nature cancer

Article

https://doi.org/10.1038/s43018-024-00890-2

Neoadjuvant atezolizumab in combination with dual HER2 blockade plus epirubicin in women with early HER2-positive breast cancer: the randomized phase 2 ABCSG-52/ATHENE trial

Received: 12 May 2024

Accepted: 4 December 2024

Published online: 16 January 2025

Check for updates

A list of authors and their affiliations appears at the end of the paper

The role of anthracyclines in the treatment of early breast cancer (EBC) is increasingly being challenged, especially in de-escalation strategies. However, owing to their immunogenic effects, anthracyclines are promising combination partners with immunotherapies. In the randomized phase 2 trial ABCSG-52 (EudraCT no. 2019-002364-27), we investigated epirubicin plus immunotherapy in women with human epidermal growth factor receptor 2 (HER2)-positive EBC. A total of 58 patients were randomized 1:1 to two cycles of a chemotherapy-free induction phase (part 1) of dual HER2 blockade with trastuzumab and pertuzumab (TP) plus the anti-programmed death ligand 1 antibody atezolizumab (TP-A) or TP alone. Thereafter, all patients received four cycles of TP-A in combination with epirubicin (part 2). The primary endpoint, pathological complete response (pCR), was met in 35 patients (60.3%; 95% confidence interval (CI) 47.5% to 71.9%), 19 patients (65.5%) in the TP-A group and 16 patients (55.2%) in the TP group. The residual cancer burden 0/I rate and objective response rate (secondary endpoints) in all patients with evaluable data were 80.0% (n = 44/55; 95% CI 67.6% to 88.4%) and 89.3% (n = 50/56; 95% CI 78.5% to 95.0%), respectively. Grade ≥3 adverse events were reported in 17 patients (29.3%). Based on our findings, we conclude that a neoadjuvant chemotherapy de-escalation immunotherapy regimen with trastuzumab, pertuzumab, atezolizumab and epirubicin is effective and safe in patients with HER2-positive EBC.

In patients with high-risk breast cancer subtypes, pathological complete response (pCR) to neoadjuvant treatment is associated with an improved long-term outcome on an individual patient level[1]. Therefore, for most patients with clinical stage II/III human epidermal growth factor receptor 2 (HER2)-positive early breast cancer (EBC), neoadjuvant dual HER2 blockade with trastuzumab and pertuzumab (TP) plus a taxane with or without an anthracycline is considered the standard of care, even when primary breast conservation seems feasible[2–4]. In phase 2 and 3 trials investigating TP plus polychemotherapy regimens in higher-risk patients (≥T2 and/or ≥N1), pCR rates of 55–62% have been reported[5–7]. In patients not achieving pCR, postneoadjuvant treatment with the HER2-directed antibody–drug conjugate trastuzumab emtansine has further improved outcomes[8]. Therefore, HER2 positivity has changed from a subtype-defining biomarker conferring poor prognosis to a positive predictive biomarker with currently available HER2-directed treatment options having vastly improved long-term outcomes.

e-mail: gabriel.rinnerthaler@medunigraz.at

https://doi.org/10.1038/s43018-024-00890-2

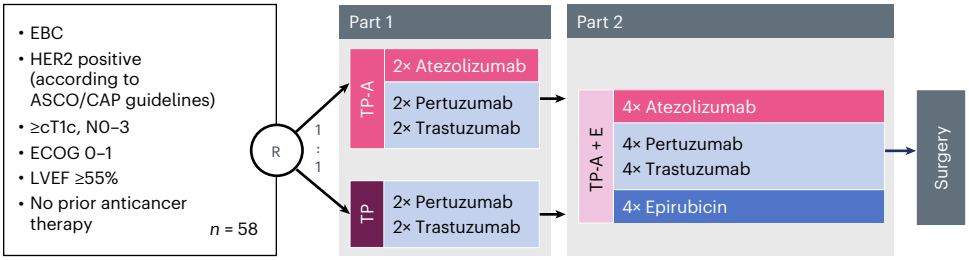

Fig. 1 | Study design. Stratification criteria: baseline TILs (<5% versus ≥5%), HR status (positive versus negative) and prognostic stage (≤IIA versus ≥IIB (American Joint Committee on Cancer staging manual version 8.0)). ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; ECOG, Eastern Cooperative Oncology Group; LVEF, left ventricular ejection fraction; R, randomization; TP-A + E, TP-A plus epirubicin.

To balance toxicity burden and treatment activity, research in recent years has focused on chemotherapy de-escalation[9]. Owing to cardiotoxicity concerns, especially in combination with anti-HER2 blockade, taxanes were considered the preferred chemotherapy backbone for de-escalation protocols over anthracyclines. In clinical trials investigating neoadjuvant monochemotherapy with a taxane plus TP, pCR rates of 39% and 56–91% were seen in patients with HER2-positive higher-risk and lower-risk EBC, respectively[10–12].

Besides their direct cytotoxic effects, conventional chemotherapeutic agents are also known to harbor immunogenic properties[13]. Doxorubicin has been shown to enhance dendritic cell maturation[14], promote the antigen-presenting abilities of mouse dendritic cells[15] and induce the expression of heat shock proteins in vitro[16]. Apart from doxorubicin, other anthracyclines (that is, epirubicin and idarubicin) can also trigger immunogenic cell death, a regulated cell death that engages the adaptive immune system[17].

These data provide a sound rationale for combining an anthracycline with immune checkpoint inhibitors (ICBs). Additionally, compared to taxanes, anthracyclines have a more favorable side-effect profile owing to their lack of neurotoxicity and hypersensitivity reactions. Atezolizumab is a monoclonal antibody targeting programmed death ligand 1 (PD-L1)[18]. Besides many other indications over a broad spectrum of neoplastic diseases, it is approved by the European Medicines Agency for the treatment of PD-L1 immune cell-positive metastatic triple-negative breast cancer[19].

The role of atezolizumab in addition to standard polychemotherapy plus dual HER2 blockade was investigated in two phase 3 trials[20,21]. In IMpassion050, atezolizumab with neoadjuvant dose-dense doxorubicin, cyclophosphamide, paclitaxel and TP did not increase pCR rates versus placebo in the intention-to-treat (ITT; pCR rate of 62.7% in the placebo group and 62.4% in the atezolizumab group, $P = 1.00$) or PD-L1-positive (pCR rate of 72.5% in the placebo group and 64.2% in the atezolizumab group, $P = 0.18$) population. In the three-arm APTneo trial, atezolizumab plus an anthracycline-containing regimen (doxorubicin, cyclophosphamide, paclitaxel, carboplatin and TP) increased the pCR rate compared to an anthracycline-free control group (paclitaxel, carboplatin and TP (HPCT); pCR rate of 61.9% versus 52.0%, $P = 0.022$). In contrast, atezolizumab plus HPCT compared to HPCT showed a similar pCR proportion (53.6% versus 52.0%, $P = 0.089$).

In the single-arm Keyriched-1 trial, the programmed death 1 inhibitor pembrolizumab was investigated in combination with TP in patients with EBC of a molecular HER2-enriched intrinsic subtype[22]. With this chemotherapy-free combination, a pCR rate of 46% was seen. Patients with HER2-enriched subtypes have a higher likelihood of achieving pCR following anti-HER2-based neoadjuvant therapy with or without chemotherapy[23]. In the single-arm Neo-PATH trial, a combination of neoadjuvant atezolizumab, docetaxel and TP was investigated in HER2-positive patients[24]. In this monochemotherapy and immunotherapy trial, a pCR rate of 61% was reported.

The results from the phase 3 IMpassion050 trial indicate that ICBs have no additive effect when combined with a standard anti-HER2 therapy plus polychemotherapy regimen[20], which has the highest assumed effectiveness in terms of pCR[5–7,10–12]. Therefore, we hypothesized that the addition of an ICB to dual HER2 blockade may add activity in regimens with a de-escalated chemotherapy backbone. The optimal cytotoxic drug in such an approach remains to be determined, and the particular role of epirubicin monochemotherapy has not been previously investigated. Therefore, in ABCSG-52/ATHENE, we investigated an anthracycline-based chemotherapy de-escalation immunotherapy regimen in patients with HER2-positive EBC.

Results

Patient and tumor characteristics

Between June 2020 and December 2021, 70 patients were screened and 58 patients (ITT population) were randomized 1:1 to TP plus atezolizumab (TP-A; $n = 29$) or TP alone ($n = 29$) at nine sites in Austria (Fig. 1). All patients received at least one dose (safety population). Because one patient in the TP-A group withdrew informed consent before surgery and one patient in the TP group received only one treatment cycle, the final efficacy assessment population consisted of 56 patients (Fig. 2).

In the ITT population, the median age was 57 years (range 33–82 years). All included patients were women, and 34 patients (59%) were postmenopausal at baseline. Of the enrolled patients, 42 (72.4%) presented with hormone receptor (HR)-positive tumors and 16 (27.6%) had HR-negative disease. According to the clinical prognostic stage, 45 patients (77.6%) were classified as having stage ≤IIA disease and 13 (22.4%) patients had stage ≥IIB disease. The detailed characteristics of the total population and by treatment arm are shown in Table 1.

Primary and secondary endpoints

In the ITT population, pCR (primary endpoint) was observed in 60.3% of patients ($n = 35/58$; 95% confidence interval (CI) 47.5% to 71.9%), 65.5% ($n = 19/29$; 95% CI 47.3% to 80.1%) in the TP-A group and 55.2% ($n = 16/29$; 95% CI 37.5% to 71.6%) in the TP group (difference: 10.3%; 95% CI −14.7% to 35.4%). In patients with an available residual cancer burden (RCB) assessment (secondary endpoint), complete or near-complete remission, defined as RCB category 0 or I, was seen in 80.0% ($n = 44/55$; 95% CI 67.6% to 88.4%), 85.7% ($n = 24/28$; 95% CI 68.5% to 94.3%) in the TP-A group and 74.1% ($n = 20/27$; 95% CI 55.3% to 86.6%) in the TP group (difference: 11.6%; 95% CI −9.4% to 32.6%). The rates of RCB category 0–III per treatment arm are shown in Supplementary Table 2.

In a univariable logistic regression model (Fig. 3), numerically lower pCR rates were observed in peri-/premenopausal patients compared to postmenopausal patients (odds ratio (OR) 0.48; 95% CI 0.16 to 1.40; two-sided $P = 0.18$), as well as in histological subtypes other than 'no special type' (OR 0.37; 95% CI 0.09 to 1.48; two-sided $P = 0.16$). Higher pCR rates were seen in patients with increased body mass index (BMI; OR per 10-unit increase 1.97; 95% CI 0.62 to 6.22;

https://doi.org/10.1038/s43018-024-00890-2

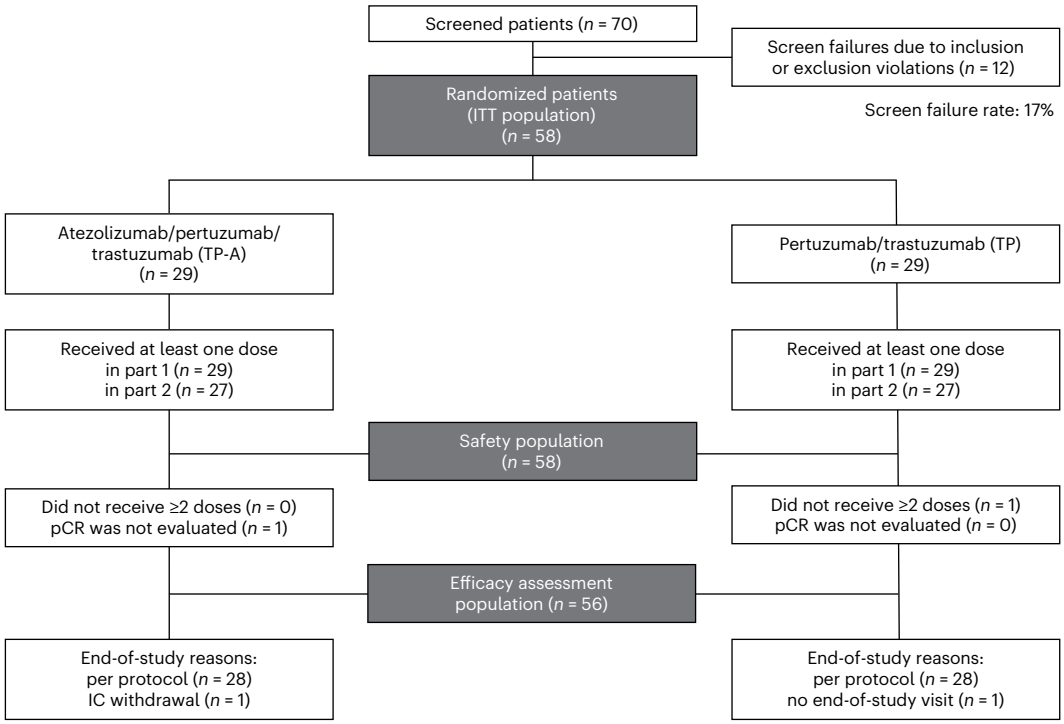

Fig. 2 | CONSORT diagram. IC, informed consent.

two-sided $P = 0.25$). None of the covariates were statistically significantly associated with pCR.

Radiological complete response, radiological partial response or radiological stable disease was detected in 21 (37.5%), 29 (51.8%) and 6 (10.7%) patients, respectively. No radiological progressive disease was seen. The overall response rate (radiological complete response + radiological partial response), the other secondary endpoint, was 89.3% (95% CI 78.5% to 95.0%).

Response according to tumor-infiltrating lymphocyte status

In a post hoc exploratory analysis, the mean proportion of stromal tumor-infiltrating lymphocytes (TILs) was 23.9% in the overall population, 22.8% in the TP group and 25.0% in the TP-A group. A lymphocytic-predominant phenotype was seen in 10.3% of patients in the TP group and 17.2% of patients in the TP-A group. No association was detected between TIL proportion at baseline and pCR (OR for a 10-percentage-point increase 1.02; 95% CI 0.78 to 1.34). A moderate positive correlation between the numeric values of TILs and PD-L1 was observed (Spearman $r = 0.57$, two-sided $P < 0.0001$).

Response according to PD-L1 status

In a post hoc exploratory analysis, the pCR rate was 69.2% ($n = 18/26$; 95% CI 50.0% to 83.5%) in PD-L1-negative patients compared to 55.2% ($n = 16/29$; 95% CI 37.5% to 71.6%) in PD-L1-positive patients. The highest pCR rates were detected in the PD-L1-negative subgroup treated in the TP-A arm (73.3%; 95% CI 48.0% to 89.1%), whereas the lowest pCR rates were observed in PD-L1-positive patients treated in the TP arm (52.9%; 95% CI 31.0% to 73.8%). pCR rates according to treatment arm and PD-L1 status are shown in Table 2.

Adverse events

Treatment-emergent adverse events (AEs) grade ≥3 were reported in 17 patients (29.3%), 9 patients (31.0%) in the TP-A group and 8 patients (27.6%) in the TP group (Table 3 and Supplementary Table 3; AEs per treatment part are listed in Supplementary Tables 4 and 5). The most frequently reported AEs in both treatment groups were nausea (69% in both groups), diarrhea (59% in TP-A, 62% in TP), fatigue (48% in TP-A, 59% in TP) and alopecia (41% in TP-A, 28% in TP; Table 3). No AEs of special interest grade ≥3 were detected (Supplementary Table 6); therefore, none of the predefined boundaries were crossed.

Discussion

The introduction of ICBs, given either alone or in combination with chemotherapy or small molecules, has considerably changed the outcome in many cancer types and therefore the landscape of standard treatments of neoplastic diseases[25]. Such positive effects of ICBs on response, disease-free survival or overall survival have been reported when used in the neoadjuvant (for example, lung cancer), adjuvant (for example, melanoma), or advanced and/or metastatic (for example, cancers of the head and neck, lung, esophagus and stomach, liver, and urinary system) setting. In breast cancer, results have been less convincing, except for triple-negative subtypes[26], which are considered to carry a higher number of mutations and thus neoepitopes allowing T cells to recognize the neoplastic cells[27]. Recently, initial promising results of the addition of ICBs to neoadjuvant chemotherapy in patients with high-risk luminal cancer have been presented[28,29]. Few data are available concerning the efficacy of ICBs in the HER2-positive subtype, particularly in the neoadjuvant setting. While this subtype can already be treated with high efficacy across all disease stages using HER2-targeted therapies, substantial room for improvement remains in two directions: further increasing pCR rates (and thus the depth of short- and long-term tumor control) and decreasing toxicity and side effects while increasing the quality of life by chemotherapy de-escalation strategies. Following the requirement for both goals, we initiated this proof-of-principle phase 2 randomized trial to test the role of atezolizumab in addition to dual HER2 blockade in an initial induction phase followed by only four cycles of a quadruple regimen combining the three antibody drugs with epirubicin monotherapy in all patients.

pCR has been widely accepted as the primary outcome parameter in the neoadjuvant treatment of breast cancer[30] and is the primary endpoint of this trial. After part 1 (two cycles) and part 2 (four cycles) of therapy, we observed a pCR rate of 60.3% (95% CI 47.5% to 71.9%) in

https://doi.org/10.1038/s43018-024-00890-2

Table 1 | Patient characteristics

Characteristic	Category	TP-A (*n*=29)	TP (*n*=29)	Total (*n*=58)
Age at randomization (years)	Median (min–max)	57 (33–77)	58 (38–82)	57 (33–82)
Sex	Female	29 (100%)	29 (100%)	58 (100%)
Menopausal status at randomization	Postmenopausal	14 (48%)	20 (69%)	34 (59%)
	Peri-/premenopausal	15 (52%)	9 (31%)	24 (41%)
TILs at randomization (stratification factor)	<5%	3 (10%)	3 (10%)	6 (10%)
	≥5%	26 (90%)	26 (90%)	52 (90%)
HR status at randomization (stratification factor)	Negative	8 (28%)	8 (28%)	16 (28%)
	Positive	21 (72%)	21 (72%)	42 (72%)
Clinical prognostic stage (stratification factor)	≤IIA	23 (79%)	22 (76%)	45 (78%)
	≥IIB	6 (21%)	7 (24%)	13 (22%)
cT stage	T1c	9 (31.0%)	7 (24%)	16 (28%)
	T2	16 (55%)	19 (66%)	35 (60%)
	T3/T4	4 (14%)	3 (10%)	7 (12%)
cN stage	N0	17 (59%)	18 (62%)	35 (60%)
	N1	11 (38%)	10 (35%)	21 (36%)
	N2	1 (3%)	1 (3%)	2 (3%)
Grade	G2	12 (41%)	16 (55%)	28 (48%)
	G3	17 (59%)	13 (45%)	30 (52%)

Table 2 | pCR rates by PD-L1 status and treatment arm

PD-L1 status	Arm	*n*	pCR rate (95% CI)
Negative (immune cells <1%)	TP-A	15	73.3% (48.0% to 89.1%)
	TP	11	63.6% (35.4% to 84.8%)
Positive (immune cells ≥1%)	TP-A	12	58.3% (32.0% to 80.7%)
	TP	17	52.9% (31.0% to 73.8%)

the overall population, which exceeded the predefined threshold for effectivity (a pCR proportion of ≥40%).

Comparing these results to previously published data suggests that chemotherapy de-escalation with an intensified immunotherapy and abbreviated chemotherapy regimen, as investigated in ABCSG-52/ATHENE, is effective. In the single-arm Neo-PATH trial[24], the combination of six cycles of atezolizumab, pertuzumab, trastuzumab and docetaxel yielded a pCR rate of 61% (90% CI 50% to 71%). This result seems comparable to the pCR rate of 65.5% (95% CI 47.3% to 80.1%) in the TP-A group in our trial, in which only four cycles of epirubicin in combination with six cycles of immunotherapy were applied. However, more patients with a higher risk for recurrence were enrolled in Neo-PATH compared to ABCSG-52/ATHENE (proportion of patients with clinical stage ≥IIB disease: 76% versus 22%). Only a few classes of cytotoxic drugs exert proimmunogenic effects that may synergize with the mechanism of ICBs and vice versa[31]. Both epirubicin and taxanes belong to these groups of drugs, although their mechanisms of interaction with the interplay between cancer cells and immune cells may differ. The differences in results between ABCSG-52 and Neo-PATH might, in part, be due to these discrepancies. The particular role of anthracyclines in combination with ICBs is supported by recently published results from the APTneo trial[21]: the addition of atezolizumab to an anthracycline-containing regimen increased pCR rates compared to an anthracycline-free regimen, whereas no additive effect of atezolizumab was observed when it was added to an anthracycline-free protocol. This is in line with findings from neoadjuvant phase 3 trials investigating combinations of an ICB plus polychemotherapy in triple-negative EBC: pCR rates were increased only with anthracycline-containing regimens[32,33] but not with anthracycline-free, taxane-including protocols[34]. This hypothesis is further supported by results from the non-comparative phase 2 TONIC trial[35]. In this study, induction therapy with a short course of anthracycline outcompeted other cytotoxic drugs (cyclophosphamide, cisplatin) in terms of the effect of a subsequent ICB in the metastatic setting of triple-negative breast cancer.

In addition, the combination of ICBs with chemotherapy may be time- and dose-sensitive. We randomized patients to induction with dual HER2 blockade alone versus a combination with atezolizumab for two cycles, followed by four cycles of the quadruple regimen. The underlying concept was that this induction therapy reveals neoepitopes for the priming and activation of antigen-presenting cells and T cells. This could help fully exploit the proimmunogenic effect of epirubicin[36]. In an exploratory analysis comparing the six versus four applications of atezolizumab (TP-A versus TP), the pCR rate was higher in the six-cycle arm (difference: 10.3%; 95% CI −15% to 35%). This finding may be considered supportive of such a priming effect and suggests that an ICB should be included upfront in the neoadjuvant setting to maximize treatment effects. As no atezolizumab-free treatment arm was included in our phase 2 trial, the quantitative effect of atezolizumab cannot be clarified in this setting. Future translational data may help clarify the biological mechanisms behind our findings.

Regarding pCR rates in subgroups, increased BMI was numerically associated with higher pCR rates on univariable analysis. This is in line with previous findings that BMI was correlated with higher responses to anthracyclines in the neoadjuvant setting[37]. In our trial, 79% of pre-/perimenopausal women had HR-positive disease compared to 68% in postmenopausal patients. As HR positivity is associated with lower pCR rates in HER2-positive tumors[5–7,10], this might explain why numerically lower pCR rates were seen in pre-/perimenopausal women enrolled in our trial.

In ABCSG-52, the numerically highest pCR rates were observed in patients with PD-L1-negative tumors at baseline biopsy and when treated within the TP-A group (pCR rate: 73.3%; 95% CI 48.0% to 89.1%). While this seems counterintuitive, it was shown that trastuzumab-sensitive cancers produce cytokines such as CCL2 (chemokine ligand 2) capable of attracting monocytes, macrophages, dendritic cells and memory T cells in the tumor tissue[38]. In such a trastuzumab-sensitive microenvironment, PD-L1 was upregulated[38]. Because of an already sufficient antitumor immune response mediated by the antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis effects of anti-HER2 antibodies, the addition of anti-PD-L1 antibodies to standard therapy may lack additional effects. Thus, PD-L1 positivity can be understood as a surrogate for a trastuzumab-sensitive microenvironment rather than a predictor of the efficacy of immune checkpoint treatment[39,40]. This is in line with our findings and those of the IMpassion050 trial (no additive effect of atezolizumab in PD-L1-positive patients)[20]. Conversely, trastuzumab-resistant microenvironments have been described as immunosuppressive[41] and having lower PD-L1 expression compared to trastuzumab-sensitive tumors[38]. In such a scenario, trastuzumab can lead to PD-L1 upregulation in immune and cancer cells, and the addition may resensitize toward HER2 targeting[42,43]. This again is in line with the findings of ABCSG-52/ATHENE and IMpassion050 (increased pCR rates in atezolizumab versus placebo in PD-L1-negative patients)[20] but in contrast to the findings of the Neo-PATH trial[24]. Therefore, further clinical and experimental investigations are required.

https://doi.org/10.1038/s43018-024-00890-2

Covariate	Category	Alternative cat. events/*n* (%)	Reference cat. events/*n* (%)	OR (95% CI)	*P* value
TILs	≥50% vs. <50%	5/8 (62.5%)	30/50 (60.0%)	1.11 (0.24 to 5.18)	0.89
TILs	≥5% vs. <5%	31/52 (59.6%)	4/6 (66.7%)	0.74 (0.12 to 4.40)	0.74
HR	Positive vs. negative	24/42 (57.1%)	11/16 (68.8%)	0.61 (0.18 to 2.06)	0.42
Prognostic stage	≥IIB vs. ≤IIA	8/13 (61.5%)	27/45 (60.0%)	1.07 (0.30 to 3.78)	0.92
Age (years)	10-year increase			1.04 (0.68 to 1.60)	0.84
BMI	10-unit increase			1.97 (0.62 to 6.22)	0.25
Grade	G3 vs. G2	19/30 (63.3%)	16/28 (57.1%)	1.30 (0.45 to 3.72)	0.63
Menopausal status	Peri-/pre- vs. postmenopausal	12/24 (50.0%)	23/34 (67.6%)	0.48 (0.16 to 1.40)	0.18
Histological type	Other vs. NST	4/10 (40.0%)	31/48 (64.6%)	0.37 (0.09 to 1.48)	0.16
PD-L1 status	Positive vs. negative	16/29 (55.2%)	18/26 (69.2%)	0.55 (0.18 to 1.66)	0.29

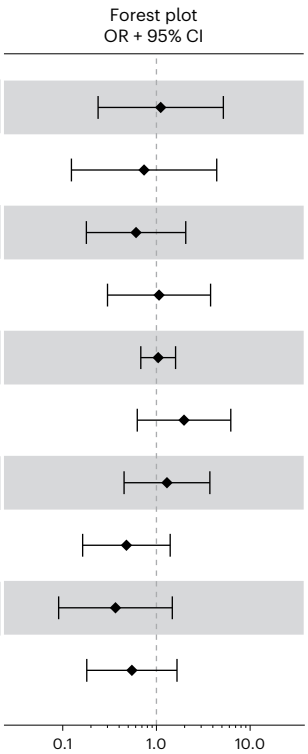

Fig. 3 | Covariate association with pCR. The prognostic stage is given according to the American Joint Committee on Cancer staging manual version 8.0. The column 'alternative cat.' refers to the alternative category, and 'reference cat.' refers to the reference category of the covariate. An OR of >1 indicates a higher pCR rate of the alternative category (left category in the 'category' column) or with higher age and BMI. Events: patients achieving pCR; sample size: 58. *P* values are from two-sided Wald tests with no adjustment for multiple testing. Covariate effects are presented as ORs including 95% CIs. NST, invasive carcinoma of no special type.

Anthracyclines not only are effective cytotoxic drugs against breast cancer but also exert cardiotoxic effects in a dose-dependent manner. The exploitation of their proimmunogenic effects is important, provided their cumulative doses can be limited. The fact that only four cycles of epirubicin were needed for a high pCR rate and no cardiotoxic effects were observed despite the combination with dual HER2 blockade and checkpoint inhibitors is promising and reassuring. In fact, the only grade 2 cardiac event occurred in the chemotherapy-free phase of the initial ICB and dual anti-HER2 therapy. Regarding immune-related side effects, continuous toxicity monitoring was included in our trial, and no grade ≥3 toxicities of special interest (immune-related AEs, cardiac disorders grade ≥2 or infusion-related reactions) were detected.

In addition to anthracyclines, taxanes, platinum compounds and alkylants (for example, cyclophosphamide) also have cardiotoxic potential. Whether a regimen with four cycles of epirubicin is more cardiotoxic than an anthracycline-free regimen consisting of six cycles of docetaxel and carboplatin is unanswered. In our view, an anthracycline-containing de-escalation protocol as in ABCSG-52/ATHENE is justified in terms of cardiotoxicities, and our toxicity data support this assumption.

The findings of this study are not without limitations. Because of the phase 2 design, this study was small and did not have an additional arm without ICB therapy over the whole treatment course. Owing to the short observation periods, no details regarding long-term outcomes, such as invasive disease-free survival, can yet be reported. We stratified patients according to the number of stromal TILs as this is an accepted prognostic marker in breast cancer and a stromal TIL proportion of ≥5% was predictive of response to an ICB combined with trastuzumab in pretreated patients in the phase 1/2b PANACEA trial[44]. Future trials should consider both the number of TILs and basal PD-L1 expression.

Table 3 | Treatment-emergent AEs in >15% of patients

AE	TP-A group (*n*=29)		TP group (*n*=29)	
	Any grade	Grade ≥3	Any grade	Grade ≥3
Nausea	20 (69.0%)	1 (3.4%)	20 (69.0%)	0
Diarrhea	17 (58.6%)	1 (3.4%)	18 (62.1%)	0
Fatigue	14 (48.3%)	0	17 (58.6%)	0
Alopecia	12 (41.4%)	1 (3.4%)	8 (27.6%)	1 (3.4%)
Chills	7 (24.1%)	0	7 (24.1%)	0
Headache	7 (24.1%)	0	8 (27.6%)	0
Decreased appetite	6 (20.7%)	0	4 (13.8%)	0
Neutropenia	6 (20.7%)	6 (20.7%)	4 (13.8%)	1 (3.4%)
Arthralgia	5 (17.2%)	0	2 (6.9%)	0
Constipation	5 (17.2%)	0	8 (27.6%)	0
Infusion-related reaction	5 (17.2%)	0	6 (20.7%)	1 (3.4%)
Mucosal inflammation	5 (17.2%)	0	5 (17.2%)	0
Pyrexia	5 (17.2%)	0	7 (24.1%)	0
Dry skin	4 (13.8%)	0	5 (17.2%)	0
Dyspepsia	2 (6.9%)	0	5 (17.2%)	0
Nasopharyngitis	1 (3.4%)	0	5 (17.2%)	0

In summary, our data provide evidence that the addition of anti-PD-L1 inhibitors to abbreviated monotherapy with an anthracycline leads to high pCR rates in HER2-positive breast cancer. Our study also raises interesting questions about the sequencing of chemotherapy and immunotherapy in a combined approach and

regarding the biological meaning of PD-L1 expression and its therapeutic inhibition in relation to the sensitivity of tumor cells against HER2 blockade.

Methods

Study design

ABCSG-52/ATHENE is a multicenter open-label, two-arm, randomized, single-stage phase 2 study (Fig. 1). Registration of the study in the European Union Clinical Trials Register was performed before the inclusion of the first patient (EudraCT no. 2019-002364-27). The study protocol was reviewed and approved by an independent ethics committee (Ethics Committee of the County of Salzburg, Austria). The first patient was enrolled on 3 July 2020, and the last patient was enrolled on 2 December 2021. The full study protocol is provided in Supplementary Information. The study design and conduct complied with all relevant regulations regarding the use of human study participants. The study was conducted in accordance with the criteria set by the Declaration of Helsinki. The CONSORT (Consolidated Standards of Reporting Trials) guidelines were followed[45]. Randomization, power calculation and statistical tests comply with the ICMJE (International Committee of Medical Journal Editors) guidelines on reporting.

Patients

Patients with previously untreated, histologically confirmed HER2-positive EBC with a clinical prognostic stage of cT1c to cT4a–d, N0–3 and M0, with adequate cardiac (ejection fraction ≥55%), renal, liver and bone marrow function were eligible for this trial. Patients with a history of malignancies other than nonmelanoma skin cancer and in situ carcinomas and those with a history of autoimmune disease, bilateral breast cancer or other concomitant serious medical conditions were excluded from trial participation. All patients signed an informed consent form before study enrollment. Patients were not compensated for clinical trial participation.

Randomization and masking

Patients were randomized 1:1 to two 3-weekly cycles of a chemotherapy-free induction phase (part 1) with TP-A or TP alone. Thereafter, all patients received four cycles of TP-A in combination with epirubicin (part 2). Randomization was done with a centralized web-based system using a minimization algorithm including the three stratification criteria: (1) baseline stromal TILs: <5% versus ≥5%; (2) HR status: HR positive versus HR negative; and (3) prognostic stage: ≤IIA versus ≥IIB (according to the clinical prognostic stage groups defined by the American Joint Committee on Cancer staging manual version 8.0). PD-L1 expression status was neither an inclusion nor a stratification factor.

Procedure

Study treatment. In the ABCSG-52/ATHENE study, treatment consisted of two parts. For part 1, both treatment groups received two 3-weekly cycles of pertuzumab (starting with 840 mg administered intravenously (IV) on cycle 1, followed by 420 mg IV for the subsequent cycles) and trastuzumab (starting with 600 mg administered subcutaneously (SC) or 8 mg kg^{-1} IV on cycle 1, followed by 600 mg SC or 6 mg kg^{-1} IV for the subsequent cycles). In the TP-A group, two 3-weekly cycles of atezolizumab (1,200 mg IV per cycle) were added.

For part 2, both groups received four 3-weekly cycles of atezolizumab (1,200 mg IV per cycle), pertuzumab (420 mg IV per cycle), trastuzumab (600 mg SC or 6 mg kg^{-1} IV per cycle) and epirubicin (90 mg m^{-2} per cycle).

Adjuvant treatment was not part of our neoadjuvant trial. If pCR was not achieved, a taxane-based adjuvant chemotherapy was recommended. After the completion of adjuvant standard anti-HER2 therapy and in the case of HR-positive patients, standard endocrine therapy was recommended.

Tumor-infiltrating lymphocytes. TILs in formalin-fixed, paraffin-embedded tissues from diagnostic biopsies performed before treatment initiation were assessed by a breast cancer pathologist. Stromal TILs were counted according to the recommendation of the International TIL Working Group[46].

PD-L1 expression. The expression of PD-L1 was assessed in formalin-fixed, paraffin-embedded tissues from diagnostic biopsies performed before the start of treatment. For PD-L1 staining, the Ventana PD-L1 SP124 assay was used according to the manufacturer's protocol (no dilution was required). PD-L1 positivity was defined as at least 1% PD-L1-expressing tumor-infiltrating immune cells[47].

Outcome parameters

The primary outcome was efficacy with regard to pCR (ypT0/is, ypN0), which was assessed in the overall study population at the time of surgery. This assessment was performed locally at each site.

The secondary outcomes were RCB and overall response rate, which were both assessed in the overall study population at the time of surgery.

Safety control. AEs were assessed and coded according to Common Terminology Criteria for AEs version 5.0. In addition, a strict, continuous safety monitoring program was designed, and the proportion of patients with at least one grade ≥3 AE of special interest, including immune-related AEs, cardiac disorders and/or infusion-related reactions (see Supplementary Table 1 for detailed definitions), was closely monitored. Prespecified Pocock-type boundaries were implemented[48], and a proportion of 20% was considered safe. The sequential boundaries were calculated such that the trial would have been stopped early with a 5% probability in case the true rate of grade ≥3 AEs of special interest was as high as 20%. Data on AEs were collected until the post-surgery visit (within 7–42 days after surgery but at least 42 days after the last dose of neoadjuvant study treatment).

Echocardiography was required at screening (within 28 days before randomization), before the first administration of epirubicin and before surgery. Additional assessments were performed as clinically indicated. At screening, patients with a left ventricular ejection fraction of <55% were not eligible for study participation. During treatment, patients whose left ventricular ejection fraction decreased to <50% had to permanently discontinue the study treatment.

Statistical analysis

Sample size assumptions were based on the pCR data reported in the NeoSphere trial[10] as well as statistical and medical expert opinions. A pCR proportion of 40% in the overall study population was assumed to indicate relevant clinical activity of this regimen, and the main goal of this trial was to estimate the true pCR proportion with a given level of precision (that is, half-width of 95% CI = 13 percentage points). With these assumptions, a sample size of 55 patients would have been required. However, given that CIs are the widest when the point estimate is 50%, the sample size calculation was based on an assumed pCR rate of 50% and yielded 57 patients. Owing to 1:1 randomization, 58 patients were randomized to achieve a balance between arms. However, the study was considered positive if at least 40% of the ITT population achieved pCR.

The primary endpoint, pCR, was assessed in the full analysis set consisting of all randomized patients. Patients were analyzed according to the ITT principle. The secondary endpoints, RCB[49] and overall response rate[50] at surgery, were assessed in all patients with a nonmissing measurement (modified ITT). Furthermore, efficacy endpoints were reanalyzed in the efficacy assessment population, which consisted of all patients who received at least two cycles of the study treatment and were assessable for pCR status.

Safety analyses were conducted based on the safety population, which comprised all patients who received at least one dose of study treatments.

Wilson score 95% CIs were derived for the primary and secondary outcomes, and 95% Wald asymptotic CIs for treatment arm differences were derived. In addition, univariable logistic regression models were used to assess potential associations of clinical covariates with pCR. Covariates used included TIL proportion, HR status, prognostic stage, age, BMI, grade, menopausal status, histological type and PD-L1 status. All indicated *P* values (Wald tests) are two-sided. No multiplicity adjustments were used.

A nonpredefined exploratory analysis of pCR rate according to PD-L1 status was conducted in the ITT population with available PD-L1 data.

Analyses were performed using SAS software version 9.4 (SAS Institute).

As the trial start coincided with the COVID-19 outbreak in 2020, site initiations were evaluated in close consultation with the trial sites and according to available resources and local circumstances. Because of an increase in the number of infections (following the peak of the COVID-19 outbreak in Austria in the spring of 2020), an evaluation of the benefit–risk balance was performed in cooperation with the ABCSG-52/ATHENE coordinating investigators and in consultation with the investigational medicinal product provider (Roche), and the results were shared with the trial site teams. According to this guidance, study conduct (including enrollment and study treatment) was continued per protocol considering site-specific and/or countrywide COVID-19 government measures and guidance documents (for example, from the Austrian Ministry of Health).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The individual clinical data, including source data, cannot be made publicly available owing to patient privacy. The individual deidentified clinical data and—if needed—statistical analysis plan as well as a data definition table file can be shared only upon approval of the analysis proposal by the steering committee and sponsor of the ABCSG-52 study and after a data-sharing agreement has been signed. Initial requests will be addressed within 6 weeks by the sponsor. Data availability after approval of the respective analysis proposal and signatory of the data-sharing agreement will depend on the analysis to be performed and agreed upon upfront in the data-sharing agreement but in no case less than 4 weeks. Please contact the corresponding author for more information. The study protocol is available in Supplementary Information.

Code availability

The SAS program code can be shared upon approval of the analysis proposal by the steering committee and sponsor of the ABCSG-52 study and after a data-sharing agreement has been signed. Please contact the corresponding author for more information.

References

1. Cortazar, P. et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* **384**, 164–172 (2014).
2. Cardoso, F. et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **30**, 1194–1220 (2019).
3. National Comprehensive Cancer Network. *Breast Cancer (Version 4.2023)* www.nccn.org/professionals/physician_gls/pdf/breast.pdf (2023).
4. Korde, L. A. et al. Neoadjuvant chemotherapy, endocrine therapy, and targeted therapy for breast cancer: ASCO guideline. *J. Clin. Oncol.* **39**, 1485–1505 (2021).
5. Hurvitz, S. A. et al. Neoadjuvant trastuzumab, pertuzumab, and chemotherapy versus trastuzumab emtansine plus pertuzumab in patients with HER2-positive breast cancer (KRISTINE): a randomised, open-label, multicentre, phase 3 trial. *Lancet Oncol.* **19**, 115–126 (2018).
6. Schneeweiss, A. et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). *Ann. Oncol.* **24**, 2278–2284 (2013).
7. Swain, S. M. et al. Pertuzumab, trastuzumab, and standard anthracycline- and taxane-based chemotherapy for the neoadjuvant treatment of patients with HER2-positive localized breast cancer (BERENICE): a phase II, open-label, multicenter, multinational cardiac safety study. *Ann. Oncol.* **29**, 646–653 (2018).
8. von Minckwitz, G. et al. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. *N. Engl. J. Med.* **380**, 617–628 (2019).
9. File, D., Curigliano, G. & Carey, L. A. Escalating and de-escalating therapy for early-stage HER2-positive breast cancer. *Am. Soc. Clin. Oncol. Educ. Book* **40**, 1–11 (2020).
10. Gianni, L. et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol.* **13**, 25–32 (2012).
11. Gluz, O. et al. Efficacy of endocrine therapy plus trastuzumab and pertuzumab vs de-escalated chemotherapy in patients with hormone receptor-positive/*ERBB2*-positive early breast cancer: the neoadjuvant WSG-TP-II randomized clinical trial. *JAMA Oncol.* **9**, 946–954 (2023).
12. Nitz, U. A. et al. De-escalation strategies in HER2-positive early breast cancer (EBC): final analysis of the WSG-ADAPT HER2+/HR− phase II trial: efficacy, safety, and predictive markers for 12 weeks of neoadjuvant dual blockade with trastuzumab and pertuzumab ± weekly paclitaxel. *Ann. Oncol.* **28**, 2768–2772 (2017).
13. Zhang, J. et al. Immunostimulatory properties of chemotherapy in breast cancer: from immunogenic modulation mechanisms to clinical practice. *Front. Immunol.* **12**, 819405 (2022).
14. Tanaka, H., Matsushima, H., Mizumoto, N. & Takashima, A. Classification of chemotherapeutic agents based on their differential in vitro effects on dendritic cells. *Cancer Res.* **69**, 6978–6986 (2009).
15. Shurin, G. V., Tourkova, I. L., Kaneno, R. & Shurin, M. R. Chemotherapeutic agents in noncytotoxic concentrations increase antigen presentation by dendritic cells via an IL-12-dependent mechanism. *J. Immunol.* **183**, 137–144 (2009).
16. Buttiglieri, S., Galetto, A., Forno, S., De Andrea, M. & Matera, L. Influence of drug-induced apoptotic death on processing and presentation of tumor antigens by dendritic cells. *Int. J. Cancer* **106**, 516–520 (2003).
17. Bezu, L. et al. Combinatorial strategies for the induction of immunogenic cell death. *Front. Immunol.* **6**, 187 (2015).
18. Herbst, R. S. et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **515**, 563–567 (2014).
19. European Medicines Agency. *Tecentriq: EPAR—Product Information* www.ema.europa.eu/documents/product-information/tecentriq-epar-product-information_en.pdf (2013).

20. Huober, J. et al. Atezolizumab with neoadjuvant anti-human epidermal growth factor receptor 2 therapy and chemotherapy in human epidermal growth factor receptor 2-positive early breast cancer: primary results of the randomized phase III IMpassion050 trial. *J. Clin. Oncol.* **40**, 2946–2956 (2022).
21. Gianni, L. et al. Abstract LBO1-02: Pathologic complete response (pCR) of neoadjuvant therapy with or without atezolizumab in HER2-positive, early high-risk and locally advanced breast cancer: APTneo Michelangelo randomized trial. *Cancer Res.* **84**, LBO1-02 (2024).
22. Kuemmel, S. et al. Abstract PD10-11: Keyriched-1—a prospective, multicenter, open label, neoadjuvant phase ii single arm study with pembrolizumab in combination with dual anti-HER2 blockade with trastuzumab and pertuzumab in early breast cancer patients with molecular HER2-enriched intrinsic subtype. *Cancer Res.* **82**, PD10-11 (2022).
23. Schettini, F. et al. HER2-enriched subtype and pathological complete response in HER2-positive breast cancer: a systematic review and meta-analysis. *Cancer Treat. Rev.* **84**, 101965 (2020).
24. Ahn, H. K. et al. Response rate and safety of a neoadjuvant pertuzumab, atezolizumab, docetaxel, and trastuzumab regimen for patients with *ERBB2*-positive stage II/III breast cancer: the Neo-PATH phase 2 nonrandomized clinical trial. *JAMA Oncol.* **8**, 1271–1277 (2022).
25. Ma, W. et al. Increasing cure rates of solid tumors by immune checkpoint inhibitors. *Exp. Hematol. Oncol.* **12**, 10 (2023).
26. Abdou, Y. et al. Immunotherapy in triple negative breast cancer: beyond checkpoint inhibitors. *NPJ Breast Cancer* **8**, 121 (2022).
27. Barroso-Sousa, R. et al. Prevalence and mutational determinants of high tumor mutation burden in breast cancer. *Ann. Oncol.* **31**, 387–394 (2020).
28. Cardoso, F. et al. LBA21 KEYNOTE-756: Phase III study of neoadjuvant pembrolizumab (pembro) or placebo (pbo)+chemotherapy (chemo), followed by adjuvant pembro or pbo+endocrine therapy (ET) for early-stage high-risk ER+/HER2− breast cancer. *Ann. Oncol.* **34**, S1260–S1261 (2023).
29. Loi, S. et al. LBA20 A randomized, double-blind trial of nivolumab (NIVO) vs placebo (PBO) with neoadjuvant chemotherapy (NACT) followed by adjuvant endocrine therapy (ET)±NIVO in patients (pts) with high-risk, ER+ HER2− primary breast cancer (BC). *Ann. Oncol.* **34**, S1259–S1260 (2023).
30. US Food and Drug Administration. Pathological complete response in neoadjuvant treatment of high-risk early-stage breast cancer: use as an endpoint to support accelerated approval. *FDA* www.fda.gov/regulatory-information/search-fda-guidance-documents/pathological-complete-response-neoadjuvant-treatment-high-risk-early-stage-breast-cancer-use (2020).
31. Galluzzi, L., Buqué, A., Kepp, O., Zitvogel, L. & Kroemer, G. Immunological effects of conventional chemotherapy and targeted anticancer agents. *Cancer Cell* **28**, 690–714 (2015).
32. Schmid, P. et al. Pembrolizumab for early triple-negative breast cancer. *N. Engl. J. Med.* **382**, 810–821 (2020).
33. Mittendorf, E. A. et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. *Lancet* **396**, 1090–1100 (2020).
34. Gianni, L. et al. Pathologic complete response (pCR) to neoadjuvant treatment with or without atezolizumab in triple-negative, early high-risk and locally advanced breast cancer: NeoTRIP Michelangelo randomized study. *Ann. Oncol.* **33**, 534–543 (2022).
35. Voorwerk, L. et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. *Nat. Med.* **25**, 920–928 (2019).
36. Mellman, I., Chen, D. S., Powles, T. & Turley, S. J. The cancer–immunity cycle: indication, genotype, and immunotype. *Immunity* **56**, 2188–2205 (2023).
37. Farr, A. et al. The effect of obesity on pathological complete response and survival in breast cancer patients receiving uncapped doses of neoadjuvant anthracycline–taxane-based chemotherapy. *Breast* **33**, 153–158 (2017).
38. Triulzi, T. et al. HER2 signaling regulates the tumor immune microenvironment and trastuzumab efficacy. *Oncoimmunology* **8**, e1512942 (2018).
39. Shang, M. et al. The therapeutic effectiveness of neoadjuvant trastuzumab plus chemotherapy for HER2-positive breast cancer can be predicted by tumor-infiltrating lymphocytes and PD-L1 expression. *Front. Oncol.* **11**, 706606 (2022).
40. Li, Y. et al. The role of programmed death ligand-1 and tumor-infiltrating lymphocytes in breast cancer overexpressing *HER2* gene. *Breast Cancer Res. Treat.* **170**, 293–302 (2018).
41. Force, J. et al. Early stage HER2-positive breast cancers not achieving a pCR from neoadjuvant trastuzumab- or pertuzumab-based regimens have an immunosuppressive phenotype. *Clin. Breast Cancer* **18**, 410–417 (2018).
42. Su, S. et al. Immune checkpoint inhibition overcomes ADCP-induced immunosuppression by macrophages. *Cell* **175**, 442–457 (2018).
43. Oyer, J. L., Gitto, S. B., Altomare, D. A. & Copik, A. J. PD-L1 blockade enhances anti-tumor efficacy of NK cells. *Oncoimmunology* **7**, e1509819 (2018).
44. Loi, S. et al. Abstract GS2-06: Phase Ib/II study evaluating safety and efficacy of pembrolizumab and trastuzumab in patients with trastuzumab-resistant HER2-positive metastatic breast cancer: results from the PANACEA (IBCSG 45-13/BIG 4-13/KEYNOTE-014) study. *Cancer Res.* **78**, GS2-06 (2018).
45. Schulz, K. F., Altman, D. G. & Moher, D.; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann. Intern. Med.* **152**, 726–732 (2010).
46. Salgado, R. et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann. Oncol.* **26**, 259–271 (2015).
47. Roche. *VENTANA PD-L1 (SP142) Assay: Interpretation Guide for Triple-Negative Breast Carcinoma (TNBC)* elabdoc-prod.roche.com/eLD/web/gb/en/documents/download/7043a6ba-c4df-ed11-1d91-005056a71a5d (2020).
48. Ivanova, A., Qaqish, B. F. & Schell, M. J. Continuous toxicity monitoring in phase II trials in oncology. *Biometrics* **61**, 540–545 (2005).
49. Symmans, W. F. et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. *J. Clin. Oncol.* **25**, 4414–4422 (2007).
50. Eisenhauer, E. A. et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur. J. Cancer* **45**, 228–247 (2009).

Acknowledgements

This research project was partially supported by Roche Austria GmbH. The funders agreed with the initial study design question but had no role in the detailed study design, data collection and analysis, decision to publish or preparation of the manuscript.

Author contributions

G.R. and R.B. contributed to the literature search. G.R., D.H. and L.S. contributed to the figures. G.R., R.B., M.B., V.B.-R., S.P.G., M.K., F.P., D.H., L.S., N.Z., M. Gnant and R.G. contributed to the study design. Z.B.-H., M.F. and N.Z. contributed to the resources. G.R., R.B., C.A.S., A.P., M.B., E.P., U.D., C.F.S., S.P.G., K.S., C.B., M. Gili, M.R., V.W., C.A.,

L.W., M.M., B.S., R.P. and R.G. contributed to the data collection. G.R., D.E., R.B., S.P.G., K.S., D.H., L.S., Z.B.-H., M.F., M. Gnant and R.G. contributed to the data analysis and interpretation. G.R., D.E., Z.B.-H., M.F., M. Gnant and R.G. contributed to the assessment and verification of data. All authors contributed to the writing of the paper.

Competing interests

G.R. reports disclosures related to consulting fees (Amgen, AstraZeneca, Daiichi Sankyo, Eli Lilly, Gilead, MSD, Novartis, Pfizer, Pierre Fabre, Roche, Stemline), payment/honoraria (Amgen, AstraZeneca, Daiichi Sankyo, Eli Lilly, Gilead, MSD, Novartis, Pfizer, Roche, Seagen, Stemline, BMS) and travel/accommodations/expenses (Amgen, Daiichi Sankyo, Eli Lilly, Gilead, Merck, Pfizer, Roche). D.E. reports disclosures related to consulting fees (AstraZeneca, Daiichi Sankyo, Gilead, Lilly, Menarini, Novartis, Pfizer, Roche, Seagen, Sirius Medical), payment/honoraria (AstraZeneca, Daiichi Sankyo, Gilead, Lilly, MSD, Novartis, Pfizer, Roche, Seagen, Sirius Medical), expert testimony (AstraZeneca, Daiichi Sankyo, Gilead, Lilly), travel/accommodations/expenses (AstraZeneca, Daiichi Sankyo, Pfizer), an advisory role (AstraZeneca, Daiichi Sankyo) and receipt of equipment/materials from Sirius Medical. R.B. reports disclosures related to grants/contracts (Daiichi Sankyo), consulting fees (AstraZeneca, Daiichi, Eisai, Eli Lilly, Gilead, Gruenenthal, MSD, Novartis, Pfizer, Pierre Fabre, Roche, Seagen, Stemline), payment/honoraria (AstraZeneca, BMS, Daiichi, Eisai, Eli Lilly, Gilead, Gruenenthal, MSD, Novartis, Pfizer, Pierre Fabre, Roche, Seagen), travel/accommodations/expenses (Daiichi, MSD, Pfizer) and an advisory role (WSG). C.A.S. reports disclosures related to support for the paper (Roche), grants/contracts (Roche), payment/honoraria (Roche) and travel/accommodations/expenses (Roche). A.P. reports disclosures related to payments for an advisory role (Novartis, Amgen, Celgene/BMS, Sandoz, Janssen, AstraZeneca, Abbvie, Takeda, Sanofi, Kite–Gilead, Roche, Pfizer, Seagen, Daiichi Sankyo) and travel/accommodations/expenses (Roche, Gilead, Daiichi Sankyo, Janssen, Eli Lilly, Pierre Fabre). M.B. reports disclosures related to grants/contracts (Amgen, AstraZeneca, Cepheid, Daiichi Sankyo, Gilead, MSD, Novartis, Seagen), consulting fees (Amgen, AstraZeneca, Celgene, Daiichi Sankyo, Eli Lilly, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Samsung, Gilead, Seagen), payment/honoraria (Amgen, AstraZeneca, Celgene, Daiichi Sankyo, Eli Lilly, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Seagen, Gilead), travel/accommodations/expenses (AstraZeneca, Daiichi Sankyo, MSD, Roche), an advisory role (Pfizer, GBG, Novartis) and a leadership/fiduciary role (OeGHO, ABCSG). E.P. reports disclosures related to support for the paper (Roche), payment/honoraria (Roche) and an advisory role (Roche). C.F.S. reports disclosures related to grants/contracts (Amgen, Roche, AstraZeneca, Daiichi), consulting fees (AstraZeneca, Novartis), payment/honoraria (Novartis, AstraZeneca, Daiichi, Seagen, Amgen), travel/accommodations/expenses (Daiichi) and an advisory role (WSG). S.P.G. reports disclosures related to consulting fees (Roche), payment/honoraria (Roche), travel/accommodations/expenses (Roche) and an advisory role (Roche). M.K. reports disclosures related to an advisory role (WSG). D.H. reports disclosures related to support for the paper (Roche). L.S. reports disclosures related to support for the paper (Roche). Z.B.-H. reports disclosures related to consulting fees (AstraZeneca, Menarini Stemline, MSD) and payment/honoraria (AstraZeneca, MSD). M. Gili reports disclosures related to support for the paper (Roche). L.W. reports disclosures related to payment/honoraria (Roche Austria) and travel/accommodations/expenses (Roche Austria). M.M. reports disclosures related to consulting fees (Roche, Eli Lilly, Novartis, AstraZeneca, Daiichi Sankyo, Pfizer, MSD), payment/honoraria (MedMedia, Roche, Eli Lilly, Novartis, AstraZeneca, Daiichi Sankyo, Pfizer, MS), travel/accommodations/expenses (Amgen, Roche, Novartis, MSD, Pierre Fabre, Daiichi Sankyo, Eisai), an advisory role (Eli Lilly, Novartis, AstraZeneca, Daiichi Sankyo, Pfizer, MSD) and receipt of equipment/materials (AstraZeneca, Astellas). B.S. reports disclosures related to travel/accommodations/expenses (Roche Austria). R.P. reports disclosures related to payment/honoraria (Myriad, Roche, Amgen, AstraZeneca, Novartis, Daiichi Sankyo, Pfizer, MedMedia), travel/accommodations/expenses (Pfizer, Novartis) and an advisory role (Novartis, Pfizer, AstraZeneca, Roche). M. Gnant reports disclosures related to consulting fees (AstraZeneca, Daiichi Sankyo, Menarini Stemline, MSD, Novartis), payment/honoraria (Amgen, Daiichi Sankyo, Eli Lilly, EPG Health (IQVIA), Menarini Stemline, Novartis, Pierre Fabre, Veracyte), expert testimony payment (Eli Lilly), travel/accommodations/expenses (Eli Lilly, Menarini Stemline, Novartis, Pierre Fabre) and an advisory role (AstraZeneca, Eli Lilly); an immediate family member is employed by Sandoz. R.G. reports disclosures related to consulting fees (Celgene, Novartis, Roche, BMS, Takeda, Abbvie, AstraZeneca, Janssen, MSD, Amgen, Merck, Gilead, Daiichi Sankyo, Sanofi), payment/honoraria (Celgene, Roche, Merck, Takeda, AstraZeneca, Novartis, Amgen, BMS, MSD, Sandoz, Abbvie, Gilead, Daiichi Sankyo, Sanofi), travel/accommodations/expenses (Roche, Amgen, Janssen, AstraZeneca, Novartis, MSD, Celgene, Gilead, BMS, Abbvie, Daiichi Sankyo), an advisory role (Celgene, Novartis, Roche, BMS, Takeda, Abbvie, AstraZeneca, Janssen, MSD, Amgen, Merck, Gilead, Daiichi Sankyo, Sanofi) and stock/stock options (Novo Nordisk, Lilly). The other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43018-024-00890-2.

Correspondence and requests for materials should be addressed to Gabriel Rinnerthaler.

Peer review information *Nature Cancer* thanks the anonymous reviewers for their contribution to the peer review of this work.

https://doi.org/10.1038/s43018-024-00890-2

Gabriel Rinnerthaler[1,2,3,18] ✉, Daniel Egle[3,4,18], Rupert Bartsch[3,5], Clemens A. Schmitt[6], Andreas Petzer[7], Marija Balic[1,3], Edgar Petru[8], Ursula Denison[9], Christian F. Singer[3,10,11], Vesna Bjelic-Radisic[3,12], Simon Peter Gampenrieder[2,3], Michael Knauer[3,13], Karl Sotlar[14], Christine Brunner[4], Florian Posch[1], Dominik Hlauschek[3], Lidija Sölkner[3], Zsuzsanna Bago-Horvath[3,15], Martin Filipits[3,16], Manuela Gili[3], Magdalena Ritter[4], Verena Wieser[3,4], Carmen Albertini[4], Nadja Zaborsky[2,17], Lukas Weiss[2,3], Maximilian Marhold[5], Bruno Schneeweiss[6], Renate Pusch[7], Michael Gnant[3,11,18] & Richard Greil[2,3,18]

[1]Division of Oncology, Department of Internal Medicine, Medical University of Graz, Graz, Austria. [2]Department of Internal Medicine III with Hematology, Medical Oncology, Hemostaseology, Infectiology and Rheumatology, Oncologic Center, Paracelsus Medical University Salzburg, Salzburg Cancer Research Institute, Center for Clinical Cancer and Immunology Trials (SCRI-CCCIT), Cancer Cluster Salzburg, Salzburg, Austria. [3]Austrian Breast and Colorectal Cancer Study Group (ABCSG), Vienna, Austria. [4]Department of Gynecology, Breast Cancer Center Tirol, Medical University of Innsbruck, Innsbruck, Austria. [5]Division of Oncology, Department of Medicine 1, Medical University of Vienna, Vienna, Austria. [6]Department of Hematology and Internal Oncology, Johannes Kepler University, Kepler University Hospital, Linz, Austria. [7]Internal Medicine I: Hematology with Stem Cell Transplantation, Hemostaseology and Medical Oncology, Ordensklinikum Linz, Linz, Austria. [8]Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria. [9]Institute for Gynecological Oncology and Senology, Karl Landsteiner Society, Hietzing Hospital, Vienna, Austria. [10]Department of Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria. [11]Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria. [12]Breast Unit, Helios University Hospital Wuppertal, University Witten/Herdecke, Wuppertal, Germany. [13]Tumor and Breast Center Eastern Switzerland, St. Gallen, Switzerland. [14]Department of Pathology, Paracelsus Medical University Salzburg, Salzburg, Austria. [15]Department of Pathology and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria. [16]Center for Cancer Research, Medical University of Vienna, Vienna, Austria. [17]Laboratory of Immunological and Molecular Cancer Research (LIMCR), Salzburg, Austria. [18]These authors contributed equally: Gabriel Rinnerthaler, Daniel Egle, Michael Gnant, Richard Greil. ✉e-mail: gabriel.rinnerthaler@medunigraz.at

nature portfolio

Corresponding author(s): Gabriel Rinnerthaler

Last updated by author(s): 2024-Nov-26

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Data Management System MACRO (Web Interface DATAPORT)
Data analysis	Analyses were performed with SAS software version 9.4 (SAS Institute Inc, Cary, NC).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The individual clinical data, including source data, cannot be made publicly available due to patient privacy. The individual de-identified clinical data and – if needed – statistical analysis plan as well as a data definition table file can be shared only upon approval of the analysis proposal by the Steering Committee and sponsor of the ABCSG 52 study, and after a data sharing agreement has been signed. Initial requests will be addressed within 6 weeks by the sponsor. Data availability after approval of the respective analysis proposal and signatory of the data sharing agreement will depend on the analysis to be performed and agreed upfront in the

data sharing agreement, but in no case less than 4 weeks. Please contact the corresponding author for more information.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Only female patients were included (information on sex at birth and sex at randomization was collected). Self-reported gender was not collected.
Reporting on race, ethnicity, or other socially relevant groupings	All included patients were White based on self-reporting. No other socially relevant groupings were performed or collected.
Population characteristics	Patients with previously untreated, histologically confirmed HER2-positive early breast cancer (EBC) with a clinical prognostic stage of cT1c-4a-d, N0–3, and M0, with adequate cardiac (ejection fraction ≥ 55%), renal, liver, and bone marrow function were eligible for this trial. Patients with a history of malignancies other than non-melanoma skin cancer and in situ carcinomas, a history of autoimmune disease, bilateral breast cancer, or other concomitant serious medical conditions were excluded from trial participation.
Recruitment	Patients presenting at clinic who fullfilled the eligiblity criteria were asked to participate in the trial. Every patient signed an informed consent before study enrollment.
Ethics oversight	Ethics Committee of the County of Salzburg, Austria

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size assumptions were based on the pCR data reported in the NeoSphere trial as well as statistical and medical expert opinions. A pCR proportion of 40% in the overall study population was assumed to indicate relevant clinical activity of this regimen and the main goal of this trial was to estimate the true pCR proportion with a given level of precision (i.e., half-width of 95% confidence interval [CI] = 13%-points). With these assumptions, a sample size of 55 patients would have been required. However, given that confidence intervals are widest when the point estimate is 50%, sample size calculation was based on an assumed pCR rate of 50% and yielded 57 patients. Due to 1:1 randomization, 58 patients were randomized to achieve balance between arms. However, the study was considered positive if at least 40% of the ITT population achieved a pCR.
Data exclusions	For the primary endpoint (pCR) no patients were excluded. For other endpoint analysis patients may have been excluded due to missing outcome data.
Replication	Not applicable
Randomization	Randomization was done using a centralized web-based randomization system on a minimization algorithm including the three stratification criteria: (1) baseline stromal tumor-infiltrating lymphocytes (TILs): < 5% vs. ≥ 5%, hormone receptor (HR) status: HR+ vs. HR-, and prognostic stage: ≤ IIA vs. ≥ IIB (according to AJCC v.8.0 clinical prognostic stage groups). Patients were randomized 1:1 to two 3-weekly cycles of a chemotherapy-free induction phase (part 1) with trastuzumab and pertuzumab (TP) plus atezolizumab (TP+A) or TP alone. Thereafter, all patients received 4 cycles of TP+A in combination with epirubicin (part 2).
Blinding	Unblinded, open-label study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used: Trastuzumab, Pertuzumab, Atezolizumab

Validation: NA

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration: EudraCT Number: 2019-002364-27

Study protocol: Supplementary Information section to manuscript

Data collection: Between June 2020 and December 2021, 70 patients were screened, and 58 patients (ITT population) were randomized 1:1 to TP-A (n=29) or TP (n=29) at 9 Austrian sites. Study database was closed in August 2022.

Outcomes: A complete pathological remission was observed in 35 pts (60·3%; 95%CI 47·5% to 71·9%), 19 (65·5%) in the TP-A group and 16 (55·2%) in TP group and primary endpoint was met. Adverse events (AEs) grade ≥ 3 were reported in 17 pts (29·3%).

Plants

Seed stocks: NA

Novel plant genotypes: NA

Authentication: NA