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PRECLINICAL STUDY

Comparison of preclinical cardiotoxic effects of different ErbB2 inhibitors

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Abstract Two novel human antitumor immunoconjugates, made up of a human anti-ErbB2 scFv, Erbicin, fused with either a human RNase or the Fc region of a human IgG1, are selectively cytotoxic for ErbB2-positive cancer cells in vitro and in vivo. The Erbicin-derived immunoagents (EDIA) target an epitope different from that of trastuzumab, the only humanized antibody currently prescribed for treatment of ErbB2-positive breast cancer (BC). As Trastuzumab has shown cardiotoxic effects, in this study, we evaluated if any side effects were exerted also by EDIA, used as single agents or in combination with anthracyclines. Furthermore, we compared the in vitro and in vivo cardiotoxic effects of EDIA with those of the other available anti-ErbB2 drugs: Trastuzumab, 2C4 (Pertuzumab), and Lapatinib. In this article, we show that EDIA, in contrast with Trastuzumab, 2C4, and Lapatinib, have no toxic effects on human fetal cardiomyocytes in vitro, and do not induce additive toxicity when combined with doxorubicin. Furthermore, EDIA do not impair cardiac function in vivo in mice, as evaluated by Color Doppler echocardiography, whereas Trastuzumab significantly reduces radial strain (RS) at day 2 and fractional shortening (FS) at day 7 of treatment in a fashion similar to doxorubicin. Also 2C4 and Lapatinib significantly reduce RS after only 2 days of treatment, even though they showed cardiotoxic effects less pronounced than those of Trastuzumab. These results strongly indicate that RS could become a reliable marker to detect early cardiac dysfunction and that EDIA could fulfill the therapeutic need of patients ineligible to Trastuzumab treatment because of cardiac dysfunction.

Keywords ErbB2/HER2 · Immunotherapy · Cardiotoxicity · Breast cancer · Herceptin/Trastuzumab

Carmine Fedele and Gennaro Riccio contributed equally to this study.

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Introduction

Overexpression of ErbB2 receptor is associated with progression of malignancy of breast cancer (BC), and is a sign of a poor prognosis [1]. Trastuzumab, the only anti-ErbB2, humanized monoclonal antibody approved by FDA for the therapy of mammary carcinoma, has proved to be effective in the immunotherapy of breast carcinoma [2]. However, large-scale clinical studies with Trastuzumab, have shown that up to 7% or 28% of patients suffer from cardiac dysfunction when Trastuzumab is used in monotherapy, or in combination with anthracyclines, respectively [3–5].

The mechanism of cardiotoxicity induced by ErbB2-inhibition is not clear. ErbB2 is thought to participate in an important pathway for growth, repair, and survival of adult cardiomyocytes [6–8]. However, ErbB2 levels in the adult heart are low when compared with the levels found in ErbB2-overexpressing BC cells, the intended targets of a

therapy based on anti-ErbB2 antibodies. Furthermore, Lapatinib, a dual kinase inhibitor of EGFR and ErbB2, has shown minimal cardiotoxicity in clinical trials [9, 10], and Pertuzumab, a new anti-ErbB2 monoclonal antibody at present tested in clinical trials, which recognizes an epitope distant from that of Trastuzumab (in the extracellular portion of ErbB2), does not show significant cardiotoxic effects in a phase I study [11]. If inhibition of ErbB2 was responsible for cardiomyocyte dysfunction, then cardiotoxicity would have been expected also with the quinazoline compound and all the other anti-ErbB2 antibodies. Thus, Trastuzumab-associated cardiotoxicity must be explained by some alternative mechanisms [12]. The hypothesis has been made that Trastuzumab cardiotoxicity is related to the inhibition of the neuregulin 1-activated pathway which directly promotes cardiac myocyte survival via ErbB2/ErbB4 heterodimerization [6].

Two novel human antitumor immunoconjugates were engineered in our laboratory by fusion of a human anti-ErbB2 single chain variable antibody fragment (scFv) [13], termed Erbicin, with either a human RNase [14] or the Fc region of a human IgG1 [15]. Both these erbicin-derived immunoagents (EDIA) are selectively cytotoxic for ErbB2-positive cancer cells in vitro and in vivo [13–16]. More interestingly, EDIA are also active on some Trastuzumab-resistant BC cells both in vitro and in vivo [17]. The sensitivity of these cells to treatment with EDIA is likely due to their distinct epitope [18, 19], since EDIA, differently from Trastuzumab, are capable of inhibiting the signaling pathway downstream ErbB2 [17].

The findings that EDIA recognize an epitope different from that of Trastuzumab led us to ascertain whether they might not present the most negative property of Trastuzumab: cardiotoxicity.

EDIA did not show in vitro adverse effects on rat cardiomyocytes and cardiomyoblasts for which Trastuzumab is severely toxic [20]. These differences have been found to be because of their diverse mechanism of action: in fact, Trastuzumab, in contrast to Erb-hcAb, induces apoptosis in cardiac cells. In vivo studies on a mouse model have shown that EDIA do not alter the cardiac function, whereas Trastuzumab significantly reduces fractional shortening (FS) in a fashion similar to that of doxorubicin [20].

Furthermore, we demonstrated that Erb-hcAb and Erb-hRNase induced in treated mice cardiac fibrosis and apoptosis at a much lower extent than treatment with Trastuzumab or doxorubicin [20].

Thus, to test whether EDIA could fulfill the therapeutic need of cancer patients ineligible to Trastuzumab treatment because of cardiac dysfunction, we implemented new preclinical protocols to assess their cardiotoxic effects in combination with anthracyclines on human fetal cardiomyocytes in vitro, and in animal models in vivo.

Moreover, we further investigated the potential therapeutic use of EDIA, by comparing their cardiotoxic effects with those of all the other available anti-ErbB2 drugs, such as Trastuzumab, Pertuzumab, and Lapatinib.

Materials and methods

Antibodies and cell lines

The hybridoma cells producing 2C4 antibody (LGC Promochem, Sesto San Giovanni, Italy) were grown in DMEM-RPMI-1640 medium in a 1:1 ratio (Sigma, St Louis, MO, USA).

The H9C2 cardiomyoblasts were cultured in DMEM containing sodium pyruvate (1.0 mM). The media were supplemented with 10% heat-inactivated fetal bovine serum, 2.0 mM L-Glutammine, 50 U/ml penicillin, and 50 μg/ml streptomycin (all from Sigma).

The Human Fetal Cardiac Myocytes (HCM) (Innoprot, Derio, Spain) were cultured according to the manufacturer's recommendations.

The antibodies and immunoconjugates used were Trastuzumab (Genentech, South San Francisco, CA, USA); Erb-hRNase, produced and purified as previously described [14] Erb-hcAb, produced from PER.C6® cells (Crucell N.V., Leiden, Netherlands) transfected with the recombinant vector and purified as previously described [15].

The antibody 2C4 was produced and purified as follows. 2C4 hybridoma cells were expanded to near confluence in complete medium, and then grown for 3–4 days in serum-free medium. The secreted antibody was purified from culture medium by affinity chromatography on a protein G Sepharose loaded with 300–500 ml of conditioned medium. Wash and elution steps were carried out as described for Erb-hcAb [15].

In vitro cardiotoxicity tests

To test the cardiotoxicity of Erb-hcAb and Erb-hRNase, HCM were seeded in 96-well microtiter plates at a density of 1×10^4 cells/wells, and allowed to adhere overnight. After the initial incubation for 16 h at 37°C, the medium was replaced with medium containing Trastuzumab, Erb-hcAb, Erb-hRNase, 2C4, Lapatinib (Tykerb®, GlaxoSmithKline, Brentford, UK), or doxorubicin at increasing concentrations. After the treatment was carried out for 24 h at 37°C, cells were washed with PBS and stained with trypan blue and tested by (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) for the determination of cell survival as previously described [20].

To evaluate the effects of the combination of Erbicin-derived immunoagents with doxorubicin, H9C2 and HCM

cells were seeded as described above, and treated for 24 h at 37°C with either doxorubicin (0.12–0.5 μM), or Erbicin-derived immunoagents (0.25–2 μM), or with a combination of the drug and EDIA at the same concentrations. Cell counts and MTT tests were carried out as described above.

Cell survival was expressed as percent of viable cells in the presence of drugs under test with respect to control cultures grown in absence of the drugs. Typically, cell survival values were obtained from at least three independent experiments in which five determinations were performed for each sample. Standard deviations were calculated on the basis of the results obtained from all the experiments.

Cell morphology analysis

HCM cells were plated at a density of 6×10^5/well in six-well plates and incubated at 37°C with Trastuzumab (2 μM), Erb-hcAb (2 μM), doxorubicin (0.5 μM), or with the combination of each antibody with doxorubicin. After 24 h, cultured cells were observed by light microscopy (Nikon ECLIPSE E1000, Melville, NY, USA), and photographed (Nikon digital camera DXM 1200F), to analyze cell morphology.

Transthoracic echocardiography

In vivo cardiac function was assessed by transthoracic echocardiography in sedated 7-week-old WT C57Bl/6 mice (Harlan Italy, San Piero al Natisone, UD, Italy) using a Vevo 2100 high-resolution imaging system (40-MHz transducer, VisualSonics, Toronto, ON, Canada). Mice were anesthetized with Tilotamine (0.09 mg/g), Zolazepam (0.09 mg/g), and 0.01% atropine (0.04 ml/g). Cardiac function was evaluated by noninvasive echocardiography in basal conditions and after intraperitoneal treatment of equimolar doses (2 nmol/mouse) of Trastuzumab, Erb-hcAb, immunoRNase, 2C4, or doxorubicin (15 mg/kg) used as a positive control. Oral treatment was carried out for the tyrosine kinase inhibitor Lapatinib (100 mg/kg/day). The left ventricular (LV) echocardiogram was assessed in both parasternal long-axis and short-axis views at a frame rate of 233 Hz. End-systole and end-diastole dimensions were defined as the phases corresponding to the ECG T wave, and to the R wave, respectively. M-mode LV end-systolic dimensions (LVESDs) and LV end-diastolic dimensions (LVEDDs) were averaged from 3 to 5 beats. LVEDD and LVESD were measured from the LV M-mode at the midpapillary muscle level. Fractional shortening percentage (%FS) was calculated as [(LVEDD−LVESD)/LVEDD] × 100 [21]. Radial strain (RS) was evaluated by speckle-tracking echocardiography, a novel technique that enables the assessment of myocardial strain (an index of myocardial deformation) through the analysis of speckle motion inherently present in a standard, 2D echocardiographic image [22].

Studies and analysis were performed blinded to heart condition. Data are presented as mean ± SD unless otherwise noted. Between-group differences were assessed by Student's t test or one-way ANOVA as appropriate. Statistical significance was defined as $P < 0.05$.

The animal experimentations described herein were conducted in accordance with the Italian regulation for experimentation on animals. All in vivo experiments were carried out with ethical committee's approval and met the standards required by the Directive 2010/63/EU of the European Parliament.

Cardiac fibrosis analysis

Interstitial fibrosis was evaluated by staining 5-μm-thick tissue sections with 1% Sirius red in picric acid (Carlo Erba Laboratories, Milan, Italy) as previously described [23]. The positively stained (red) fibrotic area was measured using a computer-assisted image analysis system (Nikon NIS ELEMENTS BRV, Melville, NY, USA) and expressed as a percentage of total area. The percentage of red staining was calculated from three samples/group, with two sections for each sample and five images for each section.

Results

In vitro effects of the anti-ErbB2 immunoagents on human cardiac cells

To test the cardiotoxicity of Erb-hcAb and Erb-hRNase on human fetal cardiomyocytes, the cells were treated in the absence or in the presence of increasing concentrations of the immunoagents. Control tests were carried out in parallel assays with Trastuzumab, 2C4 (the mouse antibody version of Pertuzumab), Lapatinib, or doxorubicin for comparison.

As shown in Fig. 1, Erb-hcAb and Erb-hRNase did not show cardiotoxic effects, whereas Trastuzumab, 2C4, Lapatinib, and doxorubicin were all found to be significantly toxic for cardiac cells.

Effects on cell survival of Erbicin-derived immunoagents in combination with doxorubicin

To evaluate the effects of the combination of Erbicin-derived compact antibody with doxorubicin, cytotoxicity assays were carried out on human fetal cardiomyocytes and on rat H9C2 cardiomyoblasts with the chemotherapeutic drug or Erb-hcAb used as single agents, or in combination.

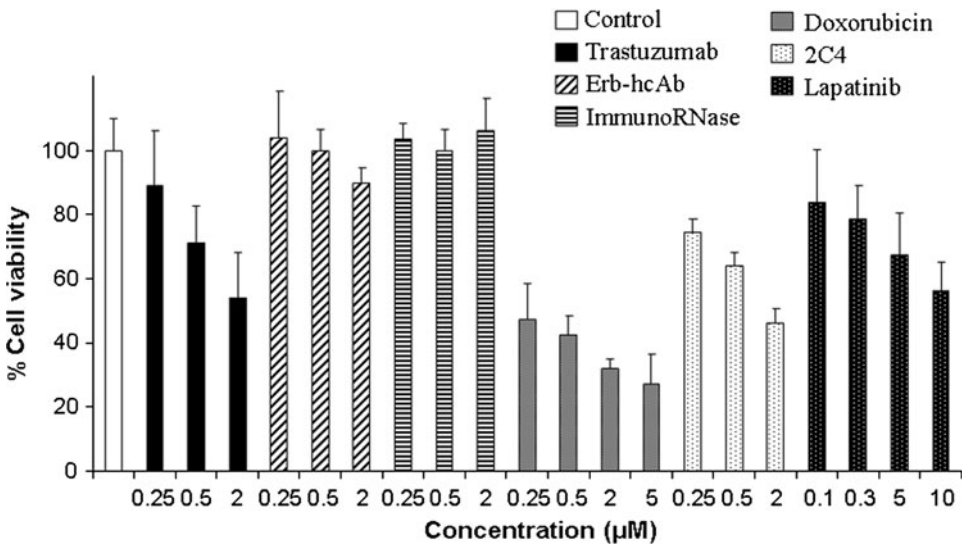

Fig. 1 Effects of the anti-ErbB2 drugs on human fetal cardiomyocytes (CFH). Dose–response tests of the indicated cells treated for 24 h with Trastuzumab (*filled square*), Erb-hcAb (▨), ImmunoRNase (▤), 2C4 (⬚), Lapatinib (▩) or doxorubicin (▩), used as a control

Results of control tests run with Trastuzumab and doxorubicin are presented for comparison. We found that the toxicity was clearly superior when the chemotherapeutic drug was given in combination with Trastuzumab (see Fig. 2), whereas no additive effects were observed for the combination of Erb-hcAb with doxorubicin.

Figure 3 shows the morphologic changes of cells treated for 24 h at 37°C with Trastuzumab (2 μM), Erb-hcAb (2 μM), doxorubicin (0.5 μM), or with the combination of each immunoagent with doxorubicin at the same concentrations. The combination of Trastuzumab and doxorubicin clearly caused an increased cell death and more marked changes in cell morphology than each drug alone, as cells appeared to lose their typical features and assume distorted, round-shaped forms. No such effects were observed for the cells treated with Erb-hcAb alone or in combination with doxorubicin.

Similar experiments were performed with the immunoRNase Erb-hRNase. Human fetal cardiomyocytes and rat H9C2 cardiomyoblasts were treated with either doxorubicin, or Erb-hRNase, or with a combination of doxorubicin with Erb-hRNase at the same concentrations. As shown in Fig. 4, doxorubicin displayed a similar cytotoxic effect when used alone or in combination with Erb-hRNase, thus suggesting that also this immunoagent, in contrast to Trastuzumab, does not increase the cardiotoxicity of the chemotherapeutic treatment.

In vivo cardiotoxic effects

We then tested the effects of combination of Trastuzumab, Erb-hcAb, or Erb-hRNase with doxorubicin, on a mouse model. For these experiments, groups of 6–10 mice were injected with equimolar doses of EDIA or Trastuzumab, used either as single agents or in combination with doxorubicin. Echocardiography measurements were performed on mouse hearts before and after treatment. Erb-hcAb and the immunoRNase, each used in monotherapy, had no effects on cardiac function: FS (Fig. 5a) and RS (Fig. 5b) were not significantly affected; only a slight reduction of RS in the group treated for 7 days with Erb-hcAb is shown, but the difference was found to be not statistically relevant. When combined with doxorubicin, EDIA showed no additive effects with respect to those of doxorubicin alone. Indeed, FS was reduced to 52 ± 0.2% early (after 2 days) also in mice treated with doxorubicin in monotherapy (2.17 mg/kg/day). On the other hand, mice treated with a combination of doxorubicin (2.17 mg/kg/day) and Trastuzumab (2.25 mg/kg/day) showed a drastic reduction of FS (49 ± 2%, vs. 60 ± 0.4% in sham), while in mice treated with Trastuzumab alone (2.25 mg/kg/day) FS decreased only at 7 days (49 ± 1.5% vs 60 ± 0.5%, $P = 0.002$). In contrast, after 2 days, myocardial strain was already reduced not only in groups of mice treated with doxorubicin or doxorubicin combined with Trastuzumab, but also in groups treated with Trastuzumab alone (43 ± 1% vs. 66 ± 0.6% in sham).

To further confirm that RS is an early predictor of future onset of cardiac dysfunction, we tested the in vivo effects on this parameter of Lapatinib and 2C4 (the mouse antibody version of Pertuzumab), previously reported [9, 11] as less cardiotoxic ErbB2-inhibitors. As shown in Fig. 6a, both FS and RS were reduced by treatment with 2C4. After 2 days of treatment with 2C4 (2.25 mg/kg/day), myocardial strain was already decreased compared to sham: 40 ± 8% versus 66 ± 0.6%, ($P = 0.02$), with FS also significantly reduced: 58 ± 1% versus 60 ± 0.4, ($P = 0.01$). LV dysfunction was exacerbated after 7 days of treatment, with strain further decreasing to 31 ± 7%, and FS to 39 ± 5% (Fig. 6a).

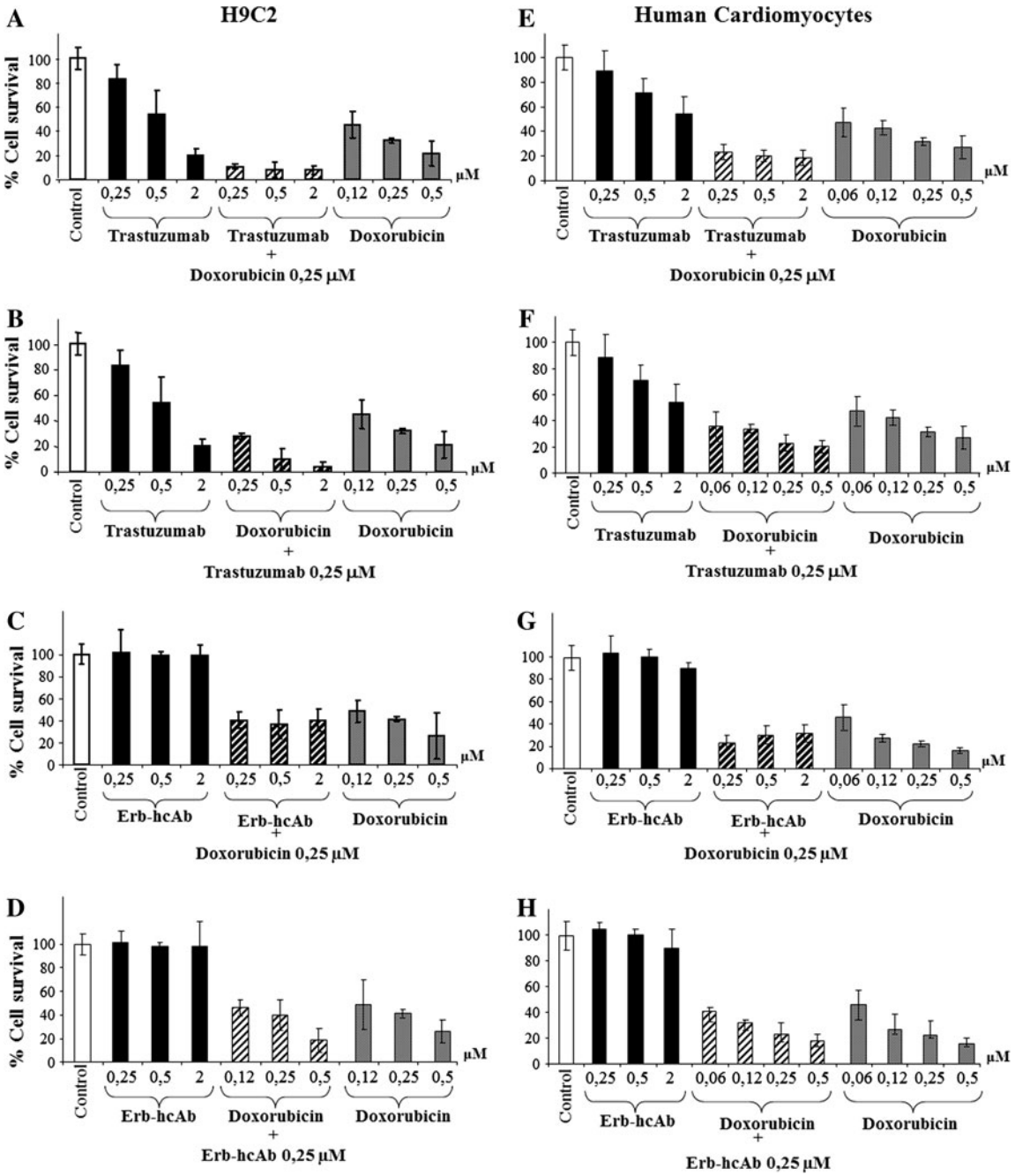

Fig. 2 Effects of combination treatment of Trastuzumab (**a**, **b**, **e**, **f**) or Erb-hcAb (**c**, **d**, **g**, **h**) with doxorubicin on human fetal cardiomyocytes (CFH) and H9C2 cardiomyoblasts. Dose–response tests of the indicated cells treated for 24 h with each antibody alone (*black bars*), doxorubicin alone (*gray bars*) or with the combination of each antibody and doxorubicin (*striped bars*)

In mice treated with Lapatinib (100 mg/kg/day) a decrease of FS was observed after 7 days (56 ± 2% vs. 60 ± 1% for sham animals, $P = 0.04$), while after 2 days FS was unaffected. At this time point, RS evaluated by speckle tracking was able to identify cardiotoxicity at a very early stage (34 ± 7% vs. 59 ± 1% in sham, $P = 0.008$) (Fig. 6b).

After treatment, mice were euthanized, and the hearts were removed and subjected to weight measurement, histological examination, and processing for detection of myocardial stress and apoptosis. Cardiac fibrosis was examined by the Sirius red staining for collagen as previously described [20]. All analyses were carried out in parallel experiments on control, untreated mice. As shown

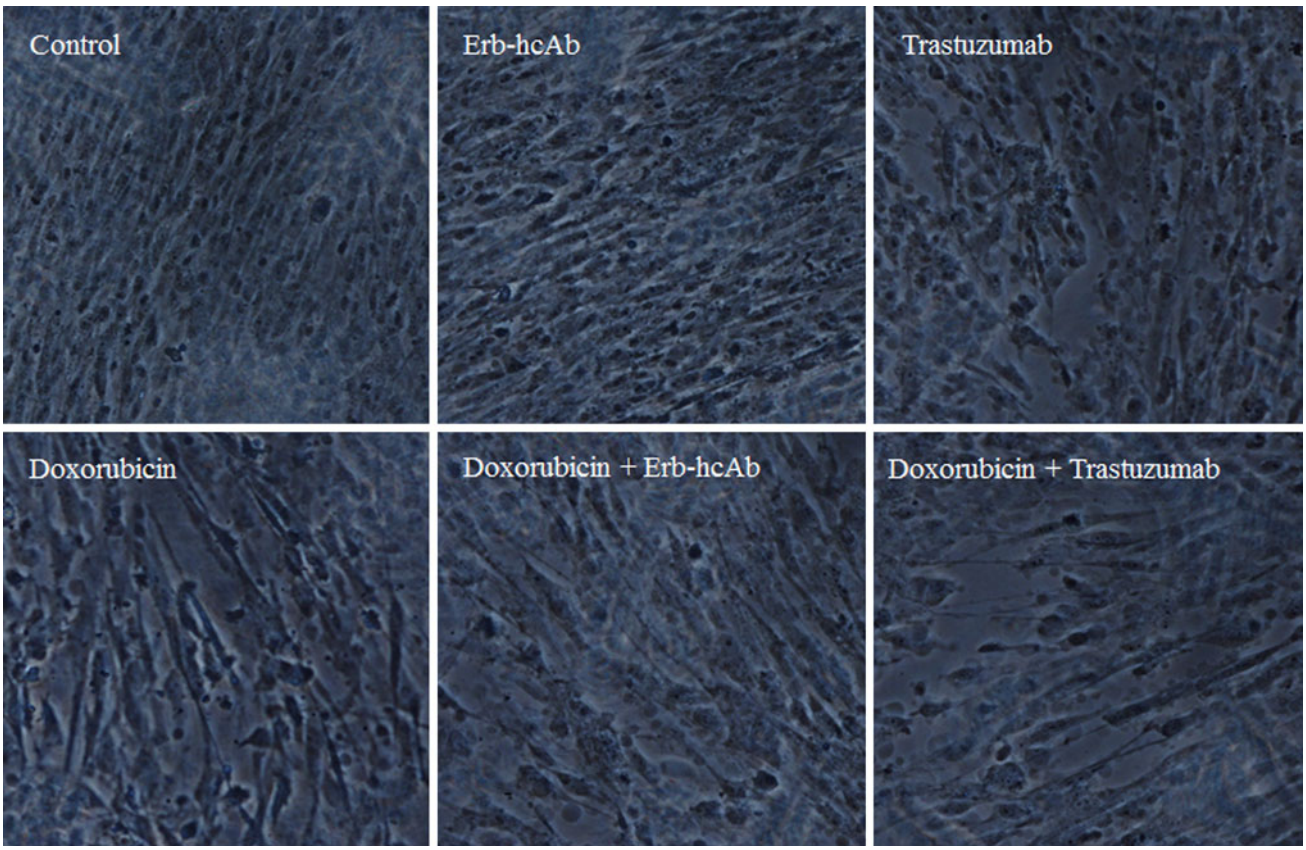

Fig. 3 Effects on cell morphology of human fetal cardiomyocytes (CFH) grown for 3 days in the absence (control) or in the presence of Trastuzumab, Erb-hcAb, doxorubicin, or with the combinations of each antibody with doxorubicin. Magnification 1:40

in Fig. 7, cardiac fibrosis was significantly increased in mouse hearts treated either with Lapatinib or 2C4 for 7 days with respect to control, untreated mice.

As previously reported [20], Trastuzumab and doxorubicin also were found to induce cardiac fibrosis at higher extent than that shown in mice untreated or treated with EDIA.

Discussion

In the past few decades, novel anticancer drugs have been successfully developed that effectively induce tumor regression or growth delay, thus prolonging patient's survival time. However, cardiovascular obnoxious side effects of the new anticancer drugs can lead to therapy-related heart failure. Although cardiac problems were predictable for anthracyclines, they were instead totally unexpected from highly targeted anticancer agents, such as Trastuzumab or tyrosine kinase inhibitors.

Since its registration by the Food and Drug Administration (FDA) in 1998, Trastuzumab (Herceptin®, Genentech, San Francisco, CA) has been administered to treat more than 450,000 women with breast BC worldwide [24]. As a monoclonal antibody directed against the human epidermal growth factor receptor-2 (HER-2), Trastuzumab was initially shown to prolong the survival of women with HER-2-positive advanced BC [4]. In 2005, landmark adjuvant studies demonstrated that adjuvant Trastuzumab either after, or in combination with, chemotherapy reduced the risk of relapse by approximately 50% and the risk of death by 33% for women with HER-2 positive early BC [25].

Cardiac toxicity was recognized as an important side effect at an early stage in the development of Trastuzumab. Manifested as symptomatic congestive heart failure (CHF) or asymptomatic left ventricular ejection fraction (LVEF) decline, Trastuzumab-induced cardiotoxicity has been attributed to blockade of HER-2 signaling in cardiac myocytes. The cardiac safety of anti-HER-2 therapy is likely to be agent specific, as the early clinical experience with Lapatinib, a dual tyrosine kinase inhibitor of the EGFR and HER-2 receptors, suggests that it may produce less cardiotoxicity compared with Trastuzumab. However, more studies are needed to better characterize the cardiac effects of Lapatinib, since the patient population studied was heterogeneous and highly selected, limiting the conclusions that can be drawn from this early data [26].

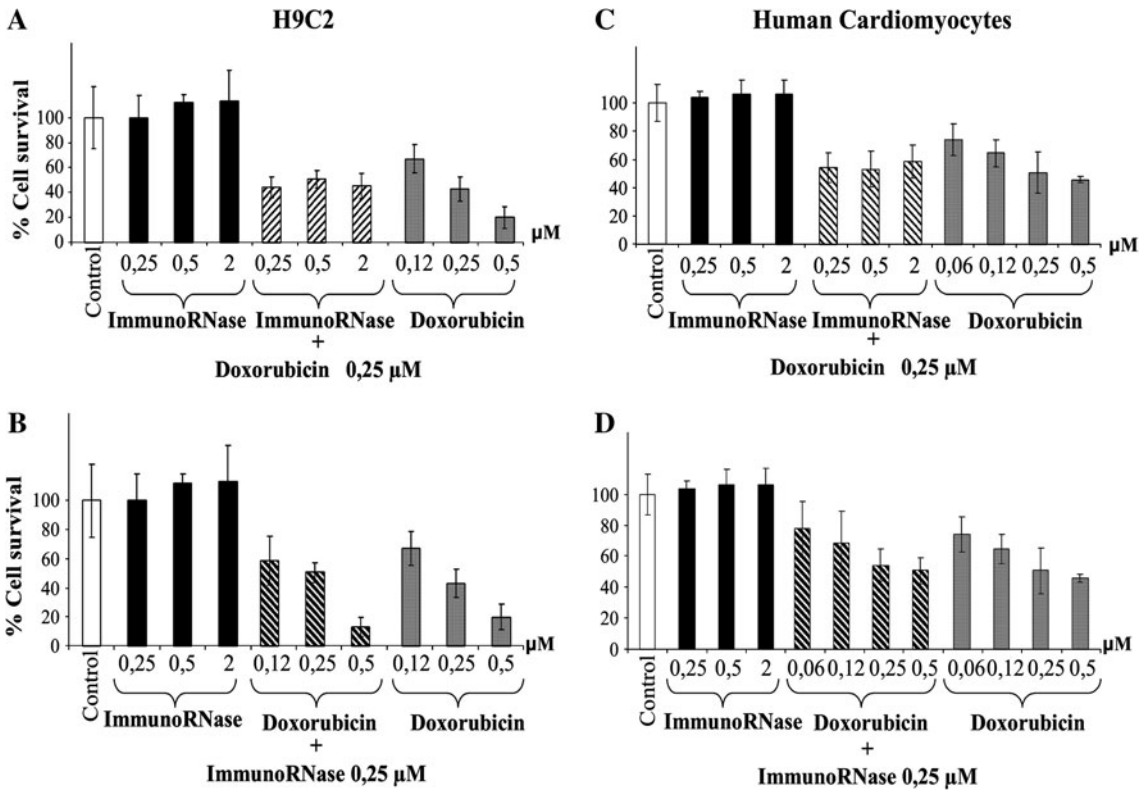

Fig. 4 Effects of combination treatment of Erb-hRNase with doxorubicin on human fetal cardiomyocytes (CFH) and H9C2 cardiomyoblasts. Dose–response tests of the indicated cells treated for 24 h with Erb-hRNase alone (*black bars*), doxorubicin alone (*gray bars*), or with the combination of Erb-hRNase and doxorubicin (*striped bars*)

A new anti-ErbB2 monoclonal antibody which recognizes an epitope distant from that of Trastuzumab (in the extracellular portion of ErbB2), Pertuzumab, does not seem to be significantly cardiotoxic [11], but data are still very preliminary, since it is still being tested in pivotal clinical trials.

This awareness has led, on the one hand, to the search for novel and safe antitumor drugs devoid of cardiac side effects, and to the development of novel noninvasive but more sensitive methods for the early screening of cardiac dysfunction, on the other.

In this article, we report on a comparative analysis of the cardiotoxic effects of EDIA, the novel human immunoagents targeting a novel epitope of ErbB2 [18, 19], different from those of other anti-ErbB2 antibodies or inhibitors currently in clinical use or trials for BC therapy.

We show here for the first time that EDIA do not show in vitro toxic effects on human fetal cardiomyocytes in stark contrast to the prototypical antibody Trastuzumab, 2C4 (Pertuzumab), and Lapatinib, an anti-ErbB2 drug which inhibits directly its intracellular tyrosine kinase domain. Similar results were obtained when EDIA were tested in combination with doxorubicin, as they did not show additive toxic effects. Different results were obtained with Trastuzumab which instead increased the toxicity of doxorubicin, according to previous studies [4].

It was also previously reported in the literature [12] that Trastuzumab is toxic to cultured human atrial cardiac microfragments maintained by co-culture with feeder cells from newborn rat heart, as it caused a complete loss of their healthy beating phenotype and tissue structure. Antibody-mediated inhibition of ErbB2 might regulate mitochondrial integrity through the Bcl-X proteins, leading to ATP depletion and contractile dysfunction without profound changes in myocyte ultrastructure [8].

As for the different effects on cardiomyocytes of not toxic Erb-hcAb, and strongly toxic Trastuzumab, our previous data on rat cardiomyocytes indicate that they can be ascribed to their different apoptotic properties. Trastuzumab induces apoptosis in cardiac cells, as it lowers the level of Bcl-XL and activates caspase 3, whereas Erb-hcAb does not induce apoptosis [20].

Another possible explanation for the lack of cardiotoxicity of EDIAs is that they recognize an epitope distinct from that of Trastuzumab [18, 19], which may differently engage the receptor to affect the basal cardiomyocyte survival pathway.

Mouse models were then employed to monitor the in vivo cardiotoxicity of the anti-ErbB2 drugs and to validate

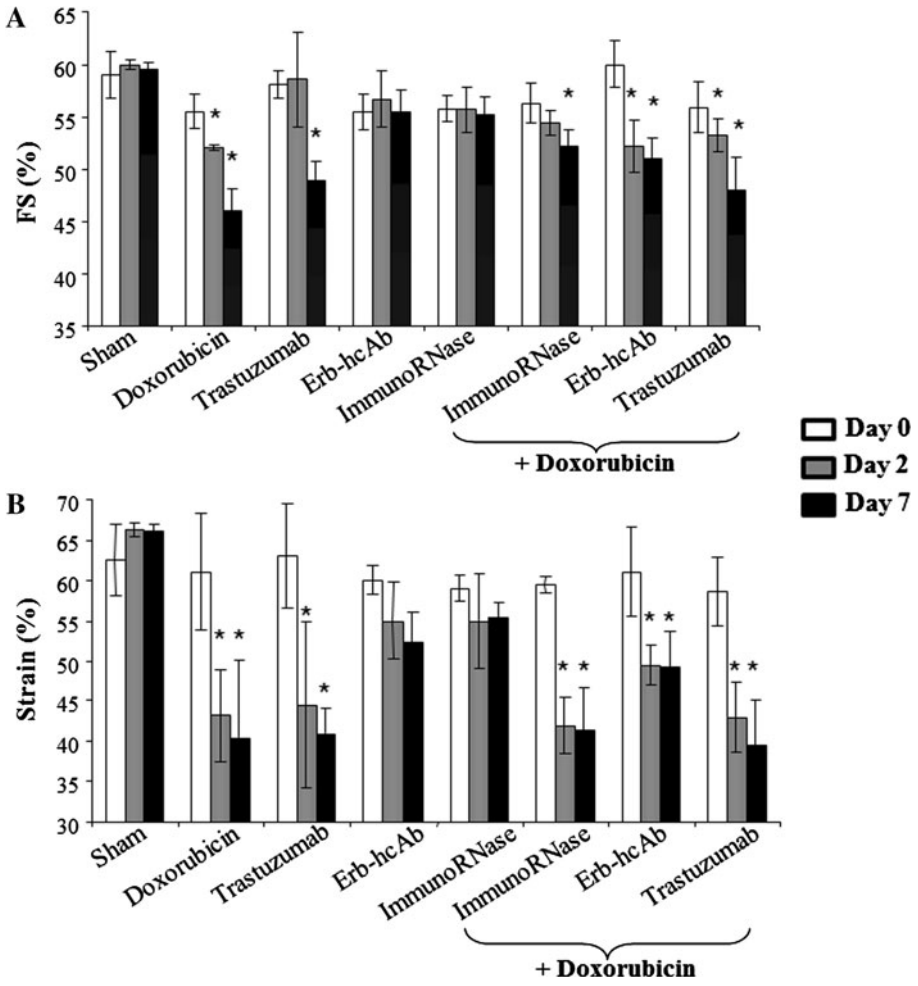

Fig. 5 In vivo effects of combinations of anti-ErbB2 immunoagents with doxorubicin on heart function. Relative FS (Fractional Shortening) and radial strain (RS) are reported before or after the treatment of mice for 2 or 7 days with immunoRNase, Trastuzumab, or Erb-hcAb, used as single agents or in combination with doxorubicin. $*P \leq 0.05$

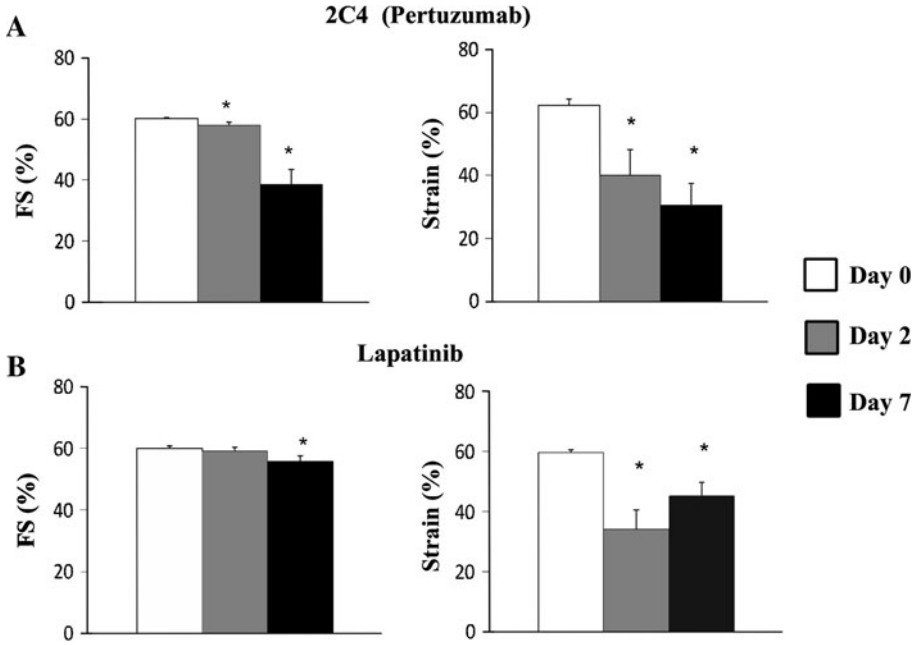

Fig. 6 In vivo effects of 2c4 (Pertuzumab) and Lapatinib on heart function. Relative FS (Fractional Shortening) and radial strain (RS) are reported before or after the treatment of mice for 2 or 7 days with the anti-ErbB2 2C4 mAb antibody (**a**, $*P \leq 0.02$) or with Lapatinib, the Tyrosine Kinase inhibitor (**b**, $*P \leq 0.04$)

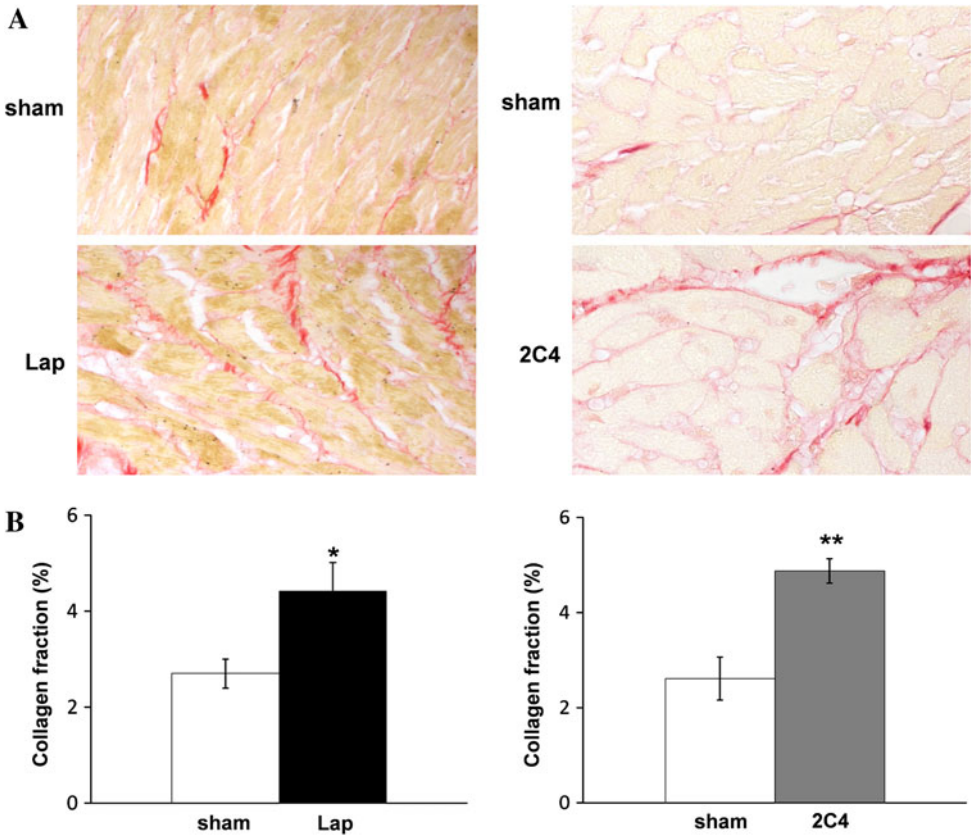

Fig. 7 Analysis of cardiac fibrosis. **a** Representative photomicrographs of LV (Left Ventricular) sections from mice treated with the indicated drugs. **b** Quantification of the interstitial fibrosis expressed as relative percentage of color intensity. (*$P = 0.02$; **$P = 0.008$ vs. sham)

speckle tracking echocardiography as a sensitive measure of myocardial function in animal models. EDIA did not impair cardiac function in vivo as they did not affect RS and FS. The latter parameter was evaluated in this study for the first time after the treatment of mice with EDIA to assess whether it could be used as a more sensitive method to predict cardiac dysfunction.

Indeed, in mice treated with Trastuzumab, 2C4, or Lapatinib alterations of RS preceded the reduction of conventional echocardiography markers, such as FS and LVEF. In particular, Trastuzumab in a fashion similar to doxorubicin reduced RS at 2 days and FS at 7 days of treatment compared to the sham group.

Similar results are shown here for 2C4 (Pertuzumab) and Lapatinib, as previously reported in the literature [9, 11] as anti-ErbB2 drugs less cardiotoxic than Trastuzumab, thus confirming that the limited number of clinical studies performed with these newer agents only provide us with partial and very preliminary results. Indeed RS, as evaluated by speckle tracking, was capable of identifying early cardiotoxicity after only 2 days of treatment with either 2C4 or Lapatinib.

As of today, traditional echocardiographic indexes of cardiac function such as FS and EF (ejection fraction) can identify patients in which Trastuzumab toxicity is already evident. Also, troponins proposed by other authors [27] could already have been elevated after anthracyclines, and therefore, the secondary administration of Trastuzumab does not act as the cause of myocardial stress, but only as a modulator of anthracyclines' stress [28]. At the moment, besides the recognition of cardiac risk factors, there is not yet a bona fide noninvasive tool that can discriminate patients who will develop cardiotoxicity by treatment with Trastuzumab. A very interesting study [29] has recently indicated that Doppler Tissue Imaging (DTI) can detect early LV dysfunction before alterations seen in conventional echocardiographic indices in an animal model of anthracycline- and Trastuzumab-mediated cardiomyopathy. Of course, further studies will be needed to validate DTI and RS as useful tools in these settings.

In conclusion, the present in vitro and in vivo studies confirm that EDIA may fulfill the therapeutic need of patients ineligible to Trastuzumab treatment because of cardiac dysfunction, and strongly indicate that RS, measured by Speckle Tracking echocardiography, could become a reliable marker for early detection of myocardial subtle changes, predicting cardiac dysfunction in advance.

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Conflict of interest The authors declare that they have no conflict of interest.

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