Neoadjuvant T-DM1/pertuzumab and paclitaxel/trastuzumab/pertuzumab for HER2+ breast cancer in the adaptively randomized I-SPY2 trial

Amy S. Clark1✉, Christina Yau2, Denise M. Wolf2, Emanuel F. Petricoin3, Laura J. van ’t Veer4, Douglas Yee4, Stacy L. Moulder5, Anne M. Wallace6, A. Jo Chien2, Claudine Isaacs7, Judy C. Boughey8, Kathy S. Albain9, Kathleen Kemmer10, Barbara B. Haley11, Hyo S. Han12, Andres Forero-Torres13, Anthony Elias14, Julie E. Lang15, Erin D. Ellis16, Rachel Yung17, Debu Tripathy15, Rita Nanda18, Julia D. Wulfkuhle5, Lamorna Brown-Swigart2, Rosa I. Gallagher5, Teresa Helsten6, Erin Roesch6, Cheryl A. Ewing2, Michael Alvarado2, Erin P. Crane7, Meredith Buxton19, Julia L. Clennell19, Melissa Paoloni19, Smita M. Asare2, Amy Wilson2, Gillian L. Hirst2, Ruby Singhrao2, Katherine Steeg2, Adam Asare2, Jeffrey B. Matthews2, Scott Berry19, Ashish Santi19, Michelle Melisko2, Jane Perlmutter20, Hope S. Rugo2, Richard B. Schwab6, W. Fraser Symmans5, Nola M. Hylton2, Donald A. Berry19, Laura J. Esserman2 & Angela M. DeMichele1

HER2-targeted therapy dramatically improves outcomes in early breast cancer. Here we report the results of two HER2-targeted combinations in the neoadjuvant I-SPY2 phase 2 adaptive platform trial for early breast cancer at high risk of recurrence: ado-trastuzumab emtansine plus pertuzumab (T-DM1/P) and paclitaxel, trastuzumab and pertuzumab (THP). Eligible women have >2.5 cm clinical stage II/III HER2+ breast cancer, adaptively randomized to T-DM1/P, THP, or a common control arm of paclitaxel/trastuzumab (TH), followed by doxorubicin/cyclophosphamide, then surgery. Both T-DM1/P and THP arms ‘graduate’ in all subtypes: predicted pCR rates are 63%, 72% and 33% for T-DM1/P (n = 52), THP (n = 45) and TH (n = 31) respectively. Toxicity burden is similar between arms. Degree of HER2 pathway signaling and phosphorylation in pretreatment biopsy specimens are associated with response to both T-DM1/P and THP and can further identify highly responsive HER2+ tumors to HER2-directed therapy. This may help identify patients who can safely de-escalate cytotoxic chemotherapy without compromising excellent outcome.
HER2-overexpressing breast cancer outcomes have improved dramatically in the last decade with the addition of HER2-directed therapy to chemotherapy for early breast cancer. In the neoadjuvant setting, pathologic complete response (pCR) rates are in the 40–50% range with taxane/trastuzumab combinations, and even higher with the addition of other HER2-targeted agents. pCR has also proven to be a strong surrogate of event-free survival for individual patients1,2, particularly in hormone receptor-negative tumors. This provides multiple opportunities: to determine whether novel investigational agents can further improve pCR rates, assess comparative toxicity, and determine how increased pCR rates affect long-term survival outcomes. As such, the I-SPY2 trial is a neoadjuvant platform trial in which serial tumor samples are collected to identify biomarkers indicative of highly responsive or non-responsive tumors. These biomarkers are essential to optimizing therapy with strategies to de-escalate toxic treatment for highly responsive tumors and escalate therapy for those that are poorly responsive.

T-DM1 (ado-trastuzumab emtansine, Kadcyla) is an intravenous drug–antibody conjugate that links the HER2-targeted monoclonal antibody trastuzumab to emtansine, an active but systemically toxic chemotherapeutic3,4. T-DM1 is currently FDA-approved as a single agent for the treatment of patients with HER2+, metastatic breast cancer who previously received trastuzumab and a taxane5. Pertuzumab (Perjeta) is a humanized monoclonal antibody that targets the extracellular dimerization domain of HER2, distinct from the binding site of trastuzumab. Pertuzumab is FDA-approved for use in combination with trastuzumab and docetaxel in metastatic breast cancer, and in combination with trastuzumab and chemotherapy as neoadjuvant or adjuvant therapy in non-metastatic disease.

This report describes the evaluation of two I-SPY2 trial arms: a neoadjuvant non-chemotherapy regimen, T-DM1 + pertuzumab (T-DM1/P), and a dual-HER2 targeting regimen, paclitaxel + trastuzumab + pertuzumab (THP), compared to paclitaxel + trastuzumab alone (TH) for HER2+ breast cancer in the I-SPY2 trial. Moreover, we examined the potential for pre-specified multi-omic biomarkers reflecting degrees of HER2 pathway activation, estrogen receptor signaling, and proliferation to predict enhanced HER2-directed treatment response in patients already deemed HER2+ by standard CLIA assays. These were examined in three signatures: the overall HER2+ group, as well as the hormone receptor (HR)+ HER2+ and HR-/HER2+ groups.

Results
Fifty-two patients were enrolled in the T-DM1/P arm at 15 clinical sites from June 6, 2013 through August 17, 2015, when the arm graduated in all HER2+ signatures (all patients, HR+/HER2+, and HR-/HER2+). During the same time period, 45 patients were enrolled in the THP arm when the arm graduated in all HER2 signatures and was converted to the HER2+ control arm. Thirty-one patients were randomized to the TH control arm across 17 sites from March 10, 2010 through to August 17, 2015 (Fig. 1). Baseline characteristics of the three arms were similar (Table 1), with the exception that the T-DM1/P arm had excess Mammaprint ultra-high (MP2) tumors (44%)
Table 1 Demographics and baseline characteristics of participants.

| Characteristic | T-DM1/P (n = 52) | THP (n = 45) | CONTROL (n = 31) |
|----------------|------------------|--------------|-----------------|
| Median age, yr (range) | 48 (33-72) | 47 (29-70) | 50 (29-71) |
| Ethnicity, n (%) | White 42 (81%) | 37 (82%) | 25 (81%) |
|                  | African American 4 (8%) | 4 (9%) | 2 (6%) |
|                  | Asian 5 (10%) | 2 (4%) | 4 (13%) |
|                  | Other/mixed 1 (2%) | 2 (4%) | 0 (0%) |
| HR Status, n (%) | Positive 35 (67%) | 29 (64%) | 19 (61%) |
|                  | Negative 17 (33%) | 16 (36%) | 12 (39%) |
| Mammaprint, n (%) | Positive 29 (56%) | 28 (62%) | 26 (84%) |
|                  | Not reported 5 (10%) | 6 (13%) | 7 (23%) |
| Median tumor size, cm (range) | 3.3 (1.5-12) | 3.4 (1.8-9) | 3.5 (1.3-11.7) |
| Baseline node status, n (%) | Palpable 18 (35%) | 17 (38%) | 10 (32%) |
|                  | Non-palpable 24 (46%) | 22 (49%) | 21 (68%) |
|                  | N/A 10 (19%) | 6 (13%) | 0 (0%) |
| HER2 qualifying test, n (%) | IHC 35 (67%) | 30 (67%) | 18 (58%) |
|                  | HER2 IHC 17 (33%) | 15 (33%) | 13 (42%) |
| IHC 3+ | 35 (67%) | 30 (67%) | 17 (55%) |
| IHC 2+ | 10 (19%) | 8 (18%) | 6 (19%) |
| IHC 1+ | 2 (4%) | 1 (2%) | 1 (3%) |
| Not reported | 5 (10%) | 6 (13%) | 7 (23%) |
| HER2 FISH, n (%) | Positive 29 (56%) | 25 (56%) | 15 (48%) |
|                  | Equivocal 0 (0%) | 0 (0%) | 1 (3%) |
|                  | Negative 1 (2%) | 0 (0%) | 1 (3%) |
|                  | Not reported 22 (42%) | 20 (44%) | 14 (45%) |
| HER2 TargetPrint, n (%) | Positive 39 (75%) | 32 (71%) | 18 (58%) |
|                  | Negative 13 (25%) | 13 (29%) | 10 (32%) |
|                  | Not reported 0 (0%) | 0 (0%) | 0 (0%) |

compared to control (16%; two-sided Fisher exact test p = 0.01). Source data is provided in Supplementary Data 1.

Efficacy. Figure 2 shows the pCR predicted probability curves (associated values are found in Supplementary Table 1). T-DM1/ pertuzumab graduated in all three HER2+ signatures. Amongst all HER2+ patients in the TDM1/P and TH arms, T-DM1/P had a higher estimated pCR rate (55%; 95% PI 41–69%) than TH (25%; 95% PI 11–38%), corresponding to a 99.9% probability that T-DM1/P was superior to TH and a 96% predictive probability of superiority in a 300-patients phase III trial. In the HR+/HER2+ and HR-/HER2+ signatures, the probability that pCR rates with T-DM1/P were superior to control were 99.7 and 98.8%, respectively.

THP also graduated in all three HER2+ signatures, as shown in Fig. 2. Amongst all HER2+ patients in the THP and TH arms, THP had a higher estimated pCR rate (56%; 95%PI 42–70%) than TH (25%; 95% PI 11–38%), corresponding to a 99.9% probability that THP was superior to TH and a 97% predictive probability of superiority in a 300-patients phase III trial. In the HR+/HER2+ and HR-/HER2+ signatures, the probability that pCR rates with THP were superior to control were 99.5 and 99.9%, respectively.

Toxicity. The toxicities of each arm are well described6–8 and no new safety signals were seen in the I-SPY2 Trial arms (Table 2; summary of all adverse events in Supplementary Data 2). Overall, adverse events are primarily grade 1/2 (see Supplementary Table 2) but one patient on the THP arm died due to respiratory failure during the THP portion of therapy and was unrelated to therapy. The majority of patients in each arm were able to receive AC after initial HER2-directed therapy (Table 2). Toxicities during AC differed between treatment arms: diarrhea and neutropenia were more frequent in the T-DM1/P arm, as was febrile neutropenia, while anemia was more frequent in the THP arm.

Event-Free Survival (EFS). Follow-up was available for 47 (90%) T-DM1/P patients, 39 (86.7%) THP patients, and 31(100%) control patients, with median follow-up of 4.1 years. Over the follow-up period, a total of 7 events were observed in the T-DM1/P arm, 3 in the THP arm, and 7 in the common TH control arm. Notably, there were five CNS recurrences during the follow up period: three in the T-DM1/P arm, two in the TH arm, and none in the THP arm (Supplementary Fig. 1). Four of the five CNS recurrences observed were HR+ (Supplementary Fig. 1).

Three-year EFS was 88% (95% CI: 79–99%) for T-DM1/P, 92% (95% CI: 84–100%) for THP and 87% (95% CI: 75–100%) for TH (Supplementary Fig. 1). In the two arms with brain metastases, EFS estimates were similar for patients who achieved pCR and those who did not: 88% regardless of pCR status for TDM1/P (95% CI: 77–100% and 74–100% for pCR and non-pCR, respectively), and 88% (67–100%) vs 86% (73–100%) for pCR vs. non-pCR, respectively, in the TH control arm. For THP, EFS was 96% (89–100%) vs 85% (68–100%) for pCR vs non-pCR.

Biomarker assessment. We assessed 10 biomarkers in the HER2, ER/PR, and proliferation pathways as predictors of response to TDM1/P and THP, hypothesizing that highly HER2-activated, non-luminal A or highly proliferative tumors may be more sensitive to anti-HER2 therapies than those that are less HER2-activated, more luminal or quiescent. HER2 and HER2 signaling was evaluated at 5 levels of resolution: IHC, quantitative protein, and phosphoprotein measurements by RPPA (total ERBB2; phosphorylated [p] pERBB2, phosphorylated [p] pEGFR), and mRNA (HER2 amplicon module Module7_ERBB2). These HER2 biomarkers were highly correlated (Supplementary Fig. 2A) and all five HER2 pathway biomarkers were significantly associated with pCR in the TDM1/P and THP arms (Fig. 3A–G, LR p < 0.05 as detailed in Table 4). Nearly all associations retained significance in a model adjusting for HR status, and in HR+/HER2+ subsets (Table 3, Supplementary Fig. 2C–H). HER family (HER2 and EGFR) activation/phosphorylation signatures clustered distinctly from total HER2 as measured by mRNA and RPPA (Supplementary Fig. 2A). Plotting pEGFR and pERBB2 expression together revealed a separation into two groups of patients: one with excellent response and one without (Fig. 3G). Since HER2 and EGFR are well-known heterodimerization partners, the fact that both are found to be highly co-activated in the pre-treatment tumor samples of responding patient population provide further evidence of functional HER2-driven pathway activation and signaling coherence.

ER/PR signaling, represented by the average expression of ESR1 and PGR, was also evaluated. Higher ER signaling levels were associated with non-response to TDM1/P and THP in the population as a whole (Fig. 4A, B, Table 3) and in the HR-/HER2+ subsets within both arms (Table 3, Supplementary Figure S2J). Consistent with the above, tumors classified Luminal A by PAM50 had a lower pCR rate relative to those of other classes (Fig. 4C, Supplementary Fig. 2).

Finally, we quantitatively assessed proliferation markers at the total protein (RPPA: Ki67), phospho-protein (pAURKA) and...
mRNA (proliferation signature Module11_Proliferation) levels as predictors of response. In the population as a whole, the mRNA proliferation signature was associated with response in both T-DM1/P and THP, whereas pAURKA was associated with response in only THP. However, all three proliferation biomarkers significantly associate with response to TDM1/P but not THP in the HR+/HER2+ subset (Fig. 4D–F; Table 3).

Of the 10 markers evaluated, the proliferation markers showed the largest predictive performance differences between arms, particularly in the HR+/HER2+ subtype. Other differences include more dramatic HER2-pathway biomarker associations with pCR in the TDM1/P arm relative to THP. Examples include HER2 3+ IHC: OR = 7.1 [1.4–50] vs. 5.2 [0.9–40]; PAM50 HER2: OR = 21 [4–219] vs 16 [0.4–6.4]; pERBB2 amplico:

![Image](https://doi.org/10.1038/s41467-021-26019-y)

**Fig. 2 Primary efficacy analysis.** pCR probability distribution curves of TDM1/P (red) vs TH (blue) and THP (orange) vs TH (blue). Arrows below x-axis indicate 95% probability interval derived from the I-SPY2 Bayesian time and covariate-adjusted logistic model described in the methods.

| Table 2 Adverse events Grade 3 and above, experienced by ≥5% of participants following TDM1, THP, TH, or AC. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Adverse event, n (%)                             | T-DM1+/P (n = 52)                               | AC (n = 49)                                     | THP arm (n = 45)                                | AC (n = 40)                                     |
| Anemia                                          | 2 (3.9%)                                       | 2 (4.1%)                                       | 1 (2.2%)                                       | 4 (10.0%)                                      |
| Diarrhea                                        | 0 (0.0%)                                       | 6 (12.2%)                                      | 0 (0.0%)                                       | 0 (0.0%)                                       |
| Febrile neutropenia                             | 0 (0.0%)                                       | 8 (16.3%)                                      | 0 (0.0%)                                       | 5 (12.5%)                                      |
| Hypertension                                    | 2 (3.8%)                                       | 0 (0.0%)                                       | 2 (4.4%)                                       | 0 (0.0%)                                       |
| Neutrophil count decreased                      | 0 (0.0%)                                       | 6 (12.2%)                                      | 2 (4.4%)                                       | 1 (2.5%)                                       |
| Vascular access complication                    | 0 (0.0%)                                       | 0 (0.0%)                                       | 0 (0.0%)                                       | 0 (0.0%)                                       |
| White blood cell count decreased                | 0 (0.0%)                                       | 2 (4.1%)                                       | 0 (0.0%)                                       | 0 (0.0%)                                       |
| Dose Reductions, n (%)                          | 1 (1.9%)                                       | 3 (5.8%)                                       | 3 (6.7%)                                       | 5 (20%)                                        |
| Early Discontinuation, n (%)                    | 3 (5.8%)                                       | 5 (10.2%)                                      | 5 (11%)                                        | 4 (10%)                                        |
| All                                             | 3 (5.8%)                                       | 5 (10.2%)                                      | 5 (11%)                                        | 4 (10%)                                        |
| Toxicity                                        | 1 (1.9%)                                       | 3 (6.1%)                                       | 3 (6.7%)                                       | 2 (5.0%)                                       |
| Progression                                     | 1 (1.9%)                                       | 0 (0.0%)                                       | 0 (0.0%)                                       | 0 (0.0%)                                       |
| Other                                           | 1 (1.9%)                                       | 2 (4.1%)                                       | 0 (0.0%)                                       | 0 (0.0%)                                       |
| Median time to surgery*, days (range)           | 170 (148–239)                                  | 176 (112–219)                                  | 171 (119–239)                                  |

*Median time to surgery: days (range)
OR/unit increase = 5.5 [2.4–16] vs 2.3 [1.2–6.4]; pERBB2: OR/unit increase = 16.3 [2.6–245] vs. 5.5 [1.5–31]) and is further illustrated by scatter plots of pERBB2 vs. pEGFR by arm (Supplementary Fig. 3). Within the common control arm, of the 10 biomarkers tested only HER2 IHC 3+ significantly associates with response (LR p = 0.046), potentially due in part to small sample size (Table 3).

**Discussion**

In HER2+ early breast cancer, neoadjuvant treatment with T-DM1/P or THP demonstrated significant improvement in predicted pCR rates over treatment with TH, when all regimens were followed by AC and surgery. Similar improvement with T-DM1/P and THP over TH was observed in both HR+ and HR- subsets, despite overall higher response rates for HR- disease.
Clinically, these results have been corroborated by several other studies and the overall findings with regard to pCR rates and toxicity are similar to what is seen in the I-SPY2 Trial. In fact, THP followed by AC became a care standard for neoadjuvant therapy in HER2+ breast cancer during its assessment in I-SPY2, with the FDA approval of pertuzumab based on results from multiple neoadjuvant trials7,9,10. T-DM-1/P alone, however, has not been adopted as a care standard largely because the KRISTINE trial showed better pCR rates with neoadjuvant taxane/platinum with HP (55.7%) over TDM-1/P (44.4%)6. Our pCR estimates in the TDM1/P arm are higher, likely because patients also received AC.

Critically, however, the I-SPY2 trial adds important and novel information to the field in the assessment of biomarkers that differentiate responsiveness to these HER2-directed therapies. Despite all tumors in these arms being classified as HER2+ by standard IHC and/or FISH, pre-treatment HER2 levels (whether measured by IHC, quantitative protein or gene expression), and activation of HER2 signaling (as measured by phosphorylation and co-activation of HER2 and EGFR, or PAM50 HER2 subtype), were all strong predictors of pathologic response to both TDM-1 and THP. Conversely, highly hormone-driven tumor biology as defined by luminal A subtype and low proliferation was inversely correlated with response in both experimental arms. Low levels of expression by protein and mRNA predict a much lower response to HER2 targeted therapy. These findings are critically important to the design of the next generation of HER2+ trials in which these biomarkers can be utilized to assess which patients can potentially de-escalate therapy (by dropping toxic anthracycline or platinum agents) from those who could benefit from treatment escalation with new and more intensive treatment approaches as illustrated in Fig. 5.

I-SPY2 is a biomarker-rich trial where we uniquely apply rigorous pre-specified statistical assessment to pre-defined mechanism-of-action biomarkers that span the DNA–RNA–protein–phosphoprotein biochemical landscape. The design helps us to learn why some patients respond and other do not. The phosphoprotein data generated here provides evidence for a phospho-HER2-EGFR cut point that, once validated, could guide escalation (those predicted to have a pCR) and de-escalation (those predicted to not have a pCR) of therapy (Fig. 3G). Our observation that HR status and estrogen signaling are associated with lower responsiveness to TDM1/P and THP is consistent with previous reports that luminal-type tumors are associated with lower responsiveness to TDM1/P and THP is potentially de-escalate therapy (by dropping toxic anthracycline or platinum agents) from those who could benefit from treatment escalation with new and more intensive treatment approaches as illustrated in Fig. 5.

The major difference in predictive signal between arms were observed in the proliferation biomarkers in the HR+/HER2+ subset, where these biomarkers associated with response in TDM1/P but not THP arm. If confirmed in independent studies, a combination of proliferation and pERBB2/pEGFR may help distinguish likely response to TDM1/P vs. THP in context of HR status. Taken together, these findings suggest that there is a great degree of heterogeneity amongst HER2-overexpressing breast cancers in the I-SPY population of patients with tumors >2.5 cm, which could potentially be exploited for patient selection strategies.

There are several limitations to the current analysis that impact interpretation, most notably the impact of small sample sizes that limit evaluation of both long-term outcomes and biomarkers. The benefit of the adaptive randomization in I-SPY2 is its efficiency in assigning the agents to the subsets of patients that benefit most, and drugs leave the trial when graduation is reached—that is, the minimum number of patients necessary can be exposed to investigational agents in order to determine signal in a statistically robust manner. With this greater degree of efficiency in evaluating pCR rates comes sample size/pow limits with respect to assessing EFS benefits or predictive biomarkers. Importantly, approximately 9% of the patients in KRISTINE who did not have a pCR on the T-DM1 arm received adjuvant chemotherapy, and there were 15 locoregional events prior to surgery in the T-DM1/ P arm, suggesting the presence of a subgroup of patients with disease that was primarily refractory to HER2 antibody–drug
**Table 3 Biomarker pCR association results summary.**

| Biomarker (sig) Type | All HER2 | HER2 IHC (3+ vs. 2+7) | HER2 IHC (3+ vs. n.a.) | ERBB2/HER2 total | EGFR Y1173 |
|----------------------|---------|------------------------|------------------------|-----------------|------------|
|                      | THP     | TH (EXP: \(n = 31\); RPPA: \(n = 29\)) | M1: pCR \(\approx\) sig Adjust for HR M2: pCR | Continuous \(\beta\) vs. reference \(\beta\) | \(95\%\) CI |
|                      | LR      | p          | OR/unit increase       | HR               | p          | HR       |
| HER2 mRNA             | \(0.0001\) | \(1.55\) | \(0.156\) - \(1.55\) | \(0.000552\) | \(0.00153\) - \(0.156\) | \(0.00153\) - \(0.156\) |
| Microarray            | -       | -          | -                      | -                | -          | -        |
| THP (EXP: \(n = 31\); RPPA: \(n = 29\)) | M1: pCR \(\approx\) sig Adjust for HR M2: pCR | Continuous \(\beta\) vs. reference \(\beta\) | \(95\%\) CI |
|                      | LR      | p          | OR/unit increase       | HR               | p          | HR       |
| HER2 mRNA             | \(0.0001\) | \(1.55\) | \(0.156\) - \(1.55\) | \(0.000552\) | \(0.00153\) - \(0.156\) | \(0.00153\) - \(0.156\) |
| Microarray            | -       | -          | -                      | -                | -          | -        |

Conjugate-based therapy alone due to the inability to internalize the antibody–toxin, as suggested by Weyergang et al. (submitted). These findings reinforce our I-SPY2 results suggesting that HER2- and HR-related biology of the primary tumor play a large role in responsiveness to T-DM1, and confirming that adjuvant therapy must be considered when examining EFS, with more aggressive adjuvant therapy in non-pCR patients potentially improving outcomes preferentially for this group of patients compared to those with pCR, creating an equalizing effect.

The similar outcomes of patients with and without a pCR that we observed is likely to be related to adjuvant therapy received. Because the primary endpoint of I-SPY2 is pCR, the type or extent of adjuvant therapy is not mandated, and was at the discretion of the treating physician. Adjuvant therapies differed significantly between the three arms and between pCR and non-pCR groups (Supplementary Table 3). For example, seven patients on the non-taxane containing T-DM1/P arm received additional chemotherapy with adjuvant taxane plus trastuzumab, and all of these were in the non-pCR group. No patients in the THP or TH arms received adjuvant chemotherapy.

The CNS is a known sanctuary site for HER2+ breast cancer, even in the setting of pCR, a finding that is further corroborated in our study: the arms where CNS recurrences were found, difference in 3-year EFS between those with pCR vs. non-pCR was not seen. It is difficult to say whether the lack of brain metastases in the THP arm was due to chance, but previous reports indicate that the addition of pertuzumab to trastuzumab may delay onset of CNS disease15. Tucatinib, a recently FDA-approved drug has shown efficacy in metastatic HER2+ breast cancer patients with brain metastases16, will be studied in the post-neoadjuvant setting combined with T-DM1 in the COMPASS-RD (NCT04457596) trial. Combining tucatinib with a less toxic regimen such as T-DM1 and avoiding the toxicity of AC could prove to be a highly effective intervention for women with tumors that highly express HER2 or fall into the HER2 molecular subtype. Development of drugs that penetrate the CNS and prevent CNS recurrence in HER2+ breast cancer is urgently needed to improve outcomes in these high-risk patients.

In summary, both T-DM1/P and THP significantly increased estimated pCR rates over TH in HER2+ patients, within both HR+ and HR- groups. Given similar high pCR rates and toxicity burden in the populations overall, it is important to develop tools that can be applied to individual patients to aid in the selection of the most active therapy in the clinic. Pretherapy gene expression and protein signaling further identifies highly sensitive tumors beyond those that are HER2+ by standard ASCO/CAP guidelines. The goal of I-SPY2 is to identify active agents, and generate useful guidance regarding the biological profiles of patients in whom further studies of an active agent or strategy should be employed. A high percentage of patients can achieve pCR with TDM1/P or THP. Without AC, these could be less toxic combinations that could be effective for HER2 molecular classification and proliferative tumors. The ability to eliminate AC altogether is under investigation in the COMPASS-pCR trial, where patients are treated to receive THP alone neoadjuvantly. The data reported here are useful in planning further definitive studies that will enable optimization of HER2-directed therapy for future phase III trials and shed important light on the heterogeneity of HER2+ breast cancer and its impact on response to targeted therapy.

**Methods**

**Study design.** The I-SPY2 Trial (NCT01042379) is an ongoing phase 2 multicenter, open-label, adaptively randomized platform trial of neoadjuvant therapy (NAT) for early breast cancer that evaluates multiple investigational agents in parallel against a common control to evaluate their response within specific breast cancer subtypes (Supplementary Fig. 4). Biomarker assessments at screening are
used to assess eligibility and classify patients into one of eight subtypes based on hormone receptor (HR), HER2-receptor, and MammaPrint status\(^2\). The adaptive randomization engine preferentially assigns patients to agents based on continually updated Bayesian probabilities of pCR rates within predefined biomarker signatures; 20% of patients are randomized to the control arm.

The primary endpoint is pCR, defined as complete resolution of invasive cancer in both breast and lymph nodes (ypT0/is and ypN0). An investigational agent “graduates” from I-SPY2 if when it achieves a ≥85% Bayesian predictive probability of demonstrating superiority to control in a hypothetical 1:1 randomized 300-patient phase 3 neoadjuvant trial in at least one of 10 clinical signatures\(^18,19\). Once the graduation threshold or a predetermined maximum enrollment is reached, accrual to the arm stops and predictive probabilities are updated after all patients complete surgery; note that the number of patients per arm is not fixed. Patients are followed for long-term outcome, including recurrence and death. Additional details on the study design have been published previously\(^20\).

**Eligibility.** Adult women with stage II or III breast cancer with primary tumors ≥ 2.5 cm clinically or ≥ 2.0 cm by imaging who have not received prior treatment for their breast cancer are eligible for I-SPY2. All patients provide written informed consent at screening and again after randomization. Only patients with HER2-positivity in their breast cancer are eligible for I-SPY2 if/when it achieves a ≥85% Bayesian predictive probability of demonstrating superiority to control in a hypothetical 1:1 randomized 300-patient phase 3 neoadjuvant trial in at least one of 10 clinical signatures\(^18,19\). Once the graduation threshold or a predetermined maximum enrollment is reached, accrual to the arm stops and predictive probabilities are updated after all patients complete surgery; note that the number of patients per arm is not fixed. Patients are followed for long-term outcome, including recurrence and death. Additional details on the study design have been published previously\(^20\).

**Assessments.** Core tumor biopsies, blood draws, and bilateral breast MRI were performed at baseline and at specified intervals during treatment (Supplementary Fig. 4). The primary endpoint was assessed using recrated tumors by local pathologists trained in the residual cancer burden (RCB) method (in which pCR is RCB 0)\(^24\). As I-SPY2 is modified intent-to-treat, patients receiving any dose of study therapy are considered evaluable; those who switch to non-protocol therapy, progress, forgo surgery, or withdraw are deemed non-pCR. Secondary endpoints were RCB, and event-free and distant relapse-free survival (EFS and DRFS).
**Fig. 5 A view to the future.** Top graphic represents the current treatment paradigm: patients with HER2+ breast cancer as determined by IHC or FISH receive the same neoadjuvant chemotherapy and HER2-directed therapy. Bottom graphic illustrates how implementation of the RPPA assay and gene expression profiling following positive IHC or FISH will enable de-escalation or escalation of chemotherapy and HER2-directed therapy. Original illustration: A. Haymond.

Qualified, pre-specified biomarkers evaluated included MammaPrint™ and TargetPrint HER2 gene expression arrays using the 44 K full genome microarray (Agenda)™, HER2 quantitative protein expression, HER2 phosphorylation (Y1248), and EGFR phosphorylation (Y1173) by RPPA. In addition, exploratory biomarker analysis used three expression signatures, PAM50 subtype, RPPA-based quantitative K67 expression, and Aurora kinase protein activation as biomarkers of TDM1/P or THP response.

**Trial oversight.** The I-SPY2 trial sponsor is QuantumLeap Healthcare Collaborative. Drug manufacturers supplied investigational agents and funding but played no role in study design, data accrual, data analysis, or manuscript preparation. The study complies with all local and national regulations regarding the use of human study participants and was conducted in accordance to the criteria set by the Declaration of Helsinki. The study received institutional review board approval at all clinical sites: University of California, San Diego Human Research Promotions Program Institutional Review Board, MedStar Health Research Institute-Georgetown University Oncology Institutional Review Board, Loyola University Chicago Health Sciences Division Institutional Review Board for the Protection of Human Subjects, UCSF Human Research Protection Program Institutional Review Board, UT Southwestern IRB, Chesapeake IRB, Oregon Health & Science University Research Integrity Office IRB, Mayo Clinic Institutional Review Boards, University of Pennsylvania Office of Regulatory Affairs Institutional Review Board, The University of Alabama at Birmingham Office of the Institutional Review Board for Human Use, University of Minnesota Human Research Protection Program, Colorado Multiple Institutional Review Board, Fred Hutchinson Cancer Research Center Institutional Review Board, University of Southern California Health Sciences Institutional Review Board, University of Texas MD Anderson Cancer Center Clinical Institutional Review Board, Western Institutional Review Board, University of Arizona Institutional Review Board, and Biological Sciences Division Chicago Biomedicine Institutional Review Board. An independent data and safety monitoring board (DSMB) convened monthly.

**Statistical analyses.** Probability distributions of pCR rates are calculated using a Bayesian time and covariate-adjusted logistic model with HR, HER2, and MammaPrint statuses as covariates used to calculate the probability that the pCR rate of the investigational arm is greater than control for each signature20–22, similarly for the predictive probabilities of success in a future trial. Experimental arms are not statistically compared to each other. During the time that both the TDM-1/P and THP arms were open, pertuzumab was approved for neoadjuvant treatment in HER2+ disease preventing further enrollment to the TH control arm as described above. In response, a revised statistical plan was adopted and approved by the DSMB which adjusts for time trends to allow comparison against a control. Having multiple arms in the trial with different time periods during which they are accruing patients enabled us to incorporate into the final analyses of the various arms.

We explicitly incorporate terms in the model to account for potential time trends in the pCR rate; we account for molecular subtype and treatment as well. This is accomplished using time-dependent offset terms in a logistic model. Time is set to 0 at each analysis. We partition time in the past into bins of 90 days each. The index of the most recent bin, that for the previous 0–90 days, is 1. The index of the bin 91–180 days in the past is 2. And so on. Let \( b \) be the index of the bin for the randomization time of patient \( i \).

We model time-trend parameters \( \delta(t) \) within each bin \( t \). These are additive parameters in the model for the log-odds ratio of pCR rate for each investigational arm compared with control. We use two sets of time-trend parameters, \( \delta(t) \) for HER2+ and \( \delta(t) \) for HER2−. Consider patient \( i \) who has subtype (HR+, HER2+, MP+) and was randomized 750 days before present. Her bin \( b \) is 9 and her time-trend offset is \( \delta(9) \).

Suppressing subscripts + and − for both HER2+ and HER2−, we set \( \delta(t) = 0 \) for \( t = 1, 2, 3, 4 \). That means the previous year’s results count fully in the analysis. Further in the past, that is, for \( t > 4 \), \( \delta(t) \) is a second-order Normal Dynamic
Linear Model (NLDLM)\(^2\). The NLDLM uses the data within bins to estimate the respective log-odds ratios, but it also serves to smooth the effect across bins. The time machine has the following structure for both HER\(^2\) and HER\(^2\), again suppressing the + and - subscripts:

\[
\delta(1) = \delta(2) = \ldots = \delta(4) = 0
\]

\[
\delta(5) \sim N(\mu_5, \tau_5)
\]

\[
\delta(6) = \delta(7) = N(\mu_6, \tau_6)
\]

\[
\delta(t) = 2\delta(t-1) + \delta(t-2) \sim N(0, \tau^2) \text{for} t > 6
\]

\[
\tau^2 = IG(\alpha, \beta)
\]

In this notation, \(N(\mu, \sigma^2)\) refers to a normal distribution with mean \(\mu\) and standard deviation \(\sigma\) and \(IG\) stands for inverse gamma. The parameters of the prior distributions are \(\mu_5 = \mu_6 = 0, \tau_5 = \tau_6 = 0.001, \alpha = 1, \) and \(\beta = 0.001.\)

An exploratory analysis of EFS was performed using patients with follow-up data as of February 26, 2019. EFS was assessed as time from treatment consent to any loco-regional or distant recurrence or death from any cause; and patients without events were censored at last follow-up. Kaplan–Meier survival curves of each arm were prepared; Cox proportional hazard modeling was used to estimate hazard ratios between T-DM1/P or THP and control arms for each signature. Statistics from this exploratory analysis are descriptive and not inferential–sample sizes are small within signatures and I-SPY\(^2\) is not powered for EFS or other survival endpoints.

### Biomarker analysis

Biomarker analyses were performed on pre-treatment biopsies, which included the Agenda 44 K full-genome microarray and reverse-phase protein array (RPPA, performed by author EP at George Mason University). We evaluated HER2 IHC, 3 expression signatures, PAM50 subtype, and 5 protein/ phospho-protein endpoints by RPPA as biomarkers of TDM1/P response.

HER2 expression by immunohistochemistry (IHC) was scored per ASCO-CAP HER2 testing guidelines (positive: 3+, equivocal: 2+, negative<1+). Pre-treatment tumor samples were assayed using Agilent 44 K (23267) or 32 K (15746) expression arrays; and these data were combined into a single gene-level dataset after batch-adjusting using ComBat\(^8\). ERBB2 amplon gene expression (Mod7 ERBB2) and proliferation (Module1_Proli) modules were scored as published\(^9\). The estrogen signature, ER_PRG_ave, was scored as the average of ESR1 and PGR expression. PAM50 ‘intrinsic subtype’ classifications were evaluated using the published method and R scripts by J. Parker\(^2\), applied to expression data from 1151 patients screened for I-SPY2 (median: 996; low-risk registry: 165), with class assignment with confidence level < 0.8 treated as N/A. These data were centered to a 1:1 ratio of HR\(^2\) to HR\(^2\) samples prior to classification.

In addition, LCM was performed to isolate tumor epithelium for signaling protein activation profiling by reverse phase protein arrays (RPPA). Protein/phospho-protein endpoints reflecting HER-family signaling (ERBB2 total protein, ERBB2 Y1248 and EGFR Y1173) and proliferation (ERBB2 total protein, and Aurora phospho-protein endpoints re...

### Data availability

Source data are provided with this paper for all outcomes except adverse events in Supplementary Data 1. A summary of all adverse events observed are provided in Supplementary Data 2. In addition, gene expression and base clinical variables have been deposited in GEO under accession number GSE181574.

### Code availability

Biomarker data analysis was performed using R version 3.6.3 and Bioconductor vers.3.10; code available upon request from ispyadmin@ucsf.edu. The randomization engine and Bayesian analytic software used in efficacy analysis are used under license from Berry Consultants, LLC; requests for code should be directed to don@berryconsultants.com.

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Author contributions

Conceptualization: L.E., D.B., L.V., D.W., J.P., W.F.S., N.H., D.B., A.D.; Methodology: L.E., D.B., C.Y., D.W., E.P., L.V., L.B.S., R.G., S.B., G.H., N.H., W.F.S.; Formal analysis: C.Y., D.W., E.P., A.S., J.W., D.B.; Investigation: A.C., C.Y., D.W., E.P., S.M., A.W., A.J.C., C.L., K.A., D.T., E.E., A.F., R.Y., H.H., J.L., R.V., B.H., K.K., A.E., R.N., J.B., J.W., L.B., R.C., T.H., E.R., C.E., M.A., E.C., P.R., M.B., S.A., M.M., H.R., R.S., W.F.S., N.H., D.Y., L.V., A.D.; Data curation: C.Y., D.W., E.P., A.S., J.W., L.B., R.C., T.H., E.R., C.E., M.A., E.C., P.R., M.B., S.A., M.M., H.R., R.S., W.F.S., N.H., D.Y., L.V., A.D.; Writing (review and editing): A.C., C.Y., D.W., E.P., J.M., H.R., L.E., A.D.; Visualization: A.C., C.Y., D.W., E.P., A.S., J.W., L.B., R.C., T.H., E.R., C.E., M.A., E.C., P.R., M.B., S.A., M.M., H.R., R.S., W.F.S., N.H., D.Y., L.V., A.D.; Project administration: M.B., J.C., M.P., S.A., G.H., R.S., K.S., A.A., L.E., A.D.; Funding acquisition: L.E.

Competing interests

Amy S. Clark has received unrelated research support from Novartis. Christina Yau has consulted for NantOmnics, LLC, part of the NantWorks network. E.F. Peticon reports: leadership roles with: Perthera and Ceres Nanosciences; stock or other ownership interests in Perthera, Ceres Nanosciences and Avant Diagnostics; consulting or advisory roles with Perthera, Ceres Nanosciences, AZGen, Avant Diagnostics; institutional research support from Ceres Nanosciences, GlassoSmithKline, Abbvie, Symphonogen, and Genentech; patents, royalties, other intellectual property from National Institutes of Health patents licensing fee distribution/royalty, co-inventor on filed George Mason University—assigned patents related to phosphorylated HER2 and EGFR response predictors for HER family-directed therapeutics, as such can receive royalties and licensing distribution on any licensed IP; travel, accommodations, expenses received from Perthera and Ceres Nanosciences. Laura J. van’t Veer is a part-time employee and stockholder in Agenda N.V. Dong Ye has received unrelated research support from Roche and Boehringer Ingelheim. Stacy Moulder has been an employee of Eli Lilly since 2021. A. Jo Chein reports institutional research support from Seagen, Merck, Amgen and Puma Biotechnology. Claudine Isaacs has received consulting fees from Seattle Genetics, Genentech, AstraZeneca, Novartis, PUMA, Pfizer, and Eisai. Judy C. Boughey has received support from Eli Lilly. Barbara Haley reports that UTSouthwestern Medical Center Dallas receives funding for her research as PI from Pfizer, Lilly, Daiichi Sankyo, Roche, Puma, AstraZeneca and Sanofi. Hyo S. Han reports institutional research support from GlaxoSmithKline, Abbvie, Prescient, GI therapeutics, Marker therapeutics, Novartis, Horizon Pharma, Pfizer, Seattle Genetics, Arvinas and Zymeworks; she has received a grant from the Department of Defence and is a member of Lilly’s Speaker’s Bureau. Andres Forero-Torres became a Seattle Genetics employee in 2018 and holds stock option from this employment. Julie Lang has previously received a research grant from ANGLE Parsortix and has received honoraria as part of the Genomic Health’s speakers bureau and a Puma Biotechnology advisory board. Debu Tripathy receives research support from Novartis, Pfizer, Polyphor and has been a paid consultant for services on steering committees or advisory boards for Novartis, Pfizer, AstraZeneca, GlaxoSmithKline, OncoPep, Gilead, Exact Sciences. Rita Nanda has received research support from Arvinas, AstraZeneca, Celgene, Corcept Therapeutics, Genentech/Roche, Immunomedics/Gilead, Merck, OBI Pharma, Inc., Odonate Therapeutics, OncoSccer, Pfizer, Taiho and SeaGen. J.D. Wulfkuhle received honoraria from DAVA Oncology and consults for Baylor College of Medicine. Melissa Paolini is an employee and stock holder in Arcus Biosciences. Donald Berry and Scott Berry are co-owners of Berry Consultants, LLC, a company that designs adaptive Bayesian clinical trials (including i-SPY 2) for pharmaceutical and medical device companies, NIH cooperative groups, patient advocacy groups, and international consortia. Hope Rugo reports institutional research support from Pfizer, Merck, Novartis, Lilly, Genentech, Odonate, Daiichi, Seattle Genetics, Eisai, Macrogenics, Sermonix, Boehringer Ingelheim, Polyphor, Astra Zeneca and Immunomedics, and has received honoraria from Puma Biotechnology, Mylan and Samsung. W. Fraser Symmans is a co-founder with equity in Delphi Diagnostics that licensed intellectual property, is a co-inventor of an issued patent for the algorithm to calculate residual cancer burden that is freely available on the internet, holds publicly traded shares in IONIS Pharmaceuticals and Eiger Biopharmaceuticals, and is an unpaid scientific advisor to Roche for translational research related to the KAITLIN trial and unpaid steering committee member for the KATHERINE trial. Laura Esserman is an unpaid member of the board of directors of Quantum Leap Healthcare Collaborative (QLHC) and received grant support from QLHC for the I-SPY2 Trial; she is a member of the Blue Cross/Blue Shield Medical Advisory Panel and receives reimbursement for her time and travel; Dr. Esserman has received unrelated research support from Merck. Angela DeMichele has received honoraria or consulting fees from Pfizer and Context Therapeutics and reports institutional research support from Novartis, Pfizer, Genentech, Calibera and Menarini. All other authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Amy S. Clark.

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