Phase II study of liposomal doxorubicin, docetaxel and trastuzumab in combination with metformin as neoadjuvant therapy for HER2-positive breast cancer

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Abstract

Background: The aim of this study was to improve activity over single human epidermal growth factor receptor 2 (HER2)-blockade sequential neaodjuvant regimens for HER2-positive breast cancer, by exploiting the concomitant administration of trastuzumab, taxane and anthracycline, while restraining cardiac toxicity with use of liposomal doxorubicin, and by adding metformin, based on preliminary evidence of antitumor activity.

Patients and methods: This multi-center, single-arm, two-stage phase II trial, assessed the safety and the activity of a new treatment regimen for HER2-positive, early or locally advanced breast cancer. Patients received six 21-day cycles of non-pegylated liposomal doxorubicin, 50 mg/m^2 intravenously (i.v.) on day 1, docetaxel, 30 mg/m^2 i.v. on days 2 and 9, trastuzumab, 2 mg/kg/week i.v. on days 2, 9, and 16 (with 4 mg/kg loading dose), in association with metformin 1000 mg orally twice daily. The primary endpoint was the rate of pathological complete response (pCR) in the breast and axilla (ypT0/is ypN0). A subgroup of patients performed a 3-deoxy-3-18F-fluorothymidine positron emission tomography (FLT-PET) at baseline and after one cycle.

Results: Among 47 evaluable patients, there were 18 pCR [38.3%, 95% confidence interval (CI) 24.5–53.6%]. A negative estrogen-receptor status, high Ki67, and histological grade 3 were related with pCR, although only grade reached statistical significance. FLT-PET maximum standardized uptake value after one cycle was inversely related to pCR in the breast (odds ratio 0.29, 95% CI 0.06–1.30, $p = 0.11$). Toxicity included grade 3–4 neutropenia in 70% and febrile neutropenia in 4% of patients, grade 1–2 nausea/vomiting in 60%/38%, and grade 3 in 4%/2%, respectively, grade 1–2 diarrhea in 72%, and grade 3 in 6%. There were two cases of reversible grade 2 left-ventricular ejection-fraction decrease, and one case of sharp troponin-T increase.

Conclusions: The concomitant administration of trastuzumab, liposomal doxorubicin, docetaxel, and metformin is safe and shows good activity, but does not appear to improve activity over conventional sequential regimens.

Keywords: HER2+ breast cancer, metformin, non-pegylated liposomal doxorubicin, neoadjuvant therapy, primary systemic therapy, trastuzumab

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Introduction

Early and locally advanced human epidermal growth factor receptor 2 (HER2)-positive breast cancer is commonly treated with neoadjuvant chemotherapy in combination with anti-HER2 monoclonal antibodies, with remarkable results, but still with a fraction of patients relapsing after a variable time lapse.[1] Attempts to improve on these results include, among others: dual HER2-blockade, intensification of the chemotherapy backbone, the concomitant, instead of sequential, administration of anthracyclines and trastuzumab, as well as the combination with other drugs targeting signaling pathways involved in resistance to anti-HER2 drugs. Our study deals with the last three issues, involving the concomitant administration of liposomal doxorubicin, docetaxel, trastuzumab, and metformin based on the rationale described in the following paragraphs.

The study had been conceived before the advent of dual HER2-blockade[2,3] as the standard neoadjuvant regimen for HER2-positive breast cancer, a regimen that is still not covered by the Italian National Health System. Nevertheless, considering that about a third of HER2-positive breast cancer cases fail to achieve pathological complete remission (pCR) even with dual HER2-blockade, the improvement of neoadjuvant treatment outcomes is still a highly relevant matter.

Combinations of anthracyclines and taxanes improve response rates compared with anthracyclines plus cyclophosphamide in metastatic breast cancer.[4] Based on these results, the concomitant administration of anthracyclines and taxanes has been studied in the adjuvant and neoadjuvant settings to improve outcomes compared with their sequential administration.[5,6] No significant differences emerged between these two strategies, apart from slightly different patterns of toxicity, and both are considered as suitable treatments.

Preclinical studies showed synergistic interactions between trastuzumab and docetaxel, and additive interactions between trastuzumab and doxorubicin.[7,8] While the concurrent administration of trastuzumab and taxanes is standard practice, the first experiences of concurrent administration of trastuzumab and doxorubicin in metastatic breast cancer resulted in prohibitive cardiac toxicity.[9] Nonetheless, shorter combination treatments with trastuzumab and anthracyclines, performed in the neoadjuvant setting, did not cause relevant cardiac side effects.[10]

The value of the concurrent administration of anthracyclines and trastuzumab in the neoadjuvant setting was addressed by the pivotal Z1041 phase III randomized trial, comparing a sequential regimen of FEC (fluorouracil, epirubicin, cyclophosphamide) followed by weekly trastuzumab plus paclitaxel, with a concomitant regimen of weekly trastuzumab plus paclitaxel, followed by trastuzumab plus FEC. The study did not show differences between the two arms, supporting the use of the sequential regimen.[11] On the other hand, a prior meta-analysis, not including Z1041, highlighted the existence of a significant benefit for the concomitant anthracycline and trastuzumab treatment in terms of pCR rates and relapse-free survival.[12] It appeared therefore useful to attempt the development of safer concomitant treatments based on liposomal doxorubicin, potentially allowing for both a higher cumulative dose of anthracycline to be administered as well as the possibility to administer it in combination with trastuzumab.

Non-pegylated liposomal doxorubicin is licensed for use only in first-line treatment of metastatic breast cancer, in combination with cyclophosphamide. In this setting, non-pegylated liposomal doxorubicin has been evaluated in comparison with conventional doxorubicin in two randomized phase III trials: as a monotherapy and in combination with cyclophosphamide. They showed equivalent response rates, no significant differences in overall survival and progression-free survival and reduced cardiotoxicity.[13,14] A meta-analysis of these two trials showed a significantly lower rate of both clinical heart failure [relative risk 0.20, 95% confidence interval (CI) 0.05–0.75] and of clinical and subclinical heart failure combined (relative risk 0.38, 95% CI 0.24–0.59) in patients treated with liposomal doxorubicin.[15] A further phase III trial comparing liposomal doxorubicin with epirubicin, either in combination with cyclophosphamide, confirmed its activity and safety.[16] Given the reduced cardiac toxicity compared with standard doxorubicin, liposomal doxorubicin has been studied in combination with docetaxel and trastuzumab in phase II clinical trials as first-line therapy in metastatic, HER2-positive breast cancer, showing promising activity and acceptable toxicity.[17,18] Cellular metabolism is deeply involved in the genesis and evolution of cancer and likely affects response and resistance to treatments.[19] Altered glucose homeostasis represents a negative prognostic factor in different breast cancer subtypes[20] including hormone receptor (HR)-positive and

HER2-positive cases. Its weight as a negative prognostic factor is amplified by insulin use while it is reduced by metformin use.[21] This has been attributed to insulin resistance and consequent hyperinsulinemia, which induces anabolic effects and stimulates cell proliferation *via* the insulin receptor and the insulin-like growth-factor 1 (IGF-1) receptor pathways, with downstream activation of the PI3K-AKT-mTOR and RAS-RAF-MEK-MAPK pathways.[22] The insulin signaling pathway is frequently co-opted in cancer cells,[23] even in the absence of hyperinsulinemia.

Administration of the biguanide metformin, one of the most widely used drugs for type 2 diabetes, has been associated with an increased rate of pCRs in diabetic patients undergoing neoadjuvant treatment for breast cancer.[24] Metformin suppresses HER2 overexpression[25] and has antitumor activity in preclinical models of HER2-positive breast cancer. And that includes trastuzumab-resistant models.[26,27] Potential mechanisms of antitumor action include inhibition of the mitochondrial electron transport chain and then of adenosine triphosphate synthesis, activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) and inhibition of mechanistic target of rapamycin (mTOR), therefore targeting some of the main master regulators of cellular metabolism, shifting it from anabolism towards catabolism.[28] Indirect effects related to the reduction of blood glucose levels and insulinemia are also invoked.

These discoveries called for us to conduct clinical trials exploring the benefit of adding metformin to standard treatments in early and advanced breast cancer.

Here, we report the results of phase II, single-arm study that assesses the activity and safety of a combination of metformin with a regimen including concomitant trastuzumab plus docetaxel and non-pegylated liposomal doxorubicin as neoadjuvant therapy for HER2-positive breast cancer. This regimen was previously developed in a phase I/II trial in advanced breast cancer, as an attempt to improve on the activity of anti-HER2 treatment by administering trastuzumab concomitantly with both anthracycline and taxane. It proved to be both active and safe.[17]

Patients and methods

Patients. Eligible patients were histologically confirmed to have HER2-positive (assessed by local pathologist and defined as 3+ staining at immunohistochemistry or as HER2-amplified at *in situ* hybridization), clinical stage cT1c with cytologically proven N1-2, or cT2–4a–d/N0–2, M0 breast cancer with any HR status, age between 18 and 75 years. They also presented an Eastern Co-operative Oncology Group performance status of 0 or 1 and adequate cardiac (left-ventricular ejection fraction, LVEF, $\geqslant$50%) as well as renal, liver, and bone marrow function. Main exclusion criteria include prior treatment for breast cancer, type 1 or 2 diabetes, and other concomitant severe comorbidities (including cardiac diseases and malignant neoplasms, except for previously treated basal-cell carcinoma and *in situ* carcinoma of the uterine cervix).

Study design. This is a multicenter, single-arm, two-stage phase II trial. It is designed to assess the safety and the activity of a combination of non-pegylated liposomal doxorubicin, docetaxel, trastuzumab, and metformin as neoadjuvant treatment for HER2-positive breast cancer.

The primary objective is to evaluate the activity of the regimen in terms of rate of pCR, defined as absence of evidence of residual invasive cancer in the breast and axillary lymph nodes (ypT0/is ypN0). The secondary objectives are the safety of the regimen, with particular attention to cardiac safety, and other activity endpoints like the clinical response rate and the rate of conservative surgery. The clinical response rate was assessed by means of breast ultrasounds according to RECIST 1.1. Follow up is ongoing to collect data on disease-free and overall survival, but these are not complete at the time of writing and will be presented separately.

The trial was approved by the ethics committee at each participating center (the list of the international review boards that approved the study are reported in a supplementary file) and was conducted in accordance with the Declaration of Helsinki and good clinical practice norms. All patients signed a written informed consent before joining the study. The trial is listed on Clinicaltrials.gov [ClinicalTrials.gov identifier: NCT02488564] and European Clinical Trials Database [EudraCT No. 2014-002602-20].

Treatment and assessments. Patients received six 21-day treatment cycles consisting of: non-pegylated liposomal doxorubicin 50 mg/m^2 intravenously over 1 h on day 1, docetaxel 30 mg/m^2

intravenously over 1 h on days 2 and 9; trastuzumab 2 mg/kg/week intravenously on days 2, 9, and 16 (with 4 mg/kg loading dose at day 2 of the first cycle). Metformin was continuously administered orally, starting 14 days prior to the beginning of the first chemotherapy cycle. Its starting dosage was 1000 mg once a day, increased to twice a day after 3 days since the beginning of therapy.

The administration of docetaxel in two divided doses on days 2 and 9 of each cycle, aimed at decreasing risk for toxicity, allowed for the reduction and in some cases the omission of the cycle's second dose based on symptoms and bloodwork.

The administration of chemotherapy drugs required a minimum absolute neutrophil count (ANC) of $\geq 1.5 \times 10^9$/l and platelets $\geq 100 \times 10^9$/l on days 1 and 2 of each cycle; in case of failure to meet those values, the treatment was to be postponed by a week. Day 9 docetaxel was reduced at 75% of the original dose in case of ANC between 1.0 and 1.5×10^9/l or platelets between 75 and 100×10^9/l. It was not administered altogether if those values were lower. A day 1 grade 2 or higher non-hematological toxicity required postponement of the treatment. On day 9, docetaxel dose was lowered to 75% of the original if toxicity grade rose to 2, and completely omitted if grade rose above 2. Primary granulocyte-colony-stimulating factor (G-CSF) prophylaxis was not required by protocol, as it had not been used in the original regimen developed in metastatic breast cancer. Secondary G-CSF prophylaxis was allowed according to clinical practice guidelines.

Baseline evaluation included breast tumor assessment with mammography and ultrasound, staging with chest X-ray, plus abdomen and pelvis ultrasound (or whole-body computerized tomography in cT4 or N2 cases) and bone scan, cardiac consultation with electrocardiogram and echocardiogram, physical examination, anthropometric evaluations, and blood tests. Breast ultrasound was repeated after two and six cycles while echocardiography was carried out every two cycles. The circulating biomarkers that were assessed periodically during treatment are glucose, lipids, hormones (insulin, C-peptide, cortisol), the inflammatory markers C-reactive protein and erythrocyte sedimentation rate, as well as cardiac markers such as high-sensitivity troponin-T (hs-TnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP).

In a subgroup of patients, all enrolled at one of the participating centers (IRST IRCCS), 3-deoxy-3-18F-fluorothymidine positron emission tomography (FLT-PET) was performed at baseline and repeated after one cycle, to study its predictive value for response to therapy.

After surgery, patients are required to undergo follow-up visits every 6 months for the first 5 years and then annually up to the 10th year. Echocardiography (or MUGA scan) and blood measurements of hs-TnT and NT-proBNP were done every 3 months during adjuvant trastuzumab treatment and are then planned annually until 5 years after surgery. Mammography and breast ultrasounds are performed annually.

Statistical analysis

This phase II clinical trial followed a Simon's two-stage optimal design, with an unremarkable rate of pCR of 0.3 and a desirable rate of pCR of 0.5. For $\alpha = 0.1$ and $1 - \beta = 0.9$, 22 patients had to be accrued in stage I, and if less than 8 pCRs had been discovered the study would have had to be shut down due to futility. If 8 or more pCRs had been discovered, an additional 24 patients would have had to be accrued for stage II, resulting in a total sample size of 46 patients. If 18 or more pCRs were observed among the 46 patients, the treatment would finally have been deemed promising. The expected trial sample size for a 0.3 true pCR rate is 30 patients, while the chance of early termination for a 0.3 true pCR rate is 0.67. We set both α and β values to 0.1 because in phase II trials false-negative results, leading to interruption of the development of a useful agent, are potentially as worrying as false-positive results, that lead to prolonging the study of an inactive drug.[29] The rate of patients lost to follow up was not formally considered for sample size calculation, but two extra patients have been enrolled (see Figure 1).

Continuous variables were summarized by medians and ranges. Normality was checked by the Shapiro–Wilk test and by inspection of density as well as quartile–quartile (q–q) plots. Comparisons between groups were made using a *t*-test or a Wilcoxon rank sum test, as appropriate. Binary variables were summarized by proportions and binomial exact CIs and compared between groups using the chi-squared test, with continuity correction or the Fisher's exact test, as appropriate. Factors predicting for pCR were assessed by

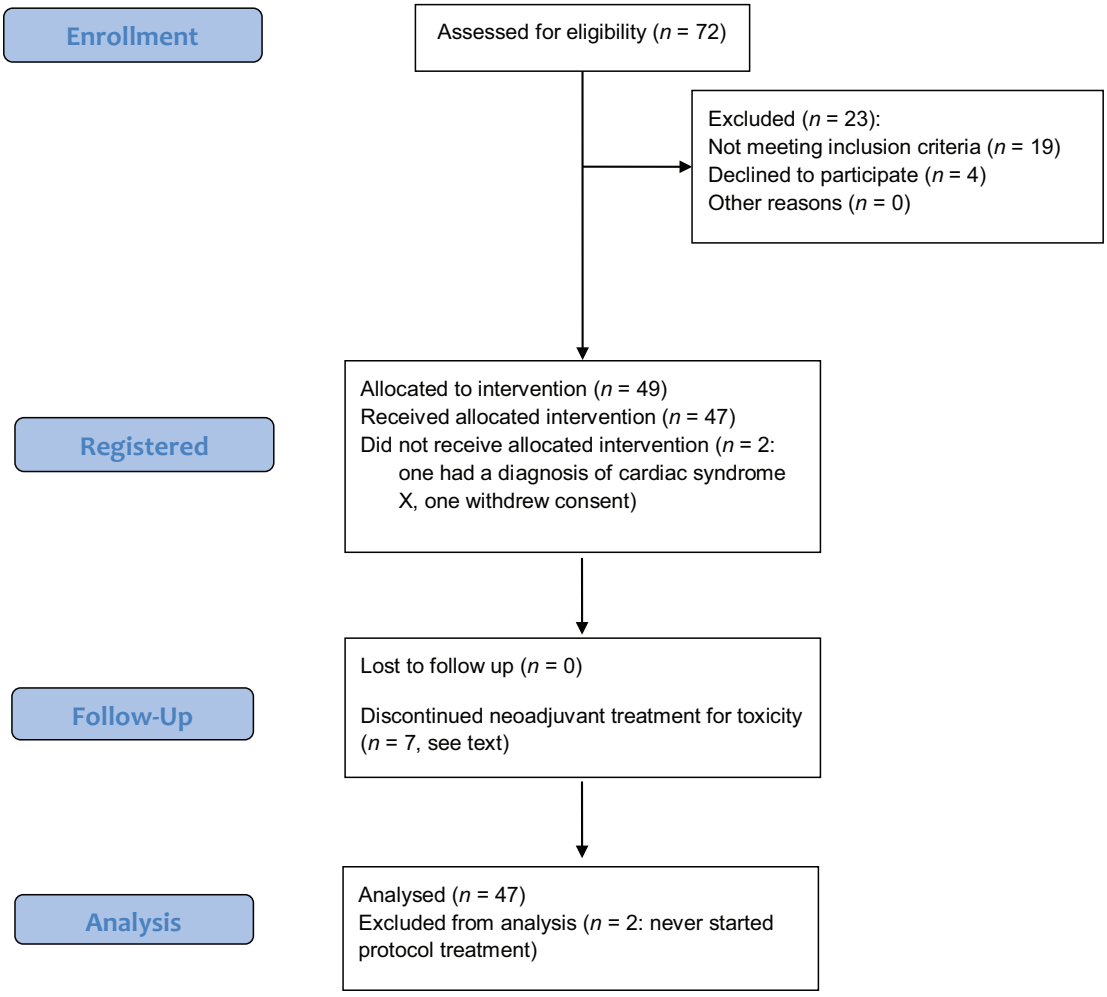

Figure 1. Study flow diagram.

means of logistic regression analysis; for continuous predictors, the linear relationship between logit and predictor values was assessed by plot inspection.

As an exploratory analysis, receiver operating characteristic (ROC) curves were constructed to assess the accuracy of FLT-PET maximum standardized uptake value (SUVmax) after one treatment cycle and of the SUVmax ratio between baseline FLT-PET and FLT-PET performed after one cycle, as predictors of pCR, with the goal of identifying the SUVmax cut-off point that maximizes the sum of sensitivity and specificity or the overall accuracy by bootstrapping estimates.

LVEF temporal trends were studied by means of mixed-effects models[30] for repeated measures, with patients as random effects and baseline covariates as fixed effects. Log transformation of LVEF values was adopted to improve normality and homoscedasticity, as shown by residuals plots and q–q plots. Significance of single fixed effects was estimated by comparing restricted maximum likelihood models with and without the fixed effect of interest, using the Kenward–Roger approximation to calculate degrees of freedom of F tests of the nested models (KRmodcomp function from the *R* pbkrtest package).[31] The likelihood-ratio test was used to compare simple linear models with mixed-effects models, which were fitted with maximum likelihood estimates, in order to test whether any random effect was warranted. To gauge the significance of specific random effects, models estimated by restricted maximum likelihood and differing for the random effect of interest were compared using type I analysis of variance and Akaike's information criterion. Analogous models were built to describe hs-TnT temporal trends.

All analyses were two-sided, with $p < 0.05$ considered significant. All analyses were conducted with R version 4.0.0 (R Core Team 2020).[32]

Results

From October 2014 to April 2018, 49 patients were registered in the study (Figure 1). Two started neither chemotherapy and trastuzumab, nor metformin (one was diagnosed with cardiac syndrome X and excluded from the protocol, and the other withdrew consent). Forty-seven patients started all drugs and were considered evaluable for activity and safety. The last two patients underwent the study screening at the same time and were both enrolled despite the planned number of patients being 46.

Baseline patient and tumor characteristics are reported in Table 1. The median age was 52 years (ranging from 31 to 73 years of age); 87% of the patients presented with infiltrating carcinoma of non-special type, 79% had stage II tumors and 21% had stage III tumors. A total of 55% showed clinical nodal involvement. Estrogen receptors were positive in 68% of the cases, and Ki67 was ⩾20% in 87% of the cases.

Baseline LVEF was ⩾55% in all patients. All 42 patients with cardiac markers available had a baseline NT-proBNP within the normal range, while 10 patients (24%) had an hs-TnT value ⩾10 ng/l (women's 99th percentile). The cardiac risk score[33] was ⩽60, corresponding to a 5-year cumulative probability of cardiac event of approximately ⩽4%, in 62% of the patients.

Forty patients underwent the complete course of neoadjuvant treatment. Of the remaining seven patients, four interrupted treatment, respectively, after one, two, three, and four treatment cycles, and three interrupted treatment after the fifth cycle, due to toxicity. All 47 evaluable patients underwent surgery after the neoadjuvant treatment.

The main study results are summarized in Table 2. Among 41 patients with measurable primary tumor, 18 (44%) achieved a complete clinical response, 14 (34%) a partial response (decrease in main diameter ⩾30%), and 9 (22%) had stable disease, for an overall objective response rate of 78.0% (95% CI 62.4–89.4%). Among all 47 patients who underwent surgery, 22 had a pCR at the primary tumor level (ypT0/is, any N; 46.8%, 95% CI 32.1–61.9%), and 18 had pCR both in the breast and in the axillary lymph nodes (ypT0/is ypN0, main endpoint; 38.3%, 95% CI 24.5–53.6%). Ki67 in the residual tumor was <20% in more than 50% of the cases, and HER2 was negative in three cases.

At univariate logistic regression (Table 3), age and clinical tumor and nodal classifications were not predictive of pathological complete response (ypT0/is ypN0). Negative estrogen-receptor status increased by 2.5 times the odds of achieving a pCR; this was not statistically significant ($p = 0.15$) due to the small sample size. Similar results were found for progestin receptors. A high Ki67 value (⩾20%) increased by over seven times the odds of achieving pCR ($p = 0.065$). Grade 3 was positively associated with pCR (Fisher's exact test $p = 0.03$), with no pCR obtained in patients with grade 2 tumors (preventing an estimate by logistic model).

In an exploratory *a priori* planned analysis conducted on 15 patients who were consecutively enrolled at the Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, FLT-PET SUVmax was computed on primary BC breast cancer) both at baseline (bFLT-SUVmax) and after the first treatment cycle (eFLT-SUVmax). The best prediction of response was provided by the value of eFLT-SUVmax: a decrease of one unit of eFLT-SUVmax increased by 71% the odds of achieving a pCR in the breast (ypT0/is, any N; $p = 0.11$), with slightly worse performance in predicting pCR in both breast and axilla. An ROC curve for eFLT-SUVmax as pCR predictor yielded an area under the curve (AUC) of 73.2%, with the best overall accuracy cut-off represented by an eFLT-SUVmax equal to 1.6.

The ratio computed between bSUVmax and eSUVmax was also informative (as was the SUVmax percent decrease, data not shown): bSUVmax/eSUVmax ratio (be-FLT-SUR) higher than 1.0 increased by 2.5 times the odds of pCR in the breast. An ROC curve for be-FLT-SUR yielded an AUC of 75%, with optimal overall accuracy cut-off represented by be-FLT-SUR of 2.5.

There was no association between bFLT-SUVmax and Ki67 measured on baseline breast tumor biopsy (Spearman correlation coefficient 0.034, $p = 0.9$) and these two variables tended to affect the likelihood of achieving a pCR in opposite directions.

Table 1. Baseline patient and tumor characteristics on 47 evaluable patients.

Variable		
Age (median, range)	52	31–73
Histotype (*n*, %)		
Non-special type	41	87.2
Lobular	3	6.4
Other	3	6.4
Tumor classification (*n*, %)		
T1	6	12.8
T2	31	66.0
T3	6	12.8
T4	4	8.5
Nodal classification (*n*, %)		
N0	19	40.4
N1	26	55.3
N2	1	2.1
N3	1	2.1
Grade (*n*, %)		
G1	0	0
G2	6	12.8 (19.4)
G3	25	53.2 (80.6)
Unknown	16	34.0 (–)
Stage (*n*, %)		
II	37	78.7
III	10	21.3
Estrogen receptor (*n*, %)		
Positive (⩾1%)	32	68.1
Negative (<1%)	15	31.9
Progesterone receptor (*n*, %)		
Positive (⩾1%)	29	61.7
Negative (<1%)	17	36.2
NA	1	2.1

(Continued)

Table 1. (Continued)

Variable		
Ki67 (*n*, %)		
<20%	6	12.8
⩾20%	41	87.2
Comorbidities (*n*, %)		
Yes	29	61.7
No	18	38.3
Baseline LVEF (% points; *n*, %)		
⩾55 and ⩽60	15	31.9
>60 and ⩽70	23	48.9
>70	9	19.1
Cardiac risk score (*n*, %)		
⩽40	9	19.1
>40 and ⩽60	20	42.6
>60 and ⩽80	16	34.0
>80 and ⩽100	2	4.3
hs-TnT		
<10 ng/l	32	68.1
⩾10 ng/l	10	21.3
NA	5	10.6
NT-proBNP		
<450 ng/l	41	87.2
450–900 ng/l	1	2.1
>900 ng/l	0	0
NA	5	10.6

hs-TnT, high-sensitivity troponin T; LVEF, left-ventricular ejection fraction; NA, not available; NT-proBNP, N-terminal pro brain-type natriuretic peptide.

Toxicity results are reported in Table 4. Most side effects were those to be expected from the cytotoxic agents involved. About 70% of the patients had grade 3 or 4 neutropenia, and 4% experienced febrile neutropenia. Anemia was mild or moderate in 34% of cases, and one patient developed grade 3 anemia. Thrombocytopenia was limited to grades 1–2 and presented itself in 6% of the patients. Mild or moderate nausea and vomiting were registered,

Table 2. Main study results.

FLT-PET SUVmax T (median, range)*		
Baseline	3.54	1.58–9.11
After 1 cycle	1.49	0.59–5.72
Clinical response on T (*n*, %)		
CR	18	38.3
PR	14	29.8
SD	9	19.1
NA	6	12.8
ORR (CR + PR)	32	68.1
Surgery (*n*, %)		
Conservative	25	53.2
Mastectomy	22	46.8
Pathological response (*n*, %)		
ypT0-is (any N)	22	46.8
ypT1-2	25	53.2
ypN0 (any T)	30	63.8
ypN+	17	36.2
pCR (ypT0-is ypN0)	18	38.3
no pCR	29	61.7
Estrogen receptor (*n*, %)**		
Positive (⩾1%)	19	76.0
Negative (<1%)	4	16.0
NA	2	8.0
Progesterone receptor (*n*, %)**		
Positive (⩾1%)	14	56.0
Negative (<1%)	10	40.0
NA	1	4.0
Ki67 (*n*, %)**		
<20%	13	52.0
⩾20%	10	40.0
NA	2	8.0
HER2 (*n*, %)**		
positive	20	80.0
negative	3	12.0
NA	2	8.0

*On 15 patients.
**On 25 patients with residual tumor.
CR, complete response; FLT-PET, 3-deoxy-3-18F-fluorothymidine positron emission tomography; HER2, human epidermal growth factor receptor 2; NA, not available; pCR, pathological complete response; ORR, overall response rate; PR, partial response; SD, stable disease; SUVmax, maximum standardized uptake value.

Table 3. Potential predictors of pCR (ypT0-is ypN0).

Univariate logistic regression analysis			
Variable	**OR**	**95% CI**	***p*****
Age (>50 *versus* ⩽50)	1.11	0.33–3.69	0.87
cT (3–4 *versus* 1–2)	1.21	0.32–4.60	0.78
cN (positive *versus* negative)	1.11	0.33–3.69	0.87
ER (negative *versus* positive)	2.51	0.71–8.86	0.15
PR (negative *versus* positive)	2.38	0.77–7.35	0.13
Ki67 (high *versus* low)	7.65	0.88–66.64	0.065
bFLT-SUVmax	0.70	0.40–1.23	0.22
bFLT-SUVmax*	0.67	0.37–1.18	0.17
eFLT-SUVmax	0.37	0.10–1.38	0.14
eFLT-SUVmax*	0.29	0.06–1.30	0.11
be-FLT-SUR	1.74	0.65–4.65	0.27
be-FLT-SUR*	2.55	0.69–9.38	0.16

*pCR on T (ypT0-is, any N).
***p* from Wald test.
95% CI, 95% confidence interval; be-FLT-SUR, bSUVmax/eSUVmax; bFLT-SUVmax, maximum standardized uptake value of FLT-PET at baseline; cN, clinical nodal classification; cT, clinical tumor classification; eFLT-SUVmax, maximum standardized uptake value of FLT-PET after cycle 1; ER, estrogen receptor status; FLT-PET, 3-deoxy-3-18F-fluorothymidine positron emission tomography; OR, odds ratio; PR, progesterone receptor status.

respectively, in 60% and 38% of the patients, while grades 3 were, respectively, limited to 4% and 2%. The incidence of diarrhea, of grade 1–2 in 72% of the patients and grade 3 in 6%, was certainly favored by the concomitant administration of metformin. Grade 3 diarrhea occurred mainly on day 9 of each chemotherapy cycles: it was found to be in relation to the recall of docetaxel in conjunction with the intake of metformin. Other prominent side effects were mucositis, asthenia, fatigue and decreased appetite.

Three patients definitely interrupted the neoadjuvant treatment because of an increase in transaminases (two grade 3 and one persistent grade 2), after one, three and five cycles respectively. Another four patients interrupted treatment respectively because of: grade 1 troponin-T increase (after two cycles), grade 3 febrile neutropenia and grade 2 diarrhea (after five cycles), worsening of grade 3 glaucoma (after five cycles), withdrawal of consent (after four cycles).

Forty-one patients completed the planned 12 administrations of adjuvant trastuzumab every 3 weeks, while six patients interrupted early: one did never start adjuvant trastuzumab due to troponin-T increase during neoadjuvant therapy, one withdrew study consent, one stopped after 3 months due to grade 2 LVEF decrease (from 67% to 50%), two stopped for grade 3 non-cardiac side effects (infection, transaminase increase), and one for disease progression.

Metformin was taken for a median of 150 days (range 21–206). Eight patients stopped metformin in advance for toxicity (diarrhea in four cases, nausea/dyspepsia in two, cardiotoxicity in two cases), while another two for personal decision.

Cardiac toxicity was assessed by measuring the LVEF by echocardiography (and in one patient with MUGA) every two cycles during neoadjuvant therapy, every 3 months during adjuvant trastuzumab, and yearly during follow up. At the same points in time, circulating hs-TnT and NT-proBNP were measured. Box-and-whisker plots of LVEF values by patient and by echocardiography times are depicted in Figure 2, and the

Table 4. Treatment-emergent adverse events are reported based on the maximum grade experienced by each patient; *n* = 47 patients).

	G1	G2	G3	G4
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
Blood disorders				
Leukopenia	2 (4.3)	3 (6.4)	11 (23.4)	3 (6.4)
Neutropenia		3 (6.4)	14 (29.8)	19 (40.4)
Febrile neutropenia				2 (4.3)
Leukocytosis		1 (2.1)		
Anemia	5 (10.6)	11 (23.4)	1 (2.1)	
Thrombocytopenia	2 (4.3)	1 (2.1)		
Cardiac disorders				
Left-ventricular systolic dysfunction	1 (2.1)	1 (2.1)		
Arrhythmia			1 (2.1)	
Palpitations	1 (2.1)	1 (2.1)		
Tachycardia	1 (2.1)			
Hypertension	1 (2.1)			
Hypotension	1 (2.1)			
Fainting	1 (2.1)			
Heart disease other		2 (4.3)		
Eye disorders				
Conjunctivitis	7 (14.9)	5 (10.6)		
Dry eye	1 (2.1)	2 (4.3)		
Glaucoma			1 (2.1)	
Gastrointestinal disorders				
Nausea	15 (31.9)	13 (27.7)	2 (4.3)	
Vomiting	14 (29.8)	4 (8.5)	1 (2.1)	
Diarrhea	14 (29.8)	20 (42.6)	3 (6.4)	
Mucositis	12 (25.5)	12 (25.5)	2 (4.3)	
Gastritis/gastric pain/dyspepsia	12 (25.5)	1 (2.1)		
Constipation	5 (10.6)			
Hemorrhoids	4 (8.5)	2 (4.3)		
General disorders/miscellanea				
Asthenia/Fatigue	17 (36.2)	8 (17.0)	2 (4.3)	

(Continued)

Table 4. (Continued)

	G1	G2	G3	G4
	n (%)	*n* (%)	*n* (%)	*n* (%)
Fever	17 (36.2)	4 (8.5)		
Pain (different sites)	7 (14.9)	3 (6.4)		
Hot flushes	1 (2.1)			
Allergic reaction	1 (2.1)			
Chills		2 (4.3)		
Edema	1 (2.1)			
Amenorrhea	1 (2.1)			
Dysuria	2 (4.3)			
Infections				
Infection (any site)	10 (21.3)	10 (21.3)	1 (2.1)	
Investigations				
Transaminase increased	8 (17.0)	2 (2.1)	3 (6.4)	
GGT increased	1 (2.1)			
Hyperbilirubinemia		1 (2.1)		
Troponin increased	1 (2.1)			
Creatinine increased		1 (2.1)		
C-reactive protein increased	1 (2.1)			
Metabolism and nutrition disorders				
Anorexia	5 (10.6)	1 (2.1)	2 (4.3)	
Weight loss	1 (2.1)	2 (4.3)		
Hypokalemia		1 (2.1)		
Hypomagnesemia	1 (2.1)			
Hyperuricemia	1 (2.1)			
Musculoskeletal disorders				
Neck stiffness	1 (2.1)			
Joint pain	1 (2.1)			
Rachis pain			1 (2.1)	
Neurological disorders				
Peripheral neuropathy	3 (6.4)			
Somnolence	1 (2.1)			

(Continued)

Table 4. (Continued)

	G1	G2	G3	G4
	n (%)	*n* (%)	*n* (%)	*n* (%)
Dysgeusia	3 (6.4)	2 (4.3)		
Syncope		1 (2.1)	1 (2.1)	
Psychiatric disorders				
Insomnia	3 (6.4)			
Anxiety	3 (6.4)			
Depression	1 (2.1)			
Respiratory disorders				
Cough	2 (4.3)	3 (6.4)		
Dyspnea	1 (2.1)	1 (2.1)		
Dysphonia	1 (2.1)			
Skin disorders				
Alopecia	3 (6.4)			
Dermatitis/erythema	7 (14.9)	5 (10.6)		
Pruritus	2 (4.3)			
Nail toxicity	4 (8.5)	2 (4.3)		
Vascular disorders				
Bleeding	3 (6.4)	1 (2.1)		
Deep vein thrombosis		3 (6.4)		
Superficial vein thrombosis	1 (2.1)			

GGT, gamma glutamyl transferase.

temporal trends of LVEF values by patient are reported in Figure 3. There were only two cases of grade 2 LVEF decrease: one led to permanent discontinuation of trastuzumab, despite subsequent LVEF recovery, while the other promptly recovered and did not require trastuzumab interruption.

In mixed-effects repeated-measures models of LVEF as a function of time (Table 5), considering time elapsed from the start of treatment (also a proxy for the number of treatment cycles) as fixed effect and patients as random effect, time never significantly affected LVEF, with beta coefficient (slope, exponentiation of coefficient of log-transformed LVEF) for time in months −0.001, $p = 0.14$. Adding random intercepts (estimating mean LVEF per patient), beyond being required by the repeated-measures design, significantly improved model quality compared with a simple linear regression model of LVEF as a function of time [Akaike information criterion (AIC) null model −512, AIC full model −542, $p < 0.0001$ by analysis of variance], while random slopes did not further improve the model ($p = 0.17$). When limiting the analysis to the neoadjuvant treatment period (first 6 months), results did not change substantially.

When single covariates were included in the model, baseline hs-TnT and NT-proBNP were significantly associated with LVEF, both when considered as continuous variables and when dichotomized as categorical variables, while age

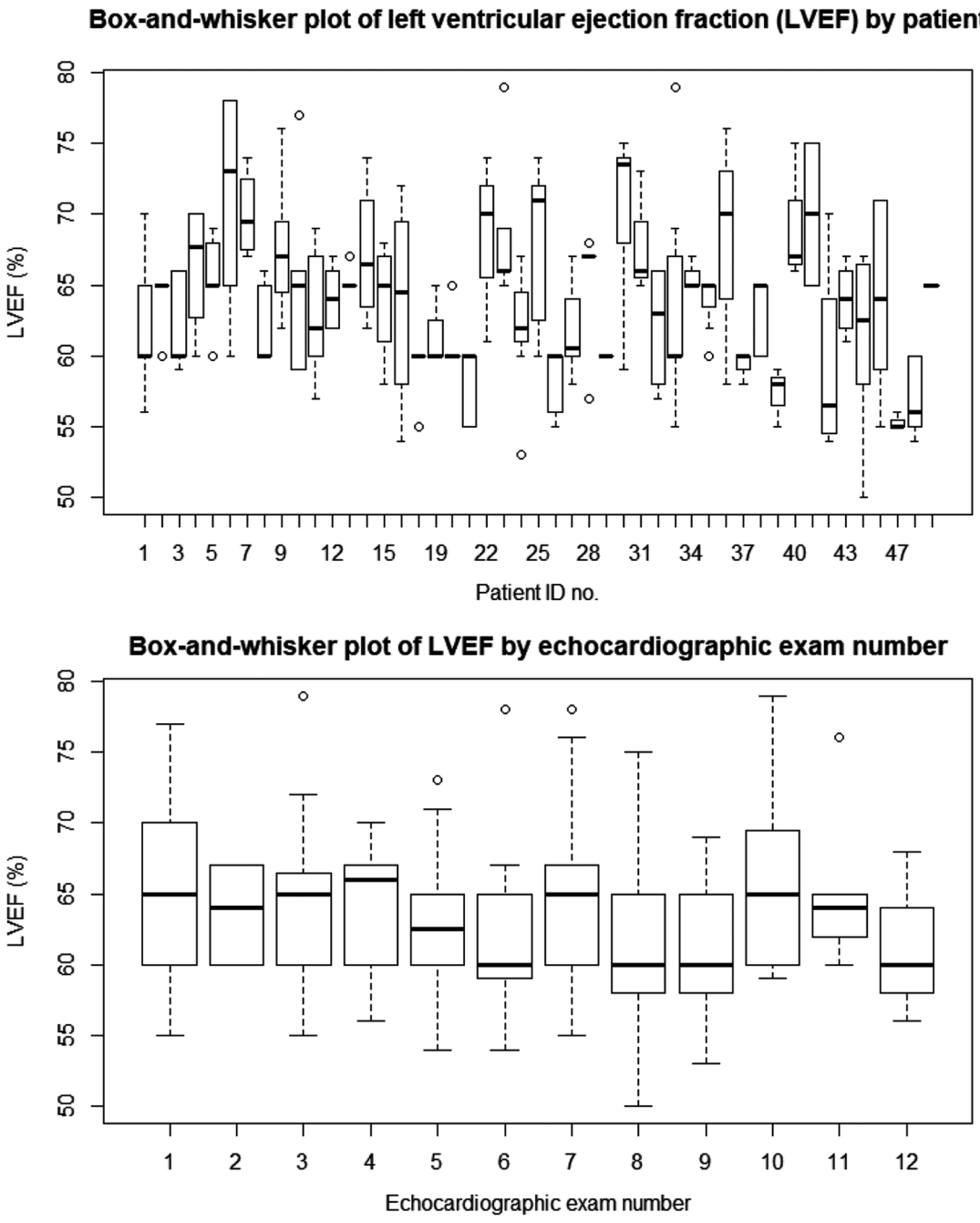

Figure 2. Left-ventricular ejection fraction (LVEF) by patient (49 patients were registered in the study, 47 of whom are evaluable) and by time point.
Boxes represent interquartile ranges [IQR, between upper (Q1) and lower (Q2) quartiles], with the median in bold in between; whiskers represent maximum and minimum values, dots represent outliers (values falling outside of the interval $Q1 - 1.5 \times IQR, Q3 + 1.5 \times IQR$).

and body mass index were not. In multivariate models, both baseline hs-TnT and NT-proBNP remained significantly associated with LVEF when considered as continuous variables, whereas only hs-TnT remained significant when the variables were dichotomized (data not shown). Interactions among variables were never significant (data not shown).

Table 5. Repeated measures mixed-effects models of LVEF.

Testing significance of random effects (on 47 patients)				
Model	**AIC**	**Comparison**	**χ^2 (d.f.)****	***p***
(a) ln(LVEF) ~ time	−512.3			
(b) ln(LVEF) ~ time + pt_int	−521.96	b *versus* a*	31.166 (1)	<0.0001
(c) ln(LVEF) ~ time + pt_int_slo	−521.49	c *versus* b	3.53 (2)	0.17
Testing significance of fixed effects (on 40 patients with all baseline covariates available)				
Model	**AIC**	**Comparison**	**F (df)*****	*p*
(a) ln(LVEF) ~ time + pt_int	−471.97			
(b) ln(LVEF) ~ time + pt_int + hs-TnT	−480.56	b *versus* a	11.38 (1)	0.002
(c) ln(LVEF) ~ time + pt_int + NT-proBNP	−476.98	c *versus* a	7.28 (1)	0.011
(d) ln(LVEF) ~ time + pt_int + age	−472.69	d *versus* a	2.68 (1)	0.110
(e) ln(LVEF) ~ time + pt_int + BMI	−469.98	e *versus* a	0.01 (1)	0.917
(f) ln(LVEF) ~ time + pt_int + hs-TnT + NT-proBNP	−487.88	f *versus* b	9.75 (1)	0.004

Models were estimated by restricted maximum likelihood, apart from model (a).
All covariates are considered as continuous variables.
*Comparison b *versus* a is based on maximum likelihood model estimates [AIC model (b) −542.47 *versus* model (a) −512.3].
**From likelihood-ratio tests.
***Kenward–Roger approximation to calculate degrees of freedom of F tests of the nested models.
AIC, Akaike information criterion; BMI, body mass index; d.f., degrees of freedom; hs-TnT, high-sensitivity troponin T; LVEF, left-ventricular ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; pt_int, patient random intercept; pt_int_slo, patient random intercept and slope.

In mixed-effects repeated-measure models of hs-TnT in function of time, considering time as fixed effect and patients as random effects, hs-TnT significantly increased over time during the neoadjuvant treatment period (Figure 4), with beta coefficient (slope, exponentiation of coefficient of log-transformed hs-TnT) 0.16 ($p < 0.001$). During the adjuvant part of treatment, hs-TnT showed a decreasing trend, such that the whole pattern during the neoadjuvant and adjuvant period was better fitted by a quadratic model. Although there was a negative correlation between LVEF and hs-TnT (Pearson correlation coefficient −0.26, $p = 0.002$), there was no clear hs-TnT cut-off predicting a grade ⩾2 LVEF decline. One patient interrupted neoadjuvant treatment (both chemotherapy and trastuzumab, undergoing surgery) after two cycles because of an increase in troponin-T, which subsequently returned to the baseline value of 10 ng/ml.

NT-proBNP almost never increased over the upper normal limit, and its temporal trends were therefore not modeled.

Discussion

Our study assessed the activity and safety of a neoadjuvant regimen for HER2-positive, early, and locally advanced breast cancer, with a strategy involving the combined administration of anthracycline and taxane, concurrently with trastuzumab and metformin. The aim was to improve the activity over single-blockade, trastuzumab-based sequential regimens, by exploiting the concomitant administration of trastuzumab and anthracycline, while restraining cardiac toxicity by using liposomal doxorubicin. Metformin was added, based on preclinical and observational clinical data of activity in HER2-positive breast cancer.[24–27]

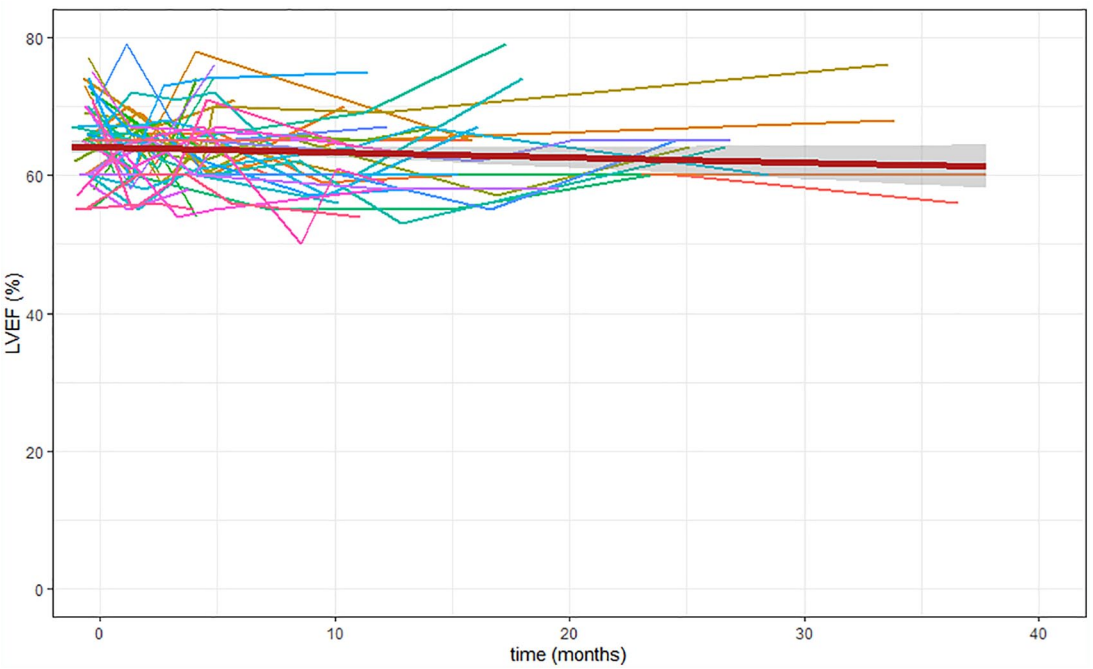

Figure 3. LVEF temporal trends by patient, with superimposed linear model fit.
LVEF, left-ventricular ejection fraction.

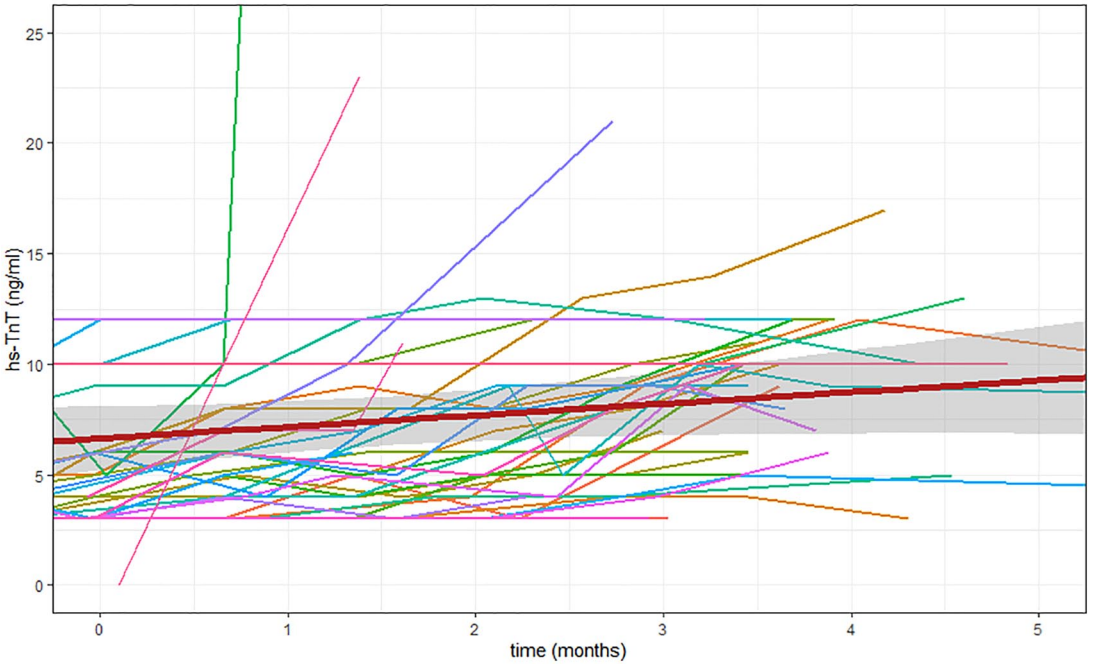

Figure 4. High-sensitivity troponin-T (hs-TnT) temporal trends by patient during the neoadjuvant treatment, with superimposed linear model fit.

We obtained a pCR rate (ypT0/is ypN0) of about 38%, which falls within the range of pCR rates reported in the literature with single HER2-blockade,[34] although there are no hints of improvement over more classical single-blockade regimens, that can achieve a pCR rate of up to about 52% in a real-world scenario.[35]

Toxicity was acceptable. In particular, the regimen showed good cardiac safety with only two cases of grade 2 LVEF decrease, both of which recovered, and one case of sharp troponin-T increase, which returned to baseline values after stopping therapy. The cardiac risk score was developed to estimate the cumulative probability of cardiac events up to 5 years in patients who started trastuzumab plus paclitaxel after four cycles of doxorubicin plus cyclophosphamide in the NSABP B-31 trial.[33] Although the cardiac risk score was >60, indicative of a 5-year cumulative probability of cardiac event approximately >4%, in 38% of our patients at baseline, there were no cases of symptomatic cardiac failure or other major cardiac events during the 1-year treatment and the follow-up assessments.

Metformin has been tested in several contexts in the breast cancer field. The effects of single-agent metformin on breast cancer proliferation, measured by Ki67, have been studied in some window of opportunity presurgical trials. Some have shown an overall decrease in Ki67,[36] while others have seen no overall changes but a modification of metformin effect according to measures of insulin resistance such as the homeostasis model assessment (HOMA) index, with decrease in Ki67 in women with HOMA >2.8 and increase in women with HOMA ⩽2.8.[37] A significant reduction in Ki67 was reported specifically in HER2-positive tumors.[38] The impact of HOMA index and other metabolic parameters on pCR in our study will be presented in a subsequent work.

Recently, the neoadjuvant randomized phase II METTEN trial reported results on the addition of metformin to a single-blockade neoadjuvant regimen for HER2-positive breast cancer. Patients were randomized to weekly paclitaxel followed by four cycles of FEC, all given concurrently with trastuzumab, either with or without metformin. There was no significant improvement in the pCR rates with the addition of metformin.[39] The study closed in advance due to slow accrual, resulting in it being underpowered to compare pCR rates between the two groups. Nonetheless, a pCR rate of 65.5% was reported for the metformin arm, higher than our finding. It must be underlined that we considered assessable for activity all patients who started treatment, although some of them interrupted after one or a few cycles. Moreover, two thirds of our patients had HR-positive disease, a condition known to reduce the probability of achieving a pCR.

Apart from the neoadjuvant METTEN study,[39] further randomized trials did not show any improvement in efficacy when metformin was added to chemotherapy in non-diabetic patients with advanced (mainly HER2-negative) breast cancer.[40,41] The same trials reported a reduced incidence of some severe side effects in the metformin arms. Both our and the METTEN study showed a contained cardiac toxicity despite the concomitant administration of trastuzumab and anthracyclines, which could partly result from a cardioprotective effect of metformin.[42] As adiposity leads to insulin resistance, hyperinsulinemia, and higher levels of IGF1, which increase breast cancer proliferation *via* activation of the PI3K-Akt-mTOR pathway, metformin has been studied in combination with exemestane and everolimus specifically in overweight and obese patients with HR-positive, HER2-negative metastatic breast cancer, showing a moderate clinical activity.

The *in vivo* mechanisms of antitumor action of metformin are still debated. While indirect, insulin- and glucose-mediated effects are supported by the differential effects according to levels of HOMA index and other conditions indicative of insulin resistance,[37] a direct effect with significant upregulation of phospho-AMPK and downregulation of phospho-Akt has been reported in a presurgical trial.[43]

The FLT-PET sub-study conducted on 15 patients found that both a low SUVmax after one cycle of therapy and a high ratio between bFLT-SUVmax and eFLT-SUVmax returned moderately accurate cut-offs for predicting pCR of primary breast cancer (eFLT-SUVmax AUC 73.2%, with overall accuracy cut-off ⩽1.6; beFLT-SUR AUC 75%, with overall accuracy cut-off ⩾2.5). No association was found between bFLT-SUVmax and Ki67, these two variables having opposing impact on the likelihood of achieving a pCR, with high Ki67 (and high grade) primary breast cancer and low bFLT-SUVmax most likely to achieve pCR (although only histological grade reached statistical significance in our

study). Our data are in line with what is reported in the literature: in a pilot study on patients with metastatic breast cancer Kenny *et al.* showed that a reduction in FLT uptake in primary and metastatic lesions after the first cycle of chemotherapy is significantly correlated with clinical response, precedes changes in tumor size and is able to discriminate between clinical response and stable disease.[44] Similar results were reported by Crippa *et al.* in a prospective study on 15 patients receiving neoadjuvant chemotherapy for locally advanced breast cancer, in which the variation of SUV between the basal study and the one obtained after one cycle of therapy significantly predicted the pathological response at the level of the primary tumor but not at the lymph node level.[45] Unlike what we found, these authors reported a significant correlation between SUVmax and Ki67 proliferation rate ($r=0.69$, $p<0.001$). Although this correlation is expected, thymidine is incorporated into deoxyribonucleic acid (DNA) during the S phase (DNA synthesis) of cell cycle, while Ki67 is expressed in all phases of the cell cycle (G1, S, G2, M), and this could explain some discordance between the two parameters. The above results need confirmation in larger and more homogeneous cohorts.

Our study has limitations, due to its single-arm design and the concomitant investigation of a non-standard chemotherapy regimen (including liposomal doxorubicin, a drug not currently licensed for use in the neoadjuvant setting) and of its combination with metformin. It is therefore impossible to ascertain the respective contribution of each single drug to the overall performance of the treatment. Nonetheless, our results show that the concomitant administration of trastuzumab and liposomal doxorubicin, as well as their association with metformin, appear safe and with acceptable activity.

Based on our and others' study results, metformin does not appear useful as a 'one size fits all' drug in HER2-positive breast cancer. Nonetheless, some patients could perhaps benefit from its use. In the METTEN study, a significant interaction was found between the presence of the C allele of the single-nucleotide polymorphism rs11212617 (known to be associated with response to metformin in type 2 diabetes) and treatment arm (with and without metformin): patients harboring the C allele achieved a higher rate of pCR with metformin than without metformin; patients with no C allele showed the same rate of pCR independently of metformin.[46] These and other biomarkers, like tumor-associated alterations in the cellular signaling pathways targeted by metformin, for example, AMPK and PI3k-Akt-mTOR, could help identify patients who might benefit from this drug.

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Conflict of interest statement

Andrea Rocca: personal fees for advisory boards from Pfizer, Novartis and Lilly and congress, travel, and accommodation fees from Roche.

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Lorenzo Gianni: travel and accommodations expenses from Roche.

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Supplemental material

Supplemental material for this article is available online.

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Harbeck N. Emerging strategies in neoadjuvant treatment of patients with HER2-positive early breast cancer. *Breast* 2019; 48: S97–S102.
2. Gianni L, Pienkowski T, Im Y, *et al.* 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. *Lancet Oncol* 2016; 17: 791–800.
3. Schneeweiss A, Chia S, Hickish T, *et al.* Long-term efficacy analysis of the randomised, phase II

TRYPHAENA cardiac safety study: evaluating pertuzumab and trastuzumab plus standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer. *Eur J Cancer* 2018; 89: 27–35.

4. Piccart-Gebhart MJ, Burzykowski T, Buyse M, *et al.* Taxanes alone or in combination with anthracyclines as first-line therapy of patients with metastatic breast cancer. *J Clin Oncol* 2008; 26: 1980–1986.
5. Eiermann W, Pienkowski T, Crown J, *et al.* Phase III study of doxorubicin/cyclophosphamide with concomitant versus sequential docetaxel as adjuvant treatment in patients with human epidermal growth factor receptor 2-normal, node-positive breast cancer: BCIRG-005 trial. *J Clin Oncol* 2011; 29: 3877–3884.
6. Vriens BEPJ, Aarts MJB, De Vries B, *et al.* Doxorubicin/cyclophosphamide with concurrent versus sequential docetaxel as neoadjuvant treatment in patients with breast cancer. *Eur J Cancer* 2013; 49: 3102–3110.
7. Pegram MD, Konecny GE, O'Callaghan C, *et al.* Rational combinations of trastuzumab with chemotherapeutic drugs used in the treatment of breast cancer. *J Natl Cancer Inst* 2004; 96: 739–749.
8. Baselga J, Norton L, Albanell J, *et al.* Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res* 1998; 58: 2825–2831.
9. Slamon DJ, Leyland-Jones B, Shak S, *et al.* Use of chemotherapy plus a monoclonal antibody against HER2. *N Engl J Med* 2001; 344: 783–792.
10. Buzdar AU, Valero V, Ibrahim NK, *et al.* Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. *Clin Cancer Res* 2007; 13: 228–233.
11. Buzdar AU, Suman VJ, Meric-Bernstam F, *et al.* Fluorouracil, epirubicin, and cyclophosphamide (FEC-75) followed by paclitaxel plus trastuzumab versus paclitaxel plus trastuzumab followed by FEC-75 plus trastuzumab as neoadjuvant treatment for patients with HER2-positive breast cancer (Z1041): a random. *Lancet Oncol* 2013; 14: 1317–1325.
12. Wu YT, Xu Z, Zhang K, *et al.* Efficacy and cardiac safety of the concurrent use of trastuzumab and anthracycline-based neoadjuvant chemotherapy for HER2-positive breast cancer: a systematic review and meta-analysis. *Ther Clin Risk Manag* 2018; 14: 1789–1797.
13. Batist BG, Ramakrishnan G, Rao CS, *et al.* Reduced cardiotoxicity and preserved antitumor eiicacy of liposome-encapsulated doxorubicin and multicenter trial of metastatic breast cancer. *J Clin Oncol* 2001; 19: 1444–1454.
14. Harris L, Batist G, Belt R, *et al.* Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. *Cancer* 2002; 94: 25–36.
15. Van Dalen EC, Michiels EM, Caron HN, *et al.* Different anthracycline derivates for reducing cardiotoxicity in cancer. *Cochrane Database Syst Rev* 2010; 3: CD005006.
16. Chan S, Davidson N, Juozaityte E, *et al.* Phase III trial of liposomal doxorubicin and cyclophosphamide compared with epirubicin and cyclophosphamide as first-line therapy for metastatic breast cancer. *Ann Oncol* 2004; 15: 1527–1534.
17. Amadori D, Milandri C, Comella G, *et al.* A phase I/II trial of non-pegylated liposomal doxorubicin, docetaxel and trastuzumab as first-line treatment in HER-2-positive locally advanced or metastatic breast cancer. *Eur J Cancer* 2011; 47: 2091–2098.
18. Venturini M, Bighin C, Puglisi F, *et al.* A multicentre phase II study of non-pegylated liposomal doxorubicin in combination with trastuzumab and docetaxel as first-line therapy in metastatic breast cancer. *Breast* 2010; 19: 333–338.
19. Vander Heiden MG and DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. *Cell* 2017; 168: 657–669.
20. Zhao XB and Ren GS. Diabetes mellitus and prognosis in women with breast cancer: a systematic review and meta-analysis. *Med (United States)* 2016; 95: e5602.
21. Sonnenblick A, Agbor-Tarh D, Bradbury I, *et al.* Impact of diabetes, insulin, and metformin use on the outcome of patients with human epidermal growth factor receptor 2-positive primary breast cancer: analysis from the ALTTO phase III randomized trial. *J Clin Oncol* 2017; 35: 1421–1429.
22. Yee LD, Mortimer JE, Natarajan R, *et al.* Metabolic health, insulin, and breast cancer:

why oncologists should care about insulin. *Front Endocrinol (Lausanne)* 2020; 11: 1–25.

23. Hopkins BD, Goncalves MD and Cantley LC. Insulin–PI3K signalling: an evolutionarily insulated metabolic driver of cancer. *Nat Rev Endocrinol*. Epub ahead of print 3 March 2020. DOI: 10.1038/s41574-020-0329-9.

24. Jiralerspong S, Palla SL, Giordano SH, *et al.* Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol* 2009; 27: 3297–3302.

25. Vazquez-Martin A, Oliveras-Ferraros C and Menendez JA. The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. *Cell Cycle* 2009; 8: 88–96.

26. Liu B, Fan Z, Edgerton SM, *et al.* Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of ErbB2/IGF-1 receptor interactions. *Cell Cycle* 2011; 10: 2959–2966.

27. Cufí S, Corominas-Faja B, Vazquez-Martin A, *et al.* Metformin-induced preferential killing of breast cancer initiating CD44+CD24-/low cells is sufficient to overcome primary resistance to trastuzumab in HER2+ human breast cancer xenografts. *Oncotarget* 2012; 3: 395–398.

28. Vancura A, Bu P, Bhagwat M, *et al.* Metformin as an anticancer agent. *Trends Pharmacol Sci* 2018; 39: 867–878.

29. Simon R. Design and analysis of clinical trials. In: DeVita VT, Lawrence TS and Rosenberg SA (eds) *Cancer: principles and practice of oncology*. Philadelphia: Lippincott Raven, 2011, pp. 705–722.

30. Bates D, Mächler M, Bolker BM, *et al.* Fitting linear mixed-effects models using lme4. *J Stat Softw*. Epub ahead of print 7 October 2015. DOI: 10.18637/jss.v067.i01.

31. Halekoh U and Højsgaard S. A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models-the R package pbkrtest. *J Stat Softw* 2014; 59: 1–32.

32. R: The R Project for Statistical Computing, https://www.r-project.org/ (accessed 15 July 2020).

33. Romond EH, Jeong JH, Rastogi P, *et al.* Seven-year follow-up assessment of cardiac function in NSABP B-31, a randomized trial comparing doxorubicin and cyclophosphamide followed by paclitaxel (ACP) with ACP plus trastuzumab as adjuvant therapy for patients with node-positive, human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol* 2012; 30: 3792–3799.

34. Dent S, Oyan B, Honig A, *et al.* HER2-targeted therapy in breast cancer: a systematic review of neoadjuvant trials. *Cancer Treat Rev* 2013; 39: 622–631.

35. Battisti NML, True V, Chaabouni N, *et al.* Pathological complete response to neoadjuvant systemic therapy in 789 early and locally advanced breast cancer patients: the Royal Marsden experience. *Breast Cancer Res Treat* 2020; 179: 101–111.

36. Niraula S, Dowling RJO, Ennis M, *et al.* Metformin in early breast cancer: a prospective window of opportunity neoadjuvant study. *Breast Cancer Res Treat* 2012; 135: 821–830.

37. Bonanni B, Puntoni M, Cazzaniga M, *et al.* Dual effect of metformin on breast cancer proliferation in a randomized presurgical trial. *J Clin Oncol* 2012; 30: 2593–2600.

38. DeCensi A, Puntoni M, Gandini S, *et al.* Differential effects of metformin on breast cancer proliferation according to markers of insulin resistance and tumor subtype in a randomized presurgical trial. *Breast Cancer Res Treat* 2014; 148: 81–90.

39. Martin-Castillo B, Pernas S, Dorca J, *et al.* A phase 2 trial of neoadjuvant metformin in combination with trastuzumab and chemotherapy in women with early HER2-positive breast cancer: the METTEN study. *Oncotarget* 2018; 9: 35687–35704.

40. Nanni O, Amadori D, De Censi A, *et al.* Metformin plus chemotherapy versus chemotherapy alone in the first-line treatment of HER2-negative metastatic breast cancer. The MYME randomized, phase 2 clinical trial. *Breast Cancer Res Treat*. Epub ahead of print 7 December 2019. DOI: 10.1007/s10549-018-05070-2.

41. Pimentel I, Lohmann AE, Ennis M, *et al.* A phase II randomized clinical trial of the effect of metformin versus placebo on progression-free survival in women with metastatic breast cancer receiving standard chemotherapy. *Breast* 2019; 48: 17–23.

42. Varjabedian L, Bourji M, Pourafkari L, *et al.* Cardioprotection by metformin: beneficial effects beyond glucose reduction. *Am J Cardiovasc Drugs* 2018; 18: 181–193.

43. Hadad SM, Coates P, Jordan LB, *et al.* Evidence for biological effects of metformin in operable breast cancer: biomarker analysis in a pre-operative window of opportunity randomized trial. *Breast Cancer Res Treat* 2015; 150: 149–155.

44. Kenny L, Coombes RC, Vigushin DM, *et al.* Imaging early changes in proliferation at 1 week post chemotherapy: a pilot study in breast cancer patients with 3′-deoxy-3′-[^{18}F] fluorothymidine positron emission tomography. *Eur J Nucl Med Mol Imaging* 2007; 34: 1339–1347.

45. Crippa F, Agresti R, Sandri M, *et al.* ^{18}F-FLT PET/CT as an imaging tool for early prediction of pathological response in patients with locally advanced breast cancer treated with neoadjuvant chemotherapy: a pilot study. *Eur J Nucl Med Mol Imaging* 2015; 42: 818–830.

46. Cuyàs E, Buxó M, Iglesias MJF, *et al.* The C allele of ATM rs11212617 associates with higher pathological complete remission rate in breast cancer patients treated with neoadjuvant metformin. *Front Oncol* 2019; 9: 1–9.