (ΔKi67) has been imputed for patients with a pCR in breast. For (B), we have categorised both baseline and surgery Ki67 into high if ≥10% or low if <10%. No patient increased Ki67 from low to high after 11 days of peri-operative treatment. Due to small number of patients in the "Low-Low" group, we have compared patients with "High" value at surgery with patients with "Low" value at surgery.


PART 1
(N = 130)
PART 2
(N = 127)
Allocated to trastuzumab (N = 57)
Allocated to lapatinib (N = 51)
Allocated to control (N = 22)
Allocated to trastuzumab (N = 32)
Allocated to combination (N = 66)
Allocated to control (N = 29)
Eligible to start trastuzumab (N = 56)
1 ineligible to start treatment (abnormal LVEF)
Eligible to start lapatinib (N = 51)
Eligible to start trastuzumab (N = 32)
Eligible to start combination (N = 65)
1 ineligible to start treatment (2 tumours, given neoCT)
Started trastuzumab (N = 55)
1 patient choice
Completed peri-operative treatment (N=54)
1 patient didn't complete due to toxicity
Started lapatinib (N = 49)
2 patient choice
Completed peri-operative treatment (N=43)
6 patients didn't complete due to toxicity
Started trastuzumab (N = 32)
Completed peri-operative treatment (N=31)
1 patient didn't complete due to toxicity
Started combination (N = 65)
Completed peri-operative treatment (N=62)
2 patients didn't complete due to toxicity
1 missed visit
Analysed for Ki67 (N=49)
7 patients without baseline and/or surgery data (incl. 1 patient with pCR)
Analysed for Ki67 (N=44)
7 patients without baseline and/or surgery data
Analysed for Ki67 (N=22)
Analysed for Ki67 (N=31)
1 patient without baseline data (with pCR)
Analysed for Ki67 (N=49)
10 patients without baseline and/or surgery data
4 pts pCR, 2 pts 0% breast cellularity & paired Ki67 data (excluded for main analysis)
Analysed for Ki67 (N=28)
1 patient without surgery data
Analysed for apoptosis (N=38)
18 without baseline and/or surgery data
Analysed for apoptosis (N=37)
14 patients without baseline and/or surgery data
Analysed for apoptosis (N=19)
3 patients without baseline and/or surgery data
Analysed for apoptosis (N=30)
2 patients without baseline and/or surgery data
Analysed for apoptosis (N=41)
22 patients without baseline and/or surgery data
2 pts 0% breast cellularity & paired apoptosis data (excluded for main analysis)
Analysed for apoptosis (N=28)
1 patient without surgery data

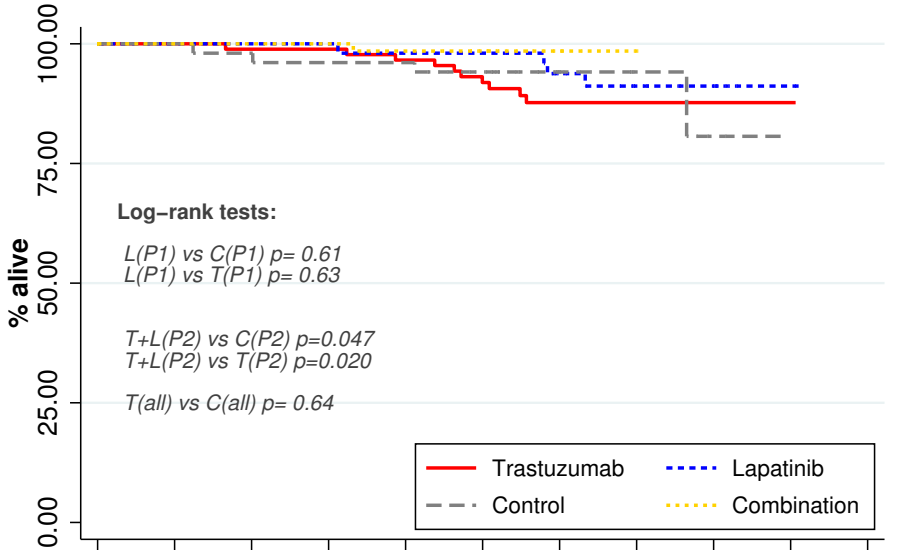

% change in Ki67
100
80
60
40
20
0
−20
−40
−60
−80
−100
pCR
in breast
Trastuzumab
Lapatinib
Control
Local rec. only
Distant rec. (+/− local)


% change in Ki67
100
80
60
40
20
0
−20
−40
−60
−80
−100
pCR
in breast
Trastuzumab
Combination
Control
Local rec. only
Distant rec. (+/− local)
RCB1



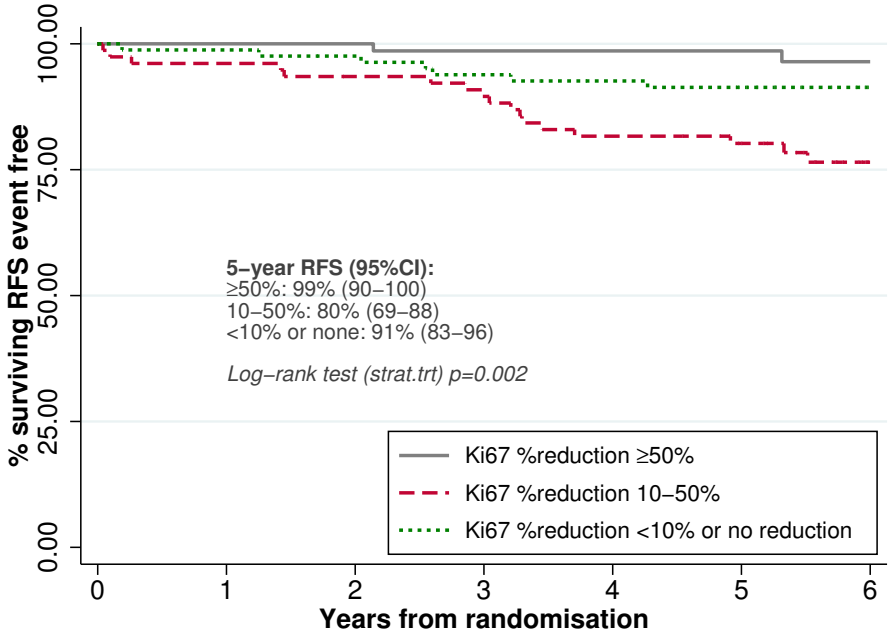
N at risk (events)

Trastuzumab:	89	(2)	87	(1)	85	(3)	82	(4)	77	(1)	68	(3)	49
Lapatinib	51	(1)	48	(0)	47	(2)	45	(2)	43	(0)	43	(0)	35
Control:	51	(0)	51	(2)	49	(1)	48	(0)	47	(1)	41	(0)	17
Combination:	66	(1)	65	(1)	64	(1)	62	(2)	60	(0)	43	(0)	8



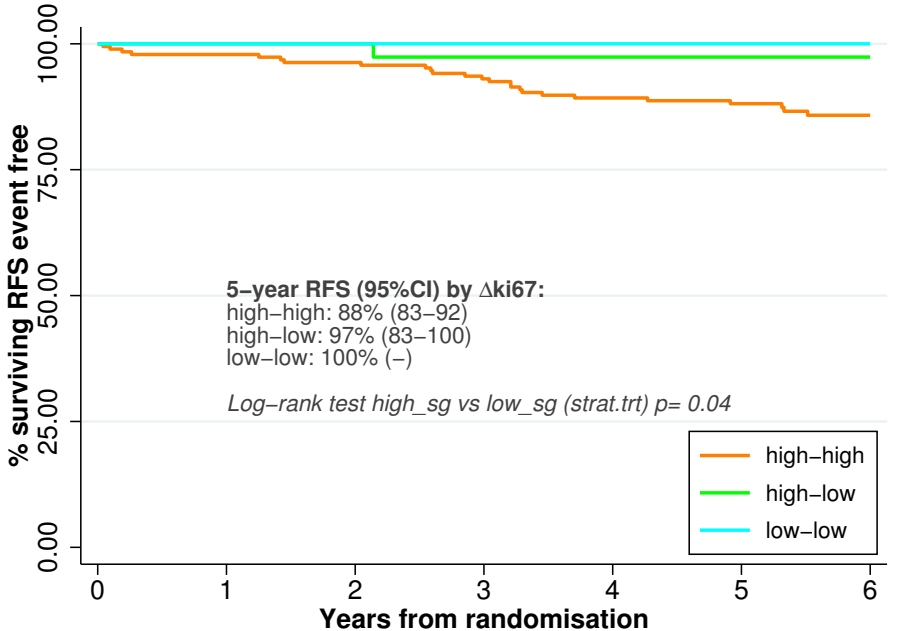
N at risk (events)

Trastuzumab:	89	(0)	89	(1)	87	(0)	87	(2)	85	(4)	73	(3)	50	(0)	37	(0)	15	(0)	4	(0)	0
Lapatinib	51	(0)	51	(0)	51	(0)	51	(1)	50	(0)	50	(2)	40	(1)	29	(0)	12	(0)	3	(0)	0
Control:	51	(0)	51	(1)	50	(1)	49	(0)	49	(1)	42	(0)	18	(0)	10	(1)	3	(0)	0	(0)	0
Combination:	66	(0)	66	(0)	66	(0)	66	(1)	65	(0)	49	(0)	11	(0)	1	(0)	0	(0)	0	(0)	0



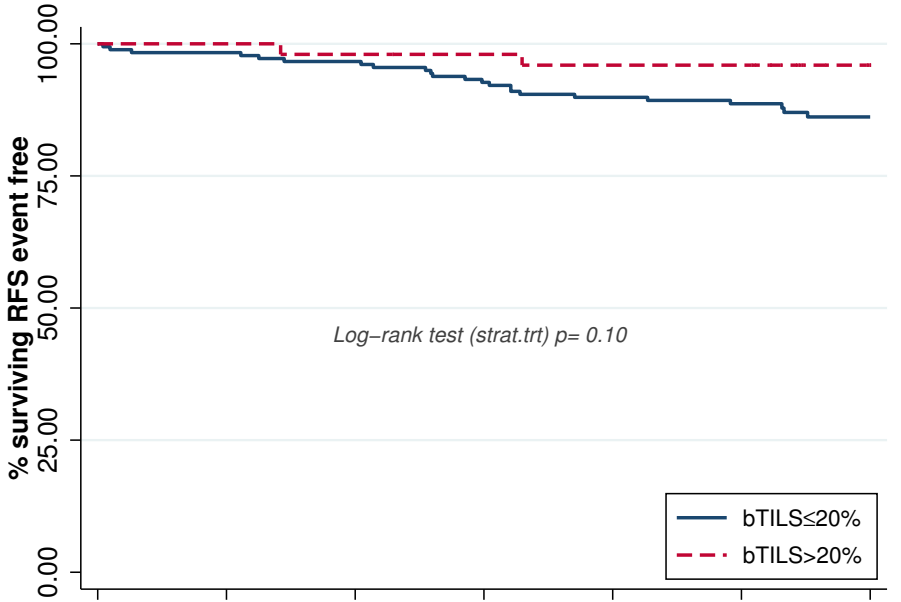
N at risk (events)

≥50%	72	(0)	71	(0)	71	(1)	69	(0)	69	(0)	57	(1)	33
10–50%	77	(3)	74	(2)	71	(3)	68	(6)	62	(1)	53	(2)	28
<10% or none	82	(1)	81	(1)	79	(3)	76	(1)	73	(1)	65	(0)	37



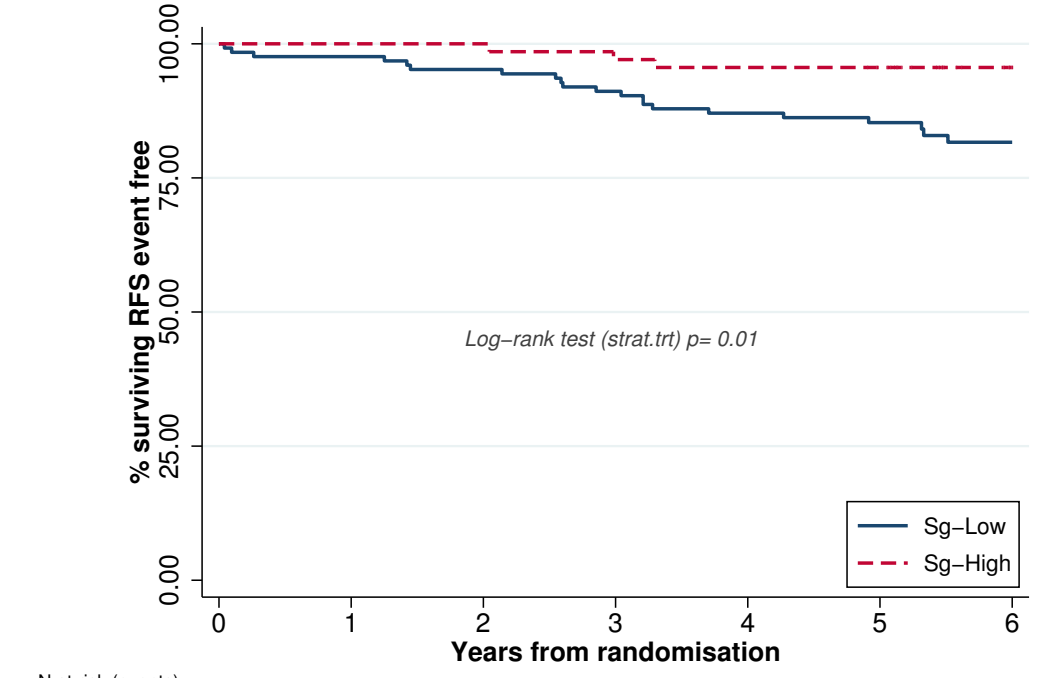
N at risk (events)

high–high	189	(4)	184	(3)	179	(6)	172	(7)	163	(2)	141	(3)	78
high–low	38	(0)	38	(0)	38	(1)	37	(0)	37	(0)	30	(0)	16
low–low	4	(0)	4	(0)	4	(0)	4	(0)	4	(0)	4	(0)	4



N at risk (events)

bTILS≤20%	180	(3)	176	(3)	171	(7)	164	(5)	157	(2)	133	(3)	70
bTILS>20%	50	(0)	50	(1)	49	(0)	48	(1)	47	(0)	44	(0)	28



N at risk (events)

Sg−Low	126	(3)	122	(3)	117	(5)	112	(5)	105	(2)	88	(3)	44
Sg−High	68	(0)	68	(0)	68	(2)	66	(1)	65	(0)	60	(0)	35