Trastuzumab Induces an Immediate, Transient Volume Increase in Humans: A Randomised Placebo-Controlled Trial

Joannes A.A. Reijers*, Jacobus Burggraaf

Centre for Human Drug Research, Leiden, The Netherlands

1. Introduction

Trastuzumab (Herceptin®) is widely and successfully used in the treatment of patients with solid tumours overexpressing the human epidermal growth factor receptor-2 (HER2, also known as ErbB2) most notably with mammary carcinoma or metastatic gastric cancer. Notwithstanding its widespread use in oncology, trastuzumab is feared for its association with cardiotoxic side effects, occurring in 1–7% of treated patients, depending on the concomitant and previous chemotherapy regimens (Garcia-Alvarez et al., 2010; Seidman et al., 2002).

The exact mechanism by which trastuzumab causes cardiac side effects is not completely unravelled. Existing evidence suggests that interaction with the HER2-signalling pathway by trastuzumab in cardiomyocytes, induces apoptosis, and interferes with cell survival mechanisms (Fuller et al., 2008; Gordon et al., 2009; Riccio et al., 2009). Compatible with these in vitro findings, electron microscopy evaluation of endocardial biopsies from patients who developed trastuzumab-associated cardiomyopathy showed ultrastructural changes in the mitochondria (Guarneri et al., 2006). It is, however, unknown how these findings translate into clinical practice. The main reason for this uncertainty is that trastuzumab is often administered in an adjuvant setting, in combination with or after previous use of radiation therapy or cytostatics with untoward cardiac effects, such as anthracyclines. Furthermore, trastuzumab is used in a heterogeneous population regarding gender, age, and co-morbidities. Seemingly, therefore, exploring trastuzumab in a homogenous population of healthy subjects could be of value to further delineate its cardiac effects and its time.

We recently performed a bio-equivalence trial in which the current-ly approved formulation of trastuzumab (Herceptin®) was compared with a trastuzumab drug product under development, code-named...
FTMB (Wisman et al., 2014). Aside from establishing bio-equivalence, serial assessments of echocardiographic measurements, body weight and laboratory parameters such as the N-terminal pro-peptide of B-type natriuretic peptide (NT-proBNP) were included in the trial design, both to safeguard the participant’s well-being and to investigate the (cardiotoxic) side effects of trastuzumab. The aim of the analysis presented in this article was to compare the registered form of trastuzumab (Herceptin®) with placebo in healthy volunteers, in terms of the assessments of cardiac function, and thus to cardiotoxicity.

2. Methods

2.1. Study Design and Population

The trial was a single-centre study of parallel design that consisted of a placebo-controlled double-blind dose escalation scheme (Fig. 1, groups 1–4), and an open-label single-dose bio-equivalence part (Fig. 1, group 5) (Wisman et al., 2014). In total, 118 male volunteers, aged 18–45 years inclusive, who were deemed healthy after a full medical screening, were enrolled sequentially in one of five groups. All had a left ventricle ejection fraction (LVEF) >55%, measured with echocardiography. The study was approved by an accredited local ethics committee (BEBO, Assen, The Netherlands) and national independent medical ethics committee (CCMO, The Hague, The Netherlands), and registered under NL37452·056·11/EudraCT 2011-002972-17. Each participant provided written informed consent.

Participants randomly received either placebo (250 mL 0·9% NaCl) or trastuzumab in 250 mL 0·9% NaCl, administered intravenously in 90 min. Two trastuzumab drug products were investigated: the registered form (Herceptin®) at a dose of 6 mg/kg (n = 46), and a biosimilar form, codenamed FTMB, in escalating doses of 0·5–6 mg/kg (n = 64). For the purpose of assessing the cardiac effects of trastuzumab, only participants who received the registered form of trastuzumab (Herceptin®, hereafter referred to as “trastuzumab”) or placebo were analysed.

2.2. Randomisation and Masking

All participants were assigned a unique number. Study medication was dispensed by a pharmacist according to a pre-established computer generated sequence, prepared using SAS® (v9·1·3, SAS Institute Inc., Cary, NC, USA) by a statistician, both of whom were not involved in the clinical conduct of the study. Concealment of treatment allocation was thus implemented. Placebo and trastuzumab treatment looked alike.

2.3. Laboratory Assessments and Body Weight

At baseline and at 1, 2, 4, 8 and 63 ± 7 days post-treatment, body weight was determined on a calibrated scale and samples for routine clinical chemistry and haematology were collected. An additional sample for troponin-T and NT-proBNP only was taken at 8 h post-dose. Samples were analysed by the clinical laboratories of the Leiden University Medical Center (LUMC). The measured laboratory parameters included electrolytes, liver panel, urea, creatinine, albumin, total protein, lipid spectrum, complete blood count and leukocyte differential, troponin-T, and NT-proBNP (N-terminal pro-peptide of B-type natriuretic peptide).

Measurement of body weight in the follow-up period and laboratory assessments at 8 h and at 1, 2, and 8 days post-administration were incorporated in the clinical trial protocol with an amendment after the

---

Fig. 1. Participant flow diagram. Flow of participants: enrolment was sequential in one of five groups (see main body); echocardiographic examinations were available for groups 1–4, laboratory results and body weight data were available for groups 2–5. Cohorts marked with an asterisk were not analysed, although baseline effects were included in the secondary analysis on the extended dataset (see main body). BS biosimilar product of trastuzumab.
study had commenced. Subsequently, laboratory and body weight data were only available in groups 2–5 (Fig. 1).

2.4. Echocardiography

Echocardiography was performed at the department of cardiology of the LUMC by trained laboratory technicians using an E-9 system (GE Healthcare, Horten, Norway) under responsibility of a cardiologist (blinded to the treatment). Two-dimensional, colour, continuous and pulsed-wave Doppler images were acquired in the parasternal and apical views. The acquired measurements were specified in a separate protocol, and focused on obtaining information on the global systolic and diastolic left ventricular function. From parasternal M-mode recordings, left ventricular ejection fraction (LVEF) and fractional shortening (FS) were measured. Additionally, from the pulsed-wave Doppler spectral signal of the mitral inflow acquired in the apical 4-chamber views the peak early (E) and late (A) velocities and deceleration time (DT) of the E wave were measured and the E/A-ratio was derived. Furthermore, from pulsed-wave tissue Doppler recordings obtained at the level of the left ventricular wall the systolic annular velocity of the lateral wall (S′; TDI) and early diastolic annular velocity of the lateral wall (E′; TDI) were measured. Finally, right ventricular systolic function was assessed by means of tricuspid annular plane systolic excursion (TAPSE).

All participants underwent echocardiographic examination at screening (within 21 days of trial drug administration). Follow-up examinations were performed at $4 \pm 1$ and $63 \pm 14$ days post-dose in the first part of the trial (Fig. 1, groups 1–4), including all placebo participants and 9 participants receiving trastuzumab.

2.5. Statistical Analysis

The trial was powered on the bio-equivalence (pharmacokinetic) endpoint; however, the calculated sample size of 46 participants in the trastuzumab arm, and 8 placebo subjects was deemed sufficient to detect clinically relevant hemodynamic changes.

Individual time profiles and treatment group averages were studied for all assessed parameters. Treatment groups were compared using mixed effect analyses of variance. This analysis incorporated participants as random factor, treatment as fixed factor, and as covariates baseline value and age. Time and treatment by time were included as well, in the delta time component and denoted mAUC and mAUCcfb respectively, served as a measure of the average post-treatment value— or average change from baseline, in case of mAUCcfb. Unless otherwise specified, hereafter, mAUC and mAUCcfb relate to the AUC at 4 days post-administration.

Values below the limit of quantification (LOQ, 0 · 003 μg/L for troponin-T and 5 ng/L for NT-proBNP) were inputted as 0 · 002 μg/L and 3 ng/L respectively. Missing baseline observations were replaced with the corresponding screening values ($\pm 21$ days before baseline).

Echocardiographic examinations not occurring on the scheduled day were excluded from analysis. To meet the assumption of normal distribution, LVEF, FS, DT, and TAPSE values were ln-transformed prior to statistical analysis.

Descriptive statistics and graphs were produced using R (v2·15·2, R Foundation for Statistical Computing, Vienna, Austria, 2012 [R Development Core Team, 2012]), and statistical analyses were performed with SAS® (v9·1·3, SAS Institute Inc., Cary, NC, USA). Results are presented as mean (standard deviation or 95% confidence interval) for continuous data and as number (percentage) for categorical data, unless otherwise specified.

2.6. Role of the Funding Source

The bioequivalence trial (Wisman et al., 2014) was funded by Synthovia BV, Nijmegen, The Netherlands; and included the measurements to investigate potential cardiotoxicity and haemodynamic effects in its design. However, Synthovia BV was not involved in the analysis as described here or in the writing of the manuscript.

3. Results

Between August and November 2011, one hundred eighteen (118) males were recruited from the community (Fig. 1). The baseline characteristics are presented in Table 1.

From all participants, who received placebo or trastuzumab (Fig. 1), 261 routine chemistry and haematology follow-up observations were included in the analysis, as well as 30 follow-up echocardiographic examinations, 259 body weights, and 316 measurements for NT-proBNP (137 < LOQ) and Troponin-T (287 < LOQ) levels. No baseline result was missing.

Table 1

| Parameter                          | All* (n = 110) | Trastuzumab (n = 46) | Placebo (n = 6) |
|-----------------------------------|---------------|----------------------|-----------------|
| **Demographics**                  |               |                      |                 |
| Age (year)                        | 25.4 (6.7)    | 24.1 (5.8)           | 24.8 (3.1)      |
| Body weight (kg)                  | 77.2 (10.4)   | 76.9 (10.2)          | 72.5 (6.1)      |
| **Routine clinical haematology and chemistry** |               |                      |                 |
| Erythrocytes (10$^12$ L$^{-1}$)   | 4.88 (0.35)   | 5.01 (0.41)          | 5.04 (0.18)     |
| Thrombocytes (10$^9$ L$^{-1}$)    | 219 (43)      | 220 (38)             | 244 (41)        |
| Haemoglobin (mm)                  | 9.2 (0.5)     | 9.1 (0.5)            | 9.6 (0.4)       |
| Haematocrit (L/L)                 | 0.435 (0.0220)| 0.434 (0.021)        | 0.446 (0.0196)  |
| Total protein (g L$^{-1}$)        | 71.1 (3.6)    | 70.9 (3.7)           | 72.8 (4.1)      |
| Albumin (g L$^{-1}$)              | 46.4 (2.2)    | 46.3 (2.0)           | 48.5 (2.8)      |
| Creatinine (μM)                   | 76.7 (9.5)    | 77.0 (9.7)           | 78.8 (8.5)      |
| **Cardiac biomarkers**            |               |                      |                 |
| NT-proBNP (ng L$^{-1}$)           | 12.1 (14.5)   | 11.6 (14.3)          | 3.0 (0.0)       |
| NT-proBNP – LOQ (n)               | 51 (46.45)    | 20 (43.53)           | 6 (100.0%)      |
| Troponin-T – LOQ (n)              | 102 (92.7)    | 42 (91.3%)           | 5 (83.3%)       |
| Mean (SD) or number of subjects (percentage). * Including also the baseline values of the participants receiving the biosimilar product. LVEF left ventricular ejection fraction; FS fractional shortening; E/A ratio of peak early (E) and late (A) velocities; DT deceleration time; S′ systolic annular velocity of the lateral wall (pulsed-wave tissue Doppler); E′ early diastolic annular velocity of the lateral wall (pulsed-wave tissue Doppler); and TAPSE tricuspid annular plane systolic excursion.

Mean (SD) or number of subjects (percentage). * Including also the baseline values of the participants receiving the biosimilar product. LVEF left ventricular ejection fraction; FS fractional shortening; E/A ratio of peak early (E) and late (A) velocities; DT deceleration time; S' systolic annular velocity of the lateral wall (pulsed-wave tissue Doppler); E' early diastolic annular velocity of the lateral wall (pulsed-wave tissue Doppler); and TAPSE tricuspid annular plane systolic excursion.
Forty participants (76 · 9%) had troponin-T levels below the LOQ during the entire follow-up period; and in the remaining participants, changes post-treatment could not be observed. Troponin-T results were therefore not analysed statistically. For NT-proBNP, the corresponding number was 4/52 (7 · 7%) participants, of whom 3/6 in the placebo group.

The time course of a selection of parameters after infusion of 6 mg/kg Herceptin® in a 22 year old healthy male is depicted in Fig. 2. A clear decline in erythrocyte concentration (and related parameters, like haemoglobin and haematocrit) characterises the first 2–4 days post-administration, with a return to baseline between 8 and 63 days post-dose. This decline was paralleled by a decrease in protein and albumin concentration, whereas body weight and NT-proBNP level increased.

Tables 2 and 3 present the results of selected laboratory parameters and body weight at 4 days post-administration. Compared to placebo, the haematocrit decreased on average with 0 · 013 L/L (mAUC, \( p \approx 0.02 \)) as did the erythrocyte, thrombocyte, and haemoglobin concentrations (Fig. 3). Similarly, the total protein and albumin concentrations declined, with a difference in mAUC of 2 g/L between placebo and trastuzumab. These differences were statistically significant (\( p < 0.05 \)), with the exception of the creatinine and erythrocyte concentrations.

Body weight increased with 0 · 7 (0 · 6) kg (Fig. 3), although compared to placebo the difference was less, mAUC 0 · 4 kg (–0 · 2–0 · 9, \( p = 0.013 \)). The only echocardiographic parameter that changed significantly was the E/A-ratio, which differed from placebo 0 · 57 (0 · 21–0 · 93, \( p = 0.0168 \)). The deceleration time (DT) and TAPSE values increased with a mean difference versus placebo of respectively 31 · 0 % and 9 · 9 %, whereas S’ and E’ values remained stable (Fig. 4).

Clinically significant changes or trends on either individual or group level could not be discerned for any of the other laboratory parameters. All participants had returned to baseline by either 8 or 63 ± 7 days post-treatment (Fig. 3).

Treatment effects on the echocardiographic parameters were less straightforward (Table 4). The LVEF increased relatively by 1 · 9% compared to placebo. Fractional shortening (FS) followed the LVEF closely. The only echocardiographic parameter that changed significantly was the E/A-ratio, which differed from placebo 0 · 57 (0 · 21–0 · 93, \( p = 0.0443 \)) at 3–5 days post-dose.

In the analysis on the extended dataset, including also the baseline effects of participants who received the biosimilar product, all contrasts between trastuzumab and placebo remained virtually unaltered (Tables 3 and 4), with the exception of the contrast for DT. Probably the baseline variability on this parameter was greater in the entire study population than in the trastuzumab arm (Table 1). Treatment
effects on other echocardiographic parameters (e.g. LVEF, E/A-ratio, TAPSE) also became less pronounced.

Age influenced the results significantly only for DT (effect $-0.0255$ ms [−0.0443, −0.0067] per annum, $p = 0.0122$). In the analysis on the extended dataset the age effect on DT disappeared, whereas a significant age effect was detected on $E\prime$ (effect $-0.0021$ m/s [−0.0036, −0.007] per annum, $p = 0.0051$). However, excluding this covariate did not improve the model, or modify the results of the analysis significantly.

4. Discussion

This is the first report of effects of trastuzumab on the cardiac function in a homogeneous healthy population, as well as the first to evaluate these effects during the first eight days after administration by means of routine laboratory and echocardiographic assessments which are commonly employed in the clinic to monitor patients with cardiomyopathy.

Fig. 2 captures the main findings following trastuzumab administration. The combination of changes, their time-profile, and the absence of signs of haemolysis or blood loss (other than the per protocol blood collections), indicate that there was haemodilution caused by fluid retention, given the concomitant increase in body weight. Such a pattern was noted in many participants treated with trastuzumab, although on a group level, the effects were less pronounced. Most importantly, none of these participants experienced cardiac symptoms of any nature (Wisman et al., 2014).

It is known that the ErbB2-receptor functions as a co-receptor which heterodimerises with other activated ErbB-class receptors. This dimerisation process is crucial for the initiation of downstream signalling of all EGF-receptors, but also decreases ligand dissociation as well as stabilises the dimer, thereby prolonging the activation of the signalling pathway (Fuller et al., 2008).
Binding of the ErbB2/ErbB4 receptors by Neuregulin-1 (NRG-1) stimulates proliferation, contractility and survival of cardiomyocytes and interference by for example trastuzumab is thought to compromise anti-apoptotic pathways, which lessens the counterbalance to noxious stimuli (Fuller et al., 2008; Force et al., 2007; Lemmens et al., 2007). Our results are in line with these preclinical findings and indeed confirm, as was suggested by in vitro experiments (Riccio et al., 2009), that already during the first days after trastuzumab administration haemodynamic effects can be noticed. Obviously, this assumes that the effect is receptor-mediated, ignoring reports which have implied other mechanisms of action (Riccio et al., 2009; Force et al., 2007; Troise et al., 2011).

The observed change in volume status can be explained by an activation of the renin-angiotensin-aldosterone system (RAAS). Whether or not this is a secondary (compensatory) response to maintain an adequate effective circulating volume, or a primary response, caused by direct interference with ErbB2/ErbB4-signalling by trastuzumab, cannot be determined based on our results. Interactions between angiotensin II and ErbB-signalling have been described previously (Fuller et al., 2008), and this observation further supports treatment of trastuzumab-associated cardiomyopathy with ACE-inhibitors.

Even though the on average modest effects were clearly distinguishable for the laboratory parameters, changes in echocardiographic parameters could not be detected, with the exception of a concise effect on the E/A-ratio. However, most likely, this observation reflects the changed volume status, and in itself does not indicate clinically relevant cardiotoxicity. This is in keeping with the finding that the TDI measurements, which are less load-dependent, remained unchanged.

A substantial and lasting decline in LVEF, as well as other earlier radiographic findings, generally first occur after months of continuous trastuzumab exposure in a population more predisposed than healthy volunteers (Fallah-Rad et al., 2011; Morris et al., 2013; Goldhirsch et al., 2013). Probably, these abnormalities already mark the progression to chronic heart failure (CHF) after the ‘normal’ compensatory mechanisms towards trastuzumab’s effects have been exhausted. We hypothesise therefore that monitoring patients in the early days after trastuzumab treatment with routine laboratory parameters and body weight measurements, as used in this trial – but also with more sensitive markers of RAAS-activation, like urinary sodium excretion and hormone levels – could be valuable in establishing an individual patient’s susceptibility to trastuzumab-induced haemodynamic effects.

Cardiospecific biomarkers are well recognised as predictors of drug-induced cardiotoxicity. For example, NT-proBNP elevation is associated with the development of cardiac dysfunction (Feola et al., 2011; Sandri et al., 2005), although others could not detect a significant change over a 12 months treatment period with trastuzumab, even in the subset of patients who developed CHF (Fallah-Rad et al., 2011; Sawaya et al., 2012). However, many of the trastuzumab reports have focused on long term effects, and did not include assessments during the first days. In individual cases, a peak in NT-proBNP concentration, coinciding with the maximal effect in terms of haemodilution and body weight (Fig. 2), was observed in this study. On a group level, this did not result in a significant difference compared to placebo, probably in consequence of the small number of participants having detectable NT-proBNP levels.

Contrary to our results, transient increases in cardiac-specific troponin have been observed in 12–50% of patients following trastuzumab treatment, most frequently after the first cycle, and troponin-I was found to be the strongest independent predictor of cardiotoxicity in multivariate analyses (Cardinale et al., 2010; Ky et al., 2014; Morris et al., 2013).
et al., 2011). However, it should be noted that in these studies trastuzumab administration was part of a treatment regime which included cytostatics.

The main strength of this trial lies in the relatively large homogeneous population and its randomised, placebo-controlled design. The fact that only healthy volunteers participated ensured that the results were not clouded by comorbidities or concomitant and previous (cardiotoxic) treatments. Furthermore, the used assessments are commonly employed to monitor patients with cardiomyopathy, which makes the findings easy implementable in clinical practice, without increasing costs.

An important weakness of the analysis is the comparison to a relatively small placebo group, which consisted of six subjects for body weight and laboratory data. This could potentially skew the placebo population, obscuring trastuzumab’s effects. Because of the placebo condition as control group, it could also be argued that the observed volume increase is a non-specific effect caused by the administration of a monoclonal antibody. However, experience with intravenous administration of (therapeutic) doses of both experimental and registered monoclonal antibodies in healthy volunteers has revealed neither elevations in body weights nor haemodilution, either in the short or long-term (data on file). Interestingly though, increases in total protein and albumin were observed during the first week after administration in both the placebo and monoclonal antibody arms of those trials, which is similar to the profiles in the placebo treated subjects as presented in Fig. 3.

In conclusion, the presented results suggest that trastuzumab administration in humans is associated with an immediate, transient volume increase, either as a primary (direct) or a secondary (compensatory) response, which can be detected easily using routine clinical assessments. Echocardiographic changes, both short and long term, could not be found after single dose administration to drug-naive patients.

With the arrival of more biosimilars, the future will likely see an increase in relatively large bio-equivalence trials in healthy volunteers. This offers unique opportunities to evaluate, in a detailed and systematic fashion, intentional and unintentional effects of existing drugs in a homogeneous population.

Contributors

JR and JB conceived the experiment and incorporated it in the bio-equivalence design. The clinical trial was executed at Centre for Human Drug Research, under responsibility of JB. JR and JB analysed the data and wrote the manuscript.

Conflicts of Interest

None to declare.

Acknowledgements

The authors wish to thank Dr. Victoria Delgado from the Department of Cardiology of the Leiden University Medical Center for her assistance in obtaining and analysing the echocardiographic results.

References

Cardinale, D., Colombo, A., Torrisi, R., et al., 2010. Trastuzumab-induced cardiotoxicity: clinical and prognostic implications of troponin I evaluation. J. Clin. Oncol. 28, 3910–3916.
Fallah-Rad, N., Walker, J.R., Wasef, A., et al., 2011. The utility of cardiac biomarkers, tissue velocity and strain imaging, and cardiac magnetic resonance imaging in predicting early left ventricular dysfunction in patients with human epidermal growth factor receptor ii-positive breast cancer treated with adjuvant trastuzumab therapy. J. Am. Coll. Cardiol. 57, 2263–2270.
Fedda, M., Garone, O., Occelli, M., et al., 2011. Cardiotoxicity after anthracycline chemotherapy in breast carcinoma: effects on left ventricular ejection fraction, troponin I and brain natriuretic peptide. Int. J. Cardiol. 148, 194–198.
Force, T., Krause, D.S., Van Etten, R.A., 2007. Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. Nat. Rev. Cancer 7, 332–344.
Fuller, S.J., Sivarajah, K., Sugden, P.H., 2008. ErbB receptors, their ligands, and the consequences of their activation and inhibition in the myocardium. J. Mol. Cell. Cardiol. 44, 831–854.
García-Alvarez, A., García-Albeniz, X., Esteve, J., Rovira, M., Bosch, X., 2010. Cardiotoxicity of tyrosine-kinase-targeting drugs. Cardiovasc. Hematol. Agents Med. Chem. 8, 11–21.
Goldhirsch, A., Gelber, R.D., Piccart-Gebhart, M.J., et al., 2013. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): an open-label, randomised controlled trial. Lancet 382, 1021–1028.
Gordon, L.L., Burke, M.A., Singh, A.T., et al., 2009. Blockade of the erbB2 receptor induces cardiomyocyte death through mitochondrial and reactive oxygen species-dependent pathways. J. Biol. Chem. 284, 2080–2087.
Guarneri, V., Lenihan, D.J., Valero, V., et al., 2006. Long-term cardiac tolerability of trastuzumab in metastatic breast cancer: the M.D. Anderson Cancer Center experience. J. Clin. Oncol. 24, 4107–4115.
Ky, B., Pert, M., Sawaya, H., et al., 2014. Early increases in multiple biomarkers predict subsequent cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab. J. Am. Coll. Cardiol. 63, 809–816.
Lemmens, K., Doggen, K., De Keulenaer, G.W., 2007. Role of neuregulin-1/ErbB signaling in cardiovascular physiology and disease: implications for therapy of heart failure. Circulation 116, 954–960.
Morris, P.G., Chen, C., Steingart, R., et al., 2011. Troponin I and C-reactive protein are commonly detected in patients with breast cancer treated with dose-dense chemotherapy incorporating trastuzumab and lapatinib. Clin. Cancer Res. 17, 3490–3499.
Morris, P.G., Iyengar, N.M., Patil, S., et al., 2013. Long-term cardiac safety and outcomes of dose-dense doxorubicin and cyclophosphamide followed by paclitaxel and trastuzumab with and without lapatinib in patients with early breast cancer. Cancer 119, 3943–3951.
Riccio, G., Esposito, G., Leoncini, E., et al., 2009. Cardiotoxic effects, or lack thereof, of anti-ErbB1 immunoagents. FASEB J. 23, 3171–3178.
Sawaya, H., Cizewski, D., Cassano, A., et al., 2006. Long-term troponin I elevation in women with early breast cancer treated with adjuvant trastuzumab therapy. J. Clin. Oncol. 24, 4107–4115.
Troise, F., Monti, M., Merlini, A., et al., 2011. A novel ErbB2 epitope targeted by human an-titumor immunoagents. FEBS J. 278, 1156–1160.
Wisman, L.A., De Cock, E.P., Reijers, J.A., et al., 2014. A phase I dose-escalation and bioequivalence study of a trastuzumab biosimilar in healthy male volunteers. Clin. Drug Investig. 34, 887–894.