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CLINICAL TRIAL

Efficacy and safety of dasatinib with trastuzumab and paclitaxel in first line HER2-positive metastatic breast cancer: results from the phase II GEICAM/2010-04 study

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Abstract

Background An important proportion of HER2-positive metastatic breast cancer patients do not respond to trastuzumab. The combination of dasatinib and trastuzumab has shown to be synergistic in preclinical models.

Methods We conducted a phase II trial combining dasatinib 100 mg once daily with trastuzumab 2 mg/kg and paclitaxel 80 mg/m^2 weekly. Primary objective was objective response rate (ORR) and secondary included safety, other efficacy parameters and pharmacodynamics in tumour tissue, blood samples and skin biopsies.

Results From June 2013 to December 2015, 29 patients were included. Median number of cycles was 12 (1–49). Only 6 patients discontinued due to adverse events. ORR was 79.3% (95% CI 60.3–92), clinical benefit rate 82.8% (95% CI 64.2–94.2). Median time to progression 23.9 months (95% CI 14.9–not reached [NR]), median progression-free survival 23.9 months (95% CI 10.3–NR). No grade 4 toxicity was seen. Grade 3 toxicities included: ejection fraction decrease, neutropenia, hyponatremia, fatigue and sensory neuropathy and one left ventricular systolic dysfunction. Phosphorylated (p)-SRC was reduced in peripheral blood mononuclear cells. Phosphorylated SRC, ERK and AKT were also reduced in epidermal keratinocytes.

Conclusions Dasatinib can be safely combined with trastuzumab and paclitaxel. The combination is active with an ORR of almost 80%. *Trial registration*: NCT01306942, EudraCT 2010-023304-27.

Keywords Phase II · HER2-positive breast cancer · Dasatinib · SRC kinase inhibitor · Trastuzumab resistance · Metastatic breast cancer

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Abbreviations

AE	Adverse events
ADC	Antibody drug conjugate
BCA	Bicinchoninic acid
CST	Cell signalling technology
CNS	Central nervous system
CBR	Clinical benefit rate
CTCAE	Common terminology criteria for adverse events
ECOG PS	Eastern Cooperative Oncology Group performance status
ECG	Electrocardiogram
FISH	Fluorescence in situ hybridization
FFPE	Formalin-fixed paraffin-embedded
IHC	Immunohistochemistry
ICH GCP	International conference on harmonization good clinical practice guidelines
LVEF	Left ventricular ejection fraction
MBC	Metastatic breast cancer
NCI	National Cancer Institute
ORR	Objective response rate
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PFS	Progression-free survival
RBC	Red blood cells
RD	Response duration
RECIST	Response evaluation criteria in solid tumours
TTP	Time to progression
T-DM1	Trastuzumab entamsine

Background

Important advances have been performed in the treatment of HER2-positive breast cancer with the incorporation of novel anti-HER2 strategies [1, 2]. These include antibodies against the receptor like trastuzumab and pertuzumab, small tyrosine kinase inhibitors such as lapatinib or neratinib, and more recently, the antibody drug conjugate (ADC) trastuzumab entamsine (T-DM1) that incorporates a potent anti-mitotic agent bind to trastuzumab [1–4]. The incorporation of all these drugs into the standard treatment has significantly improved patient clinical outcomes, with a substantial benefit in overall survival for some of them [1–3, 5].

In the metastatic setting, treatment with trastuzumab still remains a key therapy and can be administered, depending on the patient, at different time points during the evolution of the disease [2]. This antibody is usually given in combination with the standard of care chemotherapy, being paclitaxel a common partner [6].

Although treatment with trastuzumab has clearly improved the outcome of HER2-positive patients, most patients do progress during the course of their disease [1, 2]. In this context, several mechanisms related to the resistance to trastuzumab have been described including activation of downstream kinases, presence of co-activated kinase receptors and modifications of the receptor itself that makes HER2 continuously activated [7–10]. Among them our group and others, have described the presence of SRC, as an activated cytoplasmic kinase, linked with resistance to trastuzumab [3, 11, 12]. In this context, the administration of the SRC kinase inhibitor dasatinib was able to overcome resistance to this antibody in cell cultures [11].

To explore the relevance of this biological finding in the clinical setting, GEICAM, the Spanish Breast Cancer Group, designed an exploratory phase I–II study to evaluate the safety and efficacy of the combination of dasatinib and weekly trastuzumab plus paclitaxel. The phase I part evaluating the maximum tolerated dose and recommended phase to dose was recently published demonstrating that the combination had a good safety profile when using dasatinib at a dose of 100 mg once a day combined with weekly trastuzumab 2 mg/kg and paclitaxel 80 mg/m^2 [13]. In the current article we report the results of the phase II part including efficacy, long-term safety and exploratory biomarker and pharmacodynamics analyses.

Patients and methods

Study design

This was a phase II single arm, open label, multicentre study of the combination of dasatinib, trastuzumab and paclitaxel in first line treatment of HER2-positive metastatic breast cancer (MBC).

The primary objective was to evaluate the efficacy of the combination in terms of objective response rate (ORR) defined as complete plus partial response according to the response evaluation criteria in solid tumours (RECIST) version 1.1. Secondary objectives included: clinical benefit rate (CBR), defined as ORR plus stable disease for at least 6 months; progression-free survival (PFS), defined as the time from the date of the first dose to the date of objectively determined progressive disease or death from any cause whichever occurred first (for patients not known to have progressed or died as of the data cut-off date, PFS will be censored at the date of the last objective progression-free assessment prior to the initiation of a new anticancer therapy); time to progression (TTP), defined as the time from the date of the first dose to the first date of objectively determined progressive disease (for patients not known to have progressed, TTP will be censored at the date of the last objective progression-free assessment prior to the initiation of new anticancer therapy); response duration (RD), defined as the time from the date when the measurement criteria

are met for CR or PR (whichever status is recorded first) until the date of first observation of disease progression or death occurred (for patients not known to have died as of the data cut-off date, RD will be censored at the date of last visit with adequate assessment prior to the initiation of new anticancer therapy) and safety. Other secondary objectives were pharmacodynamic analyses, including changes in the expression of phosphorylated (p)-SRC and p-AKT proteins in sequential peripheral blood mononuclear cells (PBMCs) and p-SRC, p-AKT and p-ERK in formalin-fixed paraffin-embedded (FFPE) skin samples. We additionally explored the expression of p-SRC, p-ERK and p-AKT in tumour samples as prognostic biomarkers. Other exploratory objective was to correlate the early appearance of lymphocytosis with efficacy collecting lymphocyte counts from the weekly analyses within the first cycle.

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines (ICH GCP) and the Declaration of Helsinki, approved by the institutions' ethical review boards of the participating sites and health authorities in Spain and registered at ClinicalTrials.gov and EudraCT (identifiers: NCT01306942 and 2010-023304-27, respectively). Written informed consent was obtained from all patients before performing any protocol specific procedure.

Patient selection

Women aged ≥ 18 years with HER2-overexpressing MBC, determined by a central laboratory by fluorescence in situ hybridization (FISH) following ASCO/CAP guidelines [14], that fulfilled the following criteria were included in the study: measurable disease; no prior chemotherapy or anti-HER2 therapy for MBC (treatment with adjuvant chemotherapy based on taxanes or anti-HER2 therapies were allowed if at least 12 months had elapsed from the end of these therapies); Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 1; adequate bone marrow, liver and renal functions; normal cardiac function; and no concurrent medical condition; adequate contraception and a negative pregnancy test for women of child-bearing potential. Patients were excluded if they had any concurrent medical condition that may increase the risk of toxicity, including pleural or pericardial effusion of any grade. Patients with central nervous system (CNS) metastases were allowed if treated and clinically stable without medication.

Treatment plan

Treatment consisted of 28 days cycles of the combination of weekly intravenous (iv) trastuzumab (2 mg/kg, with a loading dose of 4 mg/kg in cycle 1), weekly iv paclitaxel (80 mg/m^2 for 3 weeks followed by 1 week off) and oral dasatinib (100 mg daily). Antiemetics, corticosteroids and histamine-receptor blockers were administered according to the institutions' guidelines. Treatment was continued until radiographic or symptomatic progression, unacceptable toxicity or withdrawal of the informed consent whatever occurred first. Patients with permanent discontinuation of any of the study drugs were discontinued from the study.

Efficacy and toxicity evaluation procedures

Baseline assessments were performed within 28 days of study entry. These included tumour assessment by radiological tests accepted by RECIST, thoracic X-ray, electrocardiogram (ECG), left ventricular ejection fraction (LVEF) measurement, haematology, biochemistry, pregnancy test, physical examination (including vital signs) and ECOG PS evaluation.

Tumour assessments were performed every 8 weeks until disease progression, using the same method of measurement than at baseline. In case of patient discontinuation for a reason different than disease progression, tumour assessments were performed till objective progression or initiation of new therapy. ECG was performed 4 weeks after baseline and then as clinically indicated. LVEF measurement was performed every 12 weeks. Haematology, biochemistry, physical examination and ECOG PS evaluation were performed every 4 weeks.

Adverse events (AE) were graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. The worst AE per cycle was reported.

Pharmacodynamics and biomarker analysis

Pre-treatment FFPE tumour samples, and sequential peripheral blood mononuclear cells (PBMCs) and FFPE skin samples (on Cycle 1 Day 1 [C1D1] before treatment and 8 h after treatment [0 h and 8 h]) were collected for biomarker and pharmacodynamics analyses.

Pharmacodynamic changes in the expression of p-SRC and p-AKT proteins in PBMCs samples were measured by ELISA and Western Blot. Blood samples (5 ml) were incubated with a red blood cells (RBC) lysis solution (REAL, Spain). The remaining cells were washed with phosphate-buffered saline (PBS) and lysed in ice-cold lysis buffer [3, 11, 12]. Lysates were centrifuged at 10,000×*g* at 4 °C for 10 min and the clarified cell extracts were quantified by bicinchoninic acid (BCA) assay.

The Western blot analyses were performed as described previously [3, 11, 12]. The primary and secondary antibodies used were the following: anti-p-SRC family (rabbit polyclonal SRC Tyr416 antibody, Cell Signalling Technology (CST), MA, USA), anti-GAPDH (mouse monoclonal

antibody, Santa Cruz Biotechnology, CA, USA), anti-mouse Dylight 680-conjugated and anti-rabbit Dylight 800-conjugated (Thermo Fisher Scientific, MA, USA). The bands were visualized and quantified using the Odyssey infrared scanner (LI-COR, NE, USA) and the Odyssey infrared imaging system application software version 3.0 respectively.

For ELISA analyses, a duplicate of 100 µg of the extract was used to detect p-AKT1 (Ser473) and p-SRC (Tyr416) following the manufacturer's instructions (CST, MA, USA).

Immunohistochemistry (IHC) analyses of p-SRC, p-ERK, p-AKT were performed in sequential FFPE skin samples and baseline FFPE tumour samples. Antibodies used were: anti-p-SRC (rabbit polyclonal SRC Tyr527 antibody, CST, MA, USA), anti-p-ERK1/2 (rabbit monoclonal phospho-p44/42 MAPK Thr202/Tyr204 antibody clone D13.14.4E, CST, MA, USA) and anti-p-protein kinase AKT (rabbit monoclonal AKT Ser473 antibody clone D9E, CST, MA, USA). All assays were determined on 4 µm tissue sections using a Dako Link Autostainer (Dako, CA, USA). Heat antigen retrieval was carried out in pH 9 EDTA-based buffered solution in a Dako Link platform (Dako, CA, USA). Endogenous peroxidase was quenched. Primary antibody-antigen reaction was detected by incubation with appropriate polymers coupled with peroxidase (Flex+, Dako, CA, USA). Sections were visualized with 3,3#-diaminobenzidine (DAB) and counterstained with Hematoxylin. Same sections incubated with non-immunized serum were used as negative controls. Sections of human tumours with known marker expression were used as positive controls.

Expression of markers was assessed in a blinded fashion by an experienced pathologist. For p-ERK1/2 and p-AKT, nuclear staining was required for considering a cell as positive. p-SRC was evaluated as membranous and cytoplasmic staining in target cells. A semi-quantitative histoscore (*H*-score) was calculated for all markers. The *H*-score was determined by the estimation of the percentage of tumour cells positively stained with low, medium or high staining intensity. The final score was determined after applying a weighting factor to each estimate. The formula used was $H\text{-score} = (\text{low }\%) \times 1 + (\text{medium }\%) \times 2 + (\text{high }\%) \times 3$, and the results ranged from 0 to 300.

Statistical analysis

A Simon's two-stage optimal design was used to calculate the sample size testing the null hypothesis (H0) that ORR equals 50% against the alternative hypothesis (H1) that ORR is $\geq 50\%$. With an alpha error of 0.05 and a statistical power of 80% when the true ORR is 75%, 25 evaluable patients were needed. Assuming a drop-out rate of 10%, 28 patients were required to enter the study. The time to event variables were analysed using Kaplan–Meier product limit estimator.

Biomarker changes in sequential PBMCs and skin biopsies were analysed using Wilcoxon rank sum test. Basal biomarker expression in tumour samples was correlated with ORR using Univariate Logistic Regression.

All hypotheses were tested at an alpha level of 0.05 (two sided).

The SPSS (v21.0) and SAS Enterprise 14 Guide (v5.1) were used for all analysis.

Results

From June 2013 to December 2015, 27 patients were recruited. All, plus 2 additional patients from the phase I part of the study, with measurable disease and treated with the same dasatinib doses were included in the efficacy and safety analysis, for a total of 29 patients.

Patients and disease characteristics are described in Table 1. Median age was 49 years (range 31–81), 17 patients (59%) were postmenopausal, 18 (62%) had an ECOG PS 0, 22 (76%) were hormone receptor positive and 24 (83%) had visceral involvement. Prior neo (adjuvant) chemotherapy was given to 15 patients (52%), including trastuzumab in 10 patients (34%).

Treatment exposure

A total of 404 cycles were administered with median of 12 cycles (range 1–49).

Most relevant dasatinib dose modifications observed were a dose reduction in 1 patient (1 cycle) due to fatigue grade 2, and dose omissions in 17 cycles (12 patients), in 8 of them AEs were the reason for dose omissions because of neutropenia grade 2 (2 cycles) and grade 3 (1 cycle), grade 3 fatigue, and soft tissue/skin infection (1 cycle each), grade 2 Palmar–Plantar erythrodysesthesia syndrome and respiratory infection (1 cycle each) and grade 1 colic pain (1 cycle). Paclitaxel dose was reduced due to AEs in 16 cycles (9 patients), being the main reasons neutropenia grade 2 (4 cycles), peripheral sensory neuropathy grade 2 (3 cycles) and fatigue grade 3 (2 cycles). In addition paclitaxel doses were delayed in 19 cycles (12 patients) mainly due to neutropenia grade 2 (7 cycles) and omitted in 6 patients. Trastuzumab dose was delayed in 10 cycles (10 patients), being the main reason neutropenia grade 2 (4 cycles), and omitted in 8 cycles (7 patients) mainly due to decrease in LVEF (3 cycles) and neutropenia grade 2 (2 cycles).

The median Relative Dose Intensity of trastuzumab was 98.5% (88.1–124.1), paclitaxel 94.4% (53.7–104.1) and dasatinib 100.0% (81.4–100.3).

The main reasons for treatment discontinuation included progressive disease in 11 patients (38%), AEs in 6 patients

Table 1 Patients and disease characteristics

Characteristics	$N=29$
Age, years (median; range)	49 (31–81)
Menopausal status, *n* (%)	
Premenopausal	12 (41)
Postmenopausal	17 (59)
ECOG PS, *n* (%)	
0	18 (62)
1	11 (38)
Metastatic locations, *n* (%)	
Visceral	24 (83)
Liver	19 (65)
Lung	10 (34)
Non visceral	5 (17)
Histological type, *n* (%)	
Invasive ductal carcinoma	26 (90)
Other	3 (10)
Histological grade, *n* (%)	
Grade 2	13 (45)
Grade 3	5 (17)
Unknown	11 (38)
Hormone receptor status (local), *n* (%)	
Positive	22 (76)
Negative	7 (24)
Ki67 expression (local)	
Mean, % (range)	33 (1–80)
< 20%, *n* (%)	3 (11)
≥ 20%, *n* (%)	19 (70)
Unknown	7 (19)
Prior trastuzumab treatment, *n* (%)	
Yes	10 (34)
No	19 (66)
Prior chemotherapy, *n* (%)	
Yes	15 (52)
No	14 (48)
Prior hormonotherapy, *n* (%)	
Yes	14 (48)
No	15 (52)

N number of patients, *ECOG* Eastern Cooperative Oncology Group, *PS* performance status

(21%) and investigator criteria in 7 patients (24%). Table 2 describes all reasons of treatment discontinuations.

Efficacy

The ORR was 79.3% ($n=23$; 95% Confidence Interval [CI] 60.3–92.0), with 3 (10.3%) complete responses and 20 (69.0%) partial responses. Three patients (10.3%) achieved stable disease and 3 patients (10.3%) progressed. The CBR was 82.8% ($n=24$; 95% CI 64.2–94.2).

The median PFS and TTP were 23.9 months (95% CI 10.3–not reached [NR]) (Fig. 1), and 23.9 months (95% CI 14.9-NR), respectively. The median RD was not reached.

Safety

Table 3 lists all related adverse events (toxicities), most of them were of grade 1 and 2. Grade 3 toxicities included ejection fraction decreased, neutropenia, hyponatremia, fatigue and sensory neuropathy in 2 patients each (6.9%). Seven patients experienced grade 2 ejection fraction decreased, being one of them symptomatic.

Pharmacodynamics and biomarker analysis

Western blotting and ELISA analyses in PBMCs were performed to evaluate the phosphorylation status of several proteins expected to be affected by the treatment. Western blotting studies showed that 8 h of treatment with the drug combination decreased the tyrosine phosphorylation of the different SRC family members recognized by the anti-p-SRCY416 antibody (Fig. 2a, b). In parallel, analyses of p-SRC in PBMCs by ELISA from 16 (55%) patients showed that treatment was able to reduce p-SRC by 4.4 folds ($p<0.0001$) after 8 h of administration (C1D1 0 h and 8 h). At the same time points, p-AKT was also reduced by 1.9 folds, but not reaching the statistically significance ($p=0.131$) (Fig. 2c, d).

Figure 3 shows the analyses in skin biopsies that were performed on 6 patients (3 of them from the phase I due to the limited number of patients). All of them received 100 mg of dasatinib except one patient from the phase I that was treated with 140 mg. Treatment induced a significant reduction in p-SRC expression in the membrane of keratinocyte after 8 h (C1D1 0 h and 8 h) (mean baseline *H*-score ± 95% CI: 105 ± 22 vs. 27 ± 17, $p<0.001$). We observed a similar downregulation in the nucleus of epidermal cells in p-ERK (mean baseline *H*-score ± 95% CI: 60 ± 11 vs. 16 ± 10, $p<0.001$) and p-AKT expression (mean baseline *H*-score ± 95% CI: 70 ± 30 vs. 20 ± 10, $p=0.013$).

We did not observe any significant association between p-SRC, p-AKT or p-ERK protein expression in baseline tumour samples with any of the efficacy endpoints (ORR, CBR, TTP and PFS) in 23 (79%) patients (data not shown).

Early appearance of lymphocytosis (within cycle 1) was observed in 3 patients. It was not seen any statistically significant correlation with efficacy.

Discussion

In this article we describe the clinical activity and long-term safety of dasatinib in combination with trastuzumab and paclitaxel. To our knowledge, this is the first reported study

Table 2 Reasons for treatment discontinuation

End of treatment, *n* (%)	*N* = 29
Progressive disease	11 (37.9)
Investigator/sponsor criteria	7 (24.1)
Adverse event	6 (20.7)
Consent withdrawn	2 (6.9)
Death	1 (3.4)
Protocol deviation	1 (3.4)
Treatment on going	1 (3.4)
Adverse event reasons, *n* (%)	*N* = 6
Neutropenia	3 (50.0)
Fatigue	1 (16.7)
Angor pectoris	1 (16.7)
Neurotoxicity	1 (16.7)

n number of patients

that evaluates the activity of the combination of an anti-SRC inhibitor with trastuzumab in HER2-positive patients.

Results from this study confirm the reported preclinical findings from our group and others [11, 12] in which the addition of a SRC inhibitor was able to increase the efficacy of trastuzumab [11, 12]. This was associated with a reduction of the activation of SRC and AKT, leading to an induction of apoptosis [11, 12]. The mechanism of action of dasatinib was confirmed in this study as target inhibition was demonstrated by the reduction of p-SRC and p-AKT in PBMCs in patients treated with the combination. Additionally, we identified a statistically significant reduction in the phosphorylation of ERK, SRC and AKT in keratinocytes of the epidermis after treatment.

In our study we combined dasatinib with trastuzumab and paclitaxel, as it was one of the standard of care treatments in first line, at the time the study was designed [15]. Although we acknowledge that this regimen is no longer the first option, it is still a therapeutic opportunity for subsequent lines of treatment. The dose of 100 mg daily of dasatinib was selected from the phase I study [13] due to the lack of relevant toxicities.

In this phase II trial the main grade 3 toxicities included decrease in ejection fraction, neutropenia, hyponatremia, fatigue, hypertension and sensory neuropathy in two patients each. Most frequent grade 1 toxicities included anaemia and neutropenia, AST, ALT increase and fatigue in more than half of the patients. Globally, the combination of weekly paclitaxel and trastuzumab plus daily dasatinib was safe. Its excellent tolerability, demonstrated by the high relative dose intensity (more than 90%) for all compounds and the median number of cycles administered [12], did not compromise long-term administration.

Of note, in our study the permanent discontinuation of any of the study drugs was not permitted so dasatinib or trastuzumab could not be given alone. This fact limited longer administrations of both compounds, particularly for those patients that had achieved a partial or complete response.

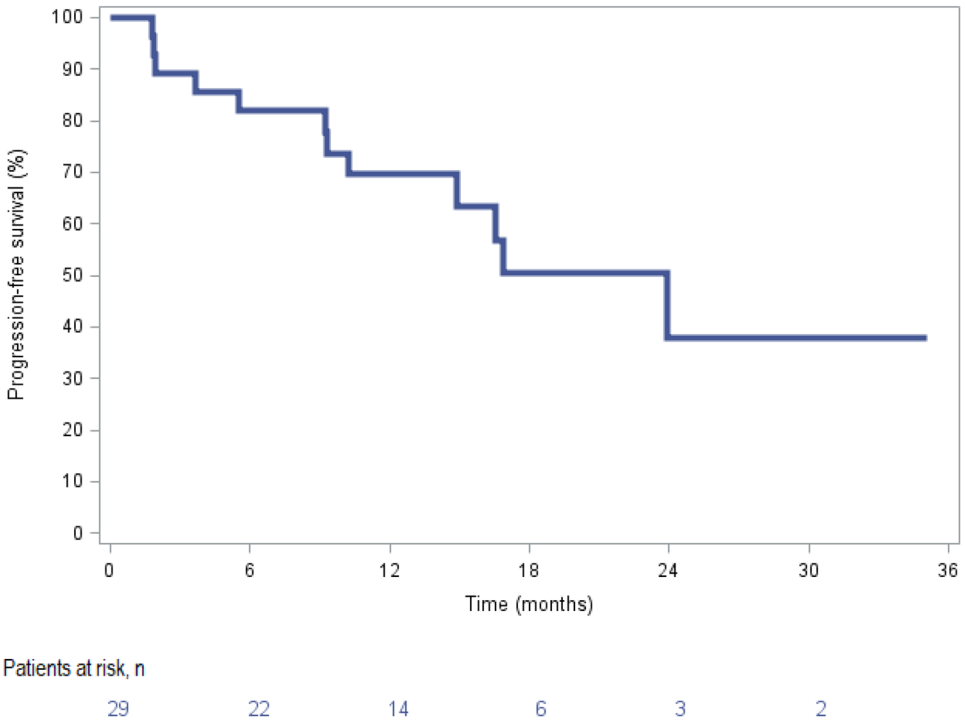

Fig. 1 Progression-free survival

Table 3 Treatment related adverse events according to National Cancer Institute Common Terminology criteria for adverse events (version 4.03)

Adverse event, *n* (%)	Grade 1	Grade 2	Grade 3
Alopecia	7 (24.1)	13 (44.8)	–
Anorexia	5 (17.2)	1 (3.4)	–
Diarrhoea	7 (24.1)	3 (10.3)	–
Ejection fraction decreased	–	7 (24.1)[a]	2 (6.9)
Fatigue	17 (58.6)	5 (17.2)	2 (6.9)
Oral mucositis	8 (27.6)	–	–
Nausea	7 (24.1)	–	–
Sensory neuropathy	11 (37.9)	5 (17.2)	2 (6.9)
aPTT prolongation	2 (6.9)	6 (20.7)	–
ALT increase	17 (58.6)	6 (20.7)	–
AP increase	9 (31.0)	2 (6.9)	–
AST increase	20 (69.0)	1 (3.4)	–
Hypocalcaemia	2 (6.9)	7 (24.1)	1 (3.4)
Hypomagnesemia	8 (27.6)	2 (6.9)	–
Hyponatremia	4 (13.8)	–	2 (6.9)
Hypophosphatemia	4 (13.8)	4 (13.8)	1 (3.4)
Anaemia	13 (44.8)	12 (41.4)	–
Leukopenia	12 (41.4)	5 (17.2)	–
Neutropenia	16 (55.2)	7 (24.1)	2 (6.9)

n number of patients, *G* grade, *aPTT* activated partial thromboplastin time, *ALT* alanine aminotransferase, *AP* alkaline phosphatase, *AST* aspartate aminotransferase

[a]One of them had a left ventricular systolic dysfunction grade 3

The efficacy observed in this study in terms of ORR and PFS is high and in line with that observed with the combination of trastuzumab, pertuzumab and taxanes as first line therapy of HER2-positive MBC [5, 16]. ORR was in the range of 80% and median PFS was longer than 23 months. Of note, compared with trastuzumab, pertuzumab and docetaxel our combination showed a favourable toxicity profile, with no bone marrow impairment.

The mechanism of action of dasatinib was confirmed in this study as target inhibition was demonstrated by the reduction of p-SRC in PBMCs and keratinocytes of the epidermis in patients treated with the combination. Additionally, we identified a statistically significant reduction in the phosphorylation of ERK and AKT in skin samples after treatment. These results confirm the reported preclinical findings from our group and others that motivated us to perform this clinical study [11, 12].

Our study has limitations. First, this is a single arm study with a limited number of patients, so a potential overestimation of benefit can be present. Secondly, this combination is not better than the double blockage with trastuzumab and pertuzumab in first line, therefore, options for its clinical development should focus on later lines of therapy, or the administration without chemotherapy for patients that do respond to combinations with chemotherapy. Finally, future combinations with pertuzumab, trastuzumab and dasatinib should be clinically explored to

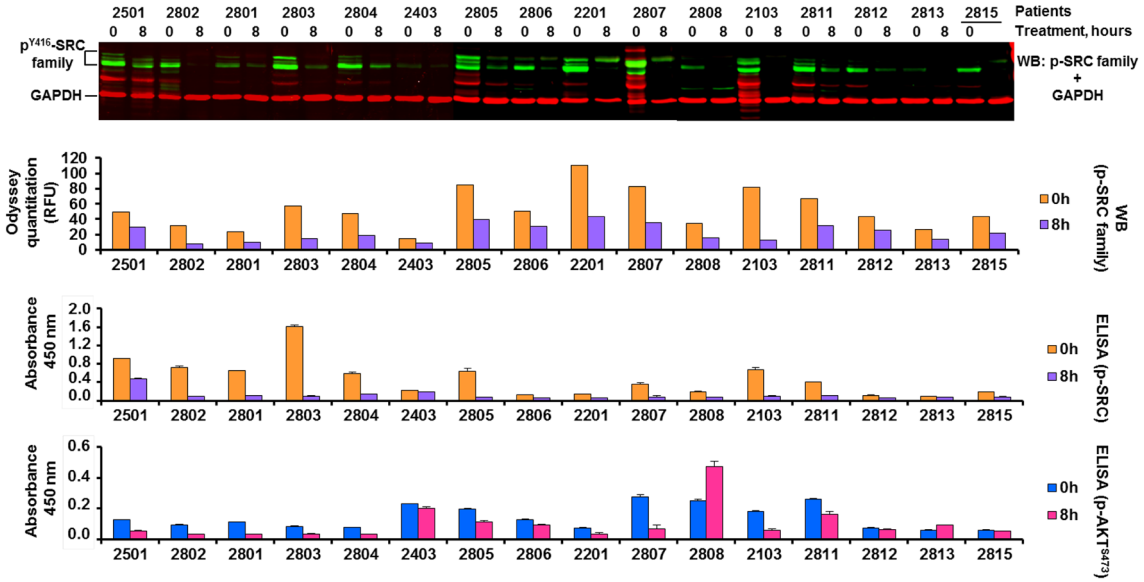

Fig. 2 Pharmacodynamics changes of p-SRC and p-AKT in sequential peripheral blood mononuclear cells (PBMCs) samples (C1D1 0 h and 8 h) by Western blot and ELISA. **a** Analysis of the levels of p-SRC family on PBMCs samples of patients before treatment (0 h) and after 8 h of treatment (8 h) by Western blot. The total level of GAPDH was used as loading control. **b** The bar graph shows the quantification of p-SRC family of the Western blot experiment in **a**. Analysis of the levels of p-SRC (Tyr416) (**c**) and p-AKT (Ser473) (**d**) on PBMCs samples of patients at 0 h and 8 h by ELISA. The bar graph shows the levels of p-SRC and p-AKT in the sequential samples of patients (0 h and 8 h)

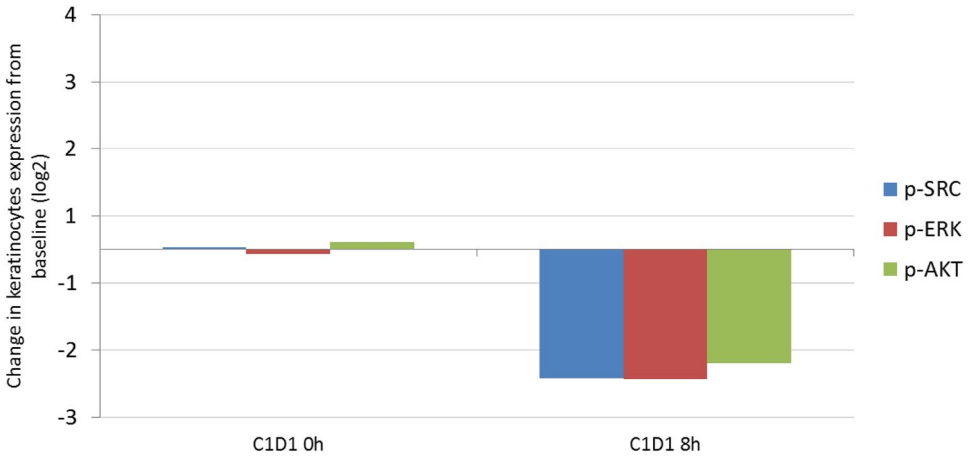

Fig. 3 Immunohistochemical expression analysis of p-SRC, p-AKT and p-ERK protein in sequential skin samples after treatment with dasatinib (C1D1, 8 h and 8 h). Bar graphs show a downregulation in p-SRC, p-ERK and p-AKT keratinocytes expression achieved after 8 h of treatment

evaluate if the inhibition of SRC can augment the efficacy of targeting HER2 with two antibodies.

In conclusion, here, we describe for the first time efficacy data from the combination of the SRC inhibitor, dasatinib, with trastuzumab and paclitaxel in HER2-positive breast cancer patients. The high efficacy observed and the good toxicity profile, pave the way for the future clinical development of this combination in metastatic breast cancer.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest Dr. A. Ocaña has received honorarium for consultant/advisory from Daiichi Sankyo, Entrechem and Servier. Dr. A. Urruticoechea has received honorarium for consultant/advisory from Roche. Dr. A. Falcón has received honorarium as speaker from Roche and Astra-Zeneca. Dr. S. Pernas has received honorarium as speaker and travel grants from Roche, she also participated as advisor for Polyphor. Dr. E. Carrasco owns Ely Lilly stock options and has received travel grants from Roche. Her husband received honoraria from Celgene, BMS, Janssen Cilag and Takeda. Dr F Rojo has received speaker honorarium from Roche, BMS, Merck and Pfizer. Dr M Ruíz-Borrego received honorarium as speaker and advisory from Roche and Astra-Zeneca. The rest of authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study are in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the institutions' ethical review boards of the participating sites and health authorities in Spain.

Informed consent Written informed consent was obtained from all individual participants included in the study.

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