Trastuzumab containing regimens for early breast cancer (Review)

Moja L, Tagliabue L, Balduzzi S, Parmelli E, Pistotti V, Guarneri V, D'Amico R

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2012, Issue 4

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[Intervention Review]

Trastuzumab containing regimens for early breast cancer

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Editorial group: Cochrane Breast Cancer Group.
Publication status and date: New, comment added to review, published in Issue 4, 2012.
Review content assessed as up-to-date: 1 February 2010.

Citation: Moja L, Tagliabue L, Balduzzi S, Parmelli E, Pistotti V, Guarneri V, D'Amico R. Trastuzumab containing regimens for early breast cancer. *Cochrane Database of Systematic Reviews* 2012, Issue 4. Art. No.: CD006243. DOI: 10.1002/14651858.CD006243.pub2.

ABSTRACT

Background

Approximately one-fifth of women who develop early breast cancer have HER2-positive tumours, which if untreated, have a worse prognosis than HER2-negative tumours. Trastuzumab is a selective treatment targeting the HER2 pathway. Although the results on efficacy seem to support its use, there are potential cardiac toxicities which need to be considered, especially for women at lower risk of recurrence, or those at increased cardiovascular risk.

Objectives

To assess the evidence on the efficacy and safety of therapy with trastuzumab, overall and in relation to its duration, concurrent or sequential administration with the standard chemotherapy regimen in patients with HER2-positive early breast cancer.

Search methods

We searched the Cochrane Breast Cancer Group's (CBCGs) Specialised Trials Register, and used the search strategy developed by the CBCG to search for randomised controlled trials (RCTs) in CENTRAL, MEDLINE, EMBASE, BIOSIS, TOXNET, and the WHO ICTRP search portal (up to February 2010).

Selection criteria

RCTs comparing the efficacy and safety of trastuzumab alone, or in combination with chemotherapy, or no treatment, or standard chemotherapy alone, in women with HER2-positive early breast cancer including women with locally advanced breast cancer.

Data collection and analysis

We collected data from published and unpublished trials. We used hazard ratios (HRs) for time-to-event outcomes and risk ratio (RRs) for binary outcomes. Subgroup analyses included duration (less or greater than six months) and concurrent or sequential trastuzumab administration.

Main results

We included eight studies involving 11,991 patients. The combined HRs for overall survival (OS) and disease-free survival (DFS) significantly favoured the trastuzumab-containing regimens (HR 0.66; 95% confidence interval (CI) 0.57 to 0.77, P < 0.00001; and HR 0.60; 95% CI 0.50 to 0.71, P < 0.00001, respectively). Trastuzumab significantly increased the risk of congestive heart failure (CHF: RR 5.11; 90% CI 3.00 to 8.72, P < 0.00001); and left ventricular ejection fraction decline (LVEF: RR 1.83; 90% CI 1.36 to 2.47, P = 0.0008). For haematological toxicities, risks did not differ. The two small trials that administered trastuzumab for less than six months did not differ in efficacy from longer studies, but found fewer cardiac toxicities. Studies with concurrent administration gave similar efficacy and toxicity results to sequential studies.

Authors' conclusions

Trastuzumab significantly improves OS and DFS in HER2-positive women with early and locally advanced breast cancer, although it also significantly increases the risk of CHF and LVEF decline. The available subgroup analyses are limited by the small number of studies. Studies that administered trastuzumab concurrently or sequentially did not differ significantly in efficacy. Shorter duration of therapy may reduce cardiotoxicity and maintain efficacy, however there is insufficient evidence at present to conclude this due to small numbers of patients in these trials.

PLAIN LANGUAGE SUMMARY

Efficacy and safety of trastuzumab in early breast cancer

Patients with early breast cancer may have HER2-positive or -negative tumours. HER2-positive cancers tend to be more aggressive. Knowing whether a cancer has high levels of the HER2 protein (about one in five breast cancers) influences the choice of treatment. Trastuzumab (brand name Herceptin) is a drug specifically available for these patients. The aim of the cancer treatment is to eliminate micrometastases at an early stage (i.e. adjuvant) so that more women survive without recurrence of the disease.

The review includes eight trials that involved 11,991 women with HER2-positive operable breast cancer who were assigned by chance to receive trastuzumab or not. Trastuzumab is always paired with a standard chemotherapy as starting treatment but it can also be continued alone or with hormone-blocking medications, such as an aromatase inhibitor or tamoxifen. Women were followed by clinicians for several years (three on average). The review found that trastuzumab significantly reduced recurrence and mortality. Some patients in treatment develop severe heart toxicity (i.e. congestive heart failure (CHF)). Breast cancer mortality is reduced by one-third but the risk of heart toxicity is five times more likely for women receiving trastuzumab than women receiving standard therapy alone. If 1000 women were given standard therapy alone (with no trastuzumab) then about 900 would survive and five would have experienced heart toxicities. If 1000 women were treated with standard chemotherapy and trastuzumab for one year, about 933 would survive (33 more women will have their lives prolonged), about 740 would be free of disease recurrence (95 more women will not experience the disease return), and 26 would have serious heart toxicity (21 more than the chemotherapy alone group) due to the drug. These heart toxicities are often reversible if the treatment is stopped straight away.

Longer treatment (one year) might involve a greater risk of severe heart toxicities than shorter treatment (six months or less), although these results are based on only two studies and few patients. In women at higher risk of recurrence and with no signs of a weak heart, trastuzumab offers far more benefits than risks. The balance of risks to benefits in patients at lower risk of recurrence (e.g. a small rather than a large tumour) must be carefully evaluated. The oncologist should share the decision with the patient concerning whether and how to start the treatment.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

Summary of findings for the main comparison. Overview: efficacy and safety outcomes for patient groups at different risks

Patient or population: HER2-positive early breast cancer patients
Settings: Early breast cancer
Intervention: Trastuzumab

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Trastuzumab				
Overall survival (OS) Follow-up: median 36 months	**Low**		**HR 0.66** (0.57 to 0.77)	9945 (8 studies)	⊕⊕⊕⊕ **high**[1]	
	100 per 1000	**67 per 1000** (58 to 78)				
	Moderate					
	300 per 1000	**210 per 1000** (184 to 240)				
	High					
	500 per 1000	**367 per 1000** (326 to 414)				
Disease free survival (DFS) Follow-up: median 36 months	**Low**		**HR 0.6** (0.5 to 0.71)	9935 (8 studies)	⊕⊕⊕⊕ **high**[1]	
	100 per 1000	**61 per 1000** (51 to 72)				
	Moderate					

	300 per 1000	**193 per 1000** (163 to 224)			
	High				
	500 per 1000	**340 per 1000** (293 to 389)			
Congestive heart failure (CHF)	**Low**		**RR 5.11** (3 to 8.72)[2]	10281 (8 studies)	⊕⊕⊕⊕ **high**[1]
	5 per 1000	**26 per 1000** (15 to 44)[2]			
	Moderate				
	20 per 1000	**102 per 1000** (60 to 174)[2]			
	High				
	50 per 1000	**256 per 1000** (150 to 436)[2]			
Left ventricular ejection fraction decline (LVEF)	**Low**		**RR 1.83** (1.36 to 2.47)[2]	7939 (7 studies)	⊕⊕⊕◯ **moderate**[1,3]
	5 per 1000	**9 per 1000** (7 to 12)[2]			
	Moderate				
	30 per 1000	**55 per 1000** (41 to 74)[2]			
	High				
	50 per 1000	**92 per 1000** (68 to 124)[2]			

Brain metastasis as first site of relapse	**13 per 1000**	**23 per 1000** (17 to 32)[2]	**RR 1.75** (1.29 to 2.38)[2]	6881 (5 studies)	⊕⊕◯◯ **low**[4]

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95%) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **RR:** risk ratio; **HR:** hazard ratio.

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] We did not downgrade although there was some concern about publication bias for some study arms.
[2] CI 90%.
[3] There is substantial heterogeneity among studies included in the analysis of LVEF decrease ($I^2 = 70\%$).
[4] The analysis for brain metastasis is limited by the design of trials which considered only the first site of relapse. High risk of bias and the number of events was less than 150. Overall we decided to downgrade by two levels when considering these issues.

BACKGROUND

Description of the condition

Breast cancer is the most common diagnosed cancer in women and the second leading cause of cancer-related death. Patients with breast cancer are classified as having cells that overexpress the human epidermal growth factor receptor 2 (known as HER2-positive) or do not overexpress the receptor (HER2-negative). Patients with HER2-positive disease typically have a worse prognosis (Gschwind 2004).

Description of the intervention

The antibody trastuzumab (Herceptin®) was developed as a means of blocking the tyrosine kinase-linked HER2 receptor (Coussens 1985). This receptor is overexpressed in 20% to 25% of women with early breast cancer (Slamon 1987). The Baselga 1996 study provided the first clinical evidence of the anti-tumour activity of trastuzumab in patients with HER2-overexpressing breast carcinomas. Trastuzumab was not associated with the most common chemotherapy-related side effects such as alopecia, neutropenia, mucositis, and vomiting, with a favourable risk-benefit profile in patients with metastatic breast cancer (Cobleigh 1999; Vogel 2001). Its potential efficacy in early breast cancer has also been investigated and three large clinical trials which involved women with early breast cancer were reported in 2005 (B31; HERA; N9831). The most common adverse events reported in these trials were fever, chills, and other acute, self-limiting symptoms that may accompany the first infusion of trastuzumab.

Cardiac dysfunction, an important side effect in patients with metastatic breast cancer when trastuzumab was used with or after anthracyclines, seemed to be of less concern in neoadjuvant or adjuvant chemotherapy (B31, see Tan-Chiu 2005). On the other hand, isolated central nervous system (CNS) progression of the tumour was more common in patients with HER2-overexpressing early breast cancer who received trastuzumab (HERA), probably as a consequence of better control of extra CNS disease by the drug (Burstein 2005).

Why it is important to do this review

Most currently available evidence supporting trastuzumab regimens relies on surrogate endpoints (i.e. disease free-survival (DFS) in patients with early breast cancer). The strength of this evidence has been questioned (Apolone 2005; Joppi 2005; Lancet 2005). Against this background, the following important clinical questions concerning trastuzumab's influence on mortality need to be tackled.

- **The risk-benefit profile in patients at low and intermediate risk of death from the neoplastic disease.** In these cases, trastuzumab might provide a limited net benefit since the recurrence and mortality risks for breast cancer are low but the drug is associated with cardiac toxicity, which might be independent of the disease stage and prognosis.
- **The optimal treatment duration.** One year is the generally accepted reference duration for trastuzumab therapy based on pivotal trial protocols (HERA). A similar clinical improvement was found in a subsequent trial in which trastuzumab was given for nine weeks (FinHer). Since cardiotoxicity may be dose-related, it is important to assess the efficacy and safety of trastuzumab administered over a shorter period.
- **The best modality of administration.** Trastuzumab can be given with chemotherapy concurrently (at the same time), or sequentially (after), and therefore it is important to compare the efficacy and safety of these two schedules.
- **The efficacy in subgroups.** We explored the effect of trastuzumab in subgroups on the basis of patients' characteristics or studies. Various prognostic factors may influence the effect of trastuzumab, i.e. tumour size or methodological characteristics of the studies (e.g. trial stopped early for benefit).

OBJECTIVES

To evaluate the efficacy and safety of trastuzumab, given with or after standard chemotherapy, in the adjuvant or neoadjuvant treatment of women with HER2-positive early and locally advanced breast cancer.

METHODS

Criteria for considering studies for this review

Types of studies

1. Randomised controlled trials (RCTs).

The following additional studies could be included in future updates of this review in the search for all available toxicity information.

- Observational studies reporting suspected trastuzumab-related adverse events.
- Case series and single case reports describing suspected adverse effects that may be due to trastuzumab. Findings from these studies will be primarily used to point out practical implications for research.
- Trials which aim to compare different dosages, durations or treatment schedules of trastuzumab, alone or in combination with other chemotherapy.

Types of participants

Women with HER2-positive breast cancer (early or locally advanced) of any age, menopausal status, nodal or hormone-receptor status.

Types of interventions

1. Intervention group: trastuzumab given following or in combination with standard chemotherapy regimen.
2. Comparator: the same chemotherapy regimen used in the intervention group without trastuzumab.

Trials could or could not specify recommended treatment upon disease progression or initial treatment failure.

Types of outcome measures

Primary outcomes

1. Overall survival (OS) using intention-to-treat (ITT) analysis.
2. Disease-free survival (DFS).

Secondary outcomes

1. Cardiac toxicity using per protocol analysis (all patients who received the experimental treatment regardless of compliance).
2. Tumour recurrences (only if data about OS and DFS were unavailable).
3. Other toxicities (defined and graded according to the World Health Organization and National Cancer Institute criteria) evaluated according to per protocol analyses.
4. Brain metastases as first site of relapse.
5. Treatment-related deaths.
6. Quality of life (QoL).

We applied the following definitions of the outcomes.

1. Overall survival (OS): time from randomisation to death (from any cause).
2. Disease-free survival (DFS): time from randomisation to date of recurrence of tumour or death from any cause.
3. Cardiac toxicity: congestive heart failure (CHF) and decline of left ventricular ejection fraction (LVEF). We considered the following definitions of CHF: New York Heart Association class III-IV; severe CHF; symptomatic CHF; or confirmed CHF. The decline of LVEF was defined as reported by the authors, as different thresholds were used.
4. Tumour recurrences: local breast cancer recurrence or development of metastatic disease. We defined 'recurrence rate' as the proportion of patients with a local breast cancer recurrence or development of metastatic disease; 'time to recurrence' (also referred to as 'disease-free interval') was the time from randomisation to first recurrence. Within distant recurrences, we considered the risk of disease progression due to metastasis of the central nervous system (CNS).
5. Other toxicities: neutropenic fever (grade 3/4); anaemia (grade 3/4); and neutropenia (grade 3/4).
6. Treatment-related death: death due to drug toxicity not to disease progression, reported as 'treatment-related', 'toxic death' or 'lethal toxicity'.
7. Quality of life (QoL): expression of well-being, measured through a validated scale (i.e. SF-36, EORTC, FACT).

Search methods for identification of studies

We limited our search to articles (conventional and grey literature) published after January 1, 1996; this is the date when Baselga and colleagues first presented data on the efficacy of trastuzumab in humans (Baselga 1996).

This systematic review shared the search methods with another Cochrane review exploring the efficacy and safety impact of trastuzumab in metastatic breast cancer (Moja 2006).

Electronic searches

For the search strategy for RCTs, please refer to the Cochrane Breast Cancer Group's (CBCG) module (Wilcken 2009).

We searched the following databases and periods.

- MEDLINE (host: OvidSP): January 1996 to February 2010 (see Appendix 1).
- EMBASE (host: Embase.com): January 1996 to December 2009 (see Appendix 2).
- Cochrane Central Register of Controlled Trials (CENTRAL): 6th December 2009 (see Appendix 3).
- BIOSIS (host: ISI Web of Knowledge): January 1996 to December 2009 (see Appendix 4).
- CBCG Specialised Register: 16th December 2009.
- TOXNET, National Library of Medicine: 1 March 2010.
- ASCO (American Society of Clinical Oncology) annual meetings: January 1996 to February 2010.

We searched MEDLINE, EMBASE, BIOSIS, TOXNET, CENTRAL, and the CBCG's Specialised Register using the medical subject headings 'Breast Neoplasms', 'Antineoplastic Agents', 'Adverse effects' and 'Toxicity', and the text words 'Trastuzumab', 'Herceptin', 'Adverse effect', 'Side effect', 'Toxic effect', 'Drug toxicity', 'Dug tolerance', 'Causality', 'Risk', 'Adverse event', 'Adverse drug reaction', 'Breast cancer', 'Breast tumour', 'Breast tumor' and 'Breast neoplasm'. We included reports irrespective of the language in which they were reported.

In addition, we searched the reference lists of other, related literature reviews.

Searching other resources

We also screened bulletins from the regulatory agencies as follows (Loke 2006).

- Current Problems in Pharmacovigilance (http://www.mhra.gov.uk/home/), UK.
- Australian Adverse Drug Reactions Bulletin (http://www.tga.gov.au/adr/aadrb.htm), Australia.
- European Public Assessment Reports from the European Medicines Evaluation Agency (http://www.ema.eu.int/#).
- MedWatch, the Food and Drug Administration Safety information and Adverse Events Reporting Program (http://www.fda.gov/medwatch/elist.htm), US.

Data collection and analysis

Selection of studies

At least two review authors (LM, LT, EP) independently screened the titles and abstracts of articles that were found for inclusion. We also assessed information available from conference proceedings on unpublished studies. We resolved disagreements by discussion. We obtained a copy of the full article for each reference reporting a potentially eligible trial. We sought further information from the authors where papers contained insufficient information to make a decision about eligibility. We applied the selection criteria described above to each trial. We recorded reasons for exclusion. We entered the characteristics and outcomes of the included trials, and details of the excluded trials into our database.

Data extraction and management

Three review authors (LT, SB, EP) independently extracted information from included trials using the pro-forma process piloted on a random sample of papers investigating other chemotherapy agents. We recorded details of study design, participants, setting, interventions, follow-up, quality components, efficacy outcomes, and side effects. (The extraction form is available from the review authors upon request). We also recorded details of previous therapies given to patients (including endocrine or other therapy). Two more review authors (LM, RD) resolved any discrepancies regarding the extraction of quantitative data or the quality assessment of RCTs. When a trial was presented in abstract form, we searched for further information using the Internet, contacting the authors, and checking for the best available resource or publication. Where possible, we sought any missing data or unclear information from the authors. For studies with more than one publication, we extracted data from all of the publications. However, we considered the final or updated version of each trial to be the primary reference for efficacy and toxicity unless otherwise specified (i.e. a large part of included patients crossed over to the other treatment arm during follow-up).

We included trials where patients crossed over to the other treatment arm at the time of progression, or received other treatment off-study and were managed according to the arm where they were originally randomised.

Assessment of risk of bias in included studies

The risk of bias assessment was based on the data provided in the publications included. If a study was reported in more than one publication, we used the publication with the most complete reporting.

Randomised controlled trials

We classified the generation of allocation sequence, allocation concealment, completeness of outcome data, and selective outcome reporting as 'adequate' (low risk of bias), 'inadequate' (high risk of bias), or 'unclear' following the criteria specified in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We considered these four items as the key domains for the risk of bias assessment. Studies with adequate procedures in all four domains were considered to have a low risk of bias; studies with inadequate procedures in one or more key domain(s) were considered to have a high risk of bias; and studies with unclear procedures in one or more key domain(s) were considered to have an unclear risk of bias. Two reviewers (SB, LT) independently assessed trials according to the predefined quality criteria. They resolved disagreements by consulting a third party (LM).

We evaluated the impact of methodological quality only on primary outcomes by considering the allocation concealment item; we assessed this by meta-regression.

Quality assessment for observational studies

In future updates of this review, we will separately assess the methodological quality of observational studies by using a component approach considering: concurrent, concomitant treatment; how allocation occurred; any attempt to balance groups by design; blinding of outcome assessment; completeness of follow-up; identification of prognostic factors (e.g. cardiovascular risk factors); and case-mix adjustment. These components are part of a list of quality items identified through a systematic review of the literature (Deeks 2003). We will not assess the quality of case series or single case reports.

Measures of treatment effect

The measure of association chosen for OS and DFS was the hazard ratio (HR). A HR less than 1.0 favoured regimens containing trastuzumab and ratios larger than 1.0 favoured regimens that do not contain trastuzumab. The measure of association chosen for combining toxicities was the risk ratio (RR). A RR greater than 1.0 indicated that the experimental treatment was more toxic than the control, and less than 1.0 suggested that the control was more toxic than treatment.

We assessed the risk/benefit profile by calculating the absolute effects using STATA 11 (Stata Corp., College Station, TX, USA). For efficacy, we obtained the absolute effect using the HR for mortality or disease progression estimated in meta-analysis and

calculated this considering the outcome at 36 months (Altman 1999). We obtained two absolute safety effects, the first using the RR for CHF and the second one using the RR for LVEF decline. We considered several hypothetical scenarios based on three patient baseline risks (low, medium, and high) for mortality, recurrence, and cardiovascular events.

We investigated the association between the occurrence of brain metastases as site of first relapse and trastuzumab by comparing the proportion of patients with a specific pattern of relapse, such as brain metastases between arms.

Assessment of heterogeneity

We assessed heterogeneity using the Chi^2 statistic and the I^2 statistic (Higgins 2011). The I^2 statistic indicated the percentage variability due to between-study (or inter-study) variability, as opposed to within-study (or intra-study) variability. Because we assumed that latent clinical heterogeneity was ubiquitous, we combined the studies using the random-effects model, regardless of statistical evidence for heterogeneity effect sizes. We classified an I^2 value greater than 50% as having substantial heterogeneity and discussed this accordingly (Higgins 2011).

We performed meta-regressions to assess whether the treatment effect was associated with the duration and concurrent or sequential administration of trastuzumab. These analyses were done with STATA 11.

Assessment of reporting biases

We evaluated the risk of outcome reporting bias for OS and DFS. In each study, we assessed the absence of these outcomes and discussed its possible impact on the overall estimates.

We assessed the impact of data from RCTs stopped early for benefit on the meta-analysis by comparing the HRs obtained from the meta-analysis which did or did not consider studies stopped early.

Data synthesis

We directly extracted the HRs and their variances for OS and DFS from the papers. If not reported, we indirectly obtained the HRs by using the methods described in Parmar 1998, employing either other available summary statistics or data extracted from published Kaplan-Meier curves. If all arms in a multi-arm trial had to be included in the meta-analysis and one treatment arm had to be considered for more than one comparisons, we decided to divide the number of events and the number of participants in that arm by the number of treatment comparisons made. This method avoided the multiple use of participants in the pooled estimate of treatment effect while retaining information from each arm of the trial. It slightly compromised the precision of the pooled estimate. For all adverse events and brain metastases treated as binary data, we used the RR as measure of association and fixed a higher type-I error (α = 0.10) (Shadish 2002).

We pooled the HRs and RRs on the log scale through the generic inverse variance approach, using the random-effects model.

Subgroup analysis and investigation of heterogeneity

We carefully reviewed the data to explore how sources of possible heterogeneity influenced the direction and magnitude of the effect and grouped studies according to the following:

- Duration of administration: we compared studies in which trastuzumab was given for less than six months, or more than six months, with regards to OS, DFS, cardiotoxicity, neutropenic fever, anaemia, neutropenia, and brain metastases as the site of first relapse.
- Modality of administration: concurrent or sequential administration of trastuzumab with regards to OS, DFS, CHF, LVEF, and brain metastases as the site of first relapse.
- Allocation concealment: studies with adequate allocation concealment or studies classified as unclear or inadequate with respect to the outcomes OS and DFS.
- Prognostic factors that could possibly modify the efficacy of trastuzumab (e.g. node-positive or negative status, positive or negative hormonal receptors, tumour size > 2 cm or < 2 cm, and age > 60 or < 60 years).
- Possible impact on the meta-analysis of the results from RCTs stopped early for benefit or non-truncated RCTs.

We explored different strategies to identify the main effect modifiers of trastuzumab. First, we assessed the consistency of treatment effect among studies, for efficacy and safety outcomes in relation to their intervention characteristics (i.e. duration and concurrent or sequential administration of trastuzumab). We followed the same approach to evaluate the consistency among studies for efficacy outcomes, with regard to the methodological characteristics (i.e. allocation concealment and early stop for benefit). We assessed consistency by testing the differences between the above subgroups using a meta-regression analysis. Second, we asssessed whether the magnitude or direction of the treatment effect was modified by patients' prognostic characteristics, i.e. lymph node status (negative or positive), hormone-receptor status (negative or positive), tumour size (> 2 cm or < 2 cm) and age (> 60 or < 60) by summarising the ratios of HRs in each study using the random-effects model. The estimated weighted mean of the ratio of HRs and its 95% CI were reported with the I^2 statistic. Since this test often has low power for identifying sources of heterogeneity, we fixed a higher type-I error (α = 0.10) (Hardy 1998).

RESULTS

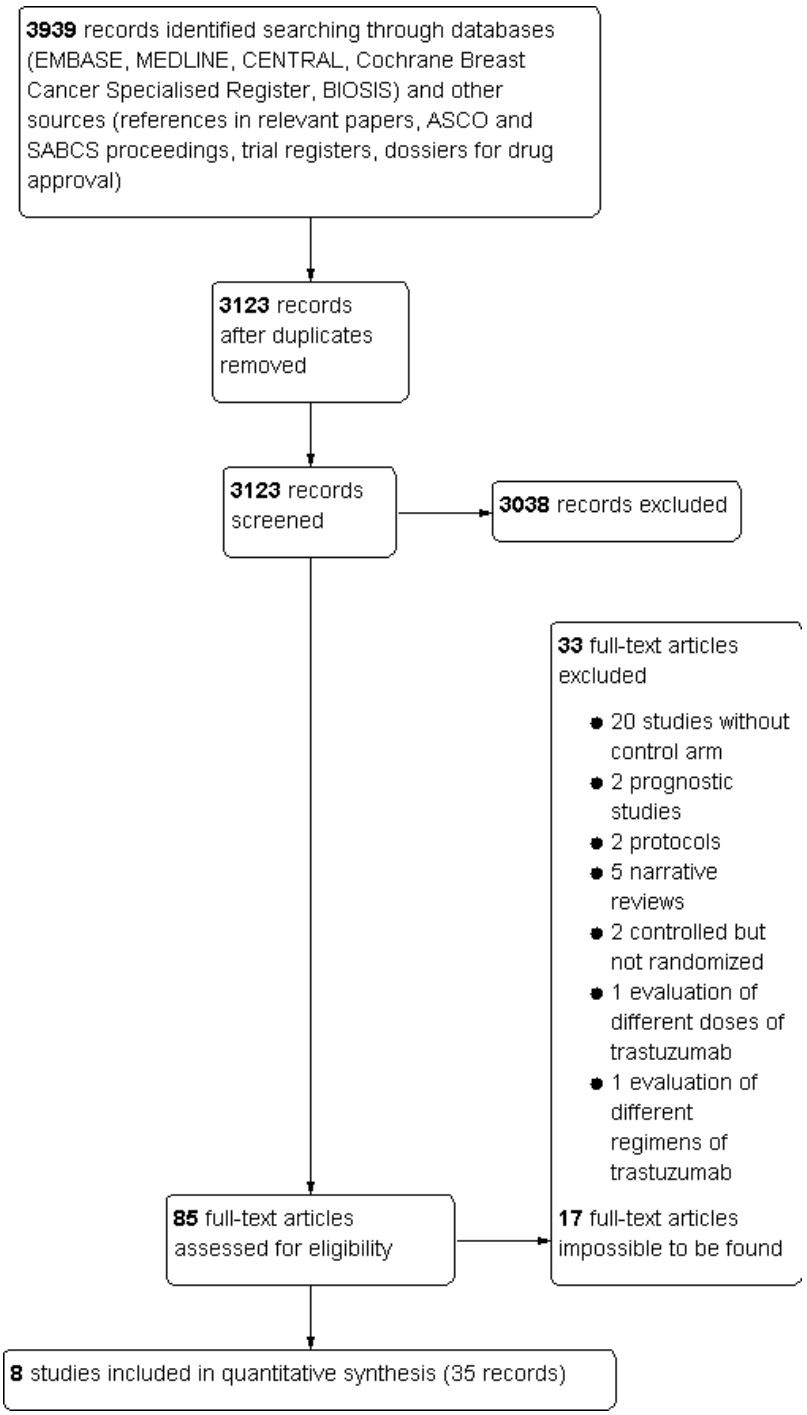
Description of studies

See: Characteristics of included studies; Characteristics of excluded studies.

Results of the search

Randomised trials evaluating the efficacy of trastuzumab for metastatic breast cancer started accruing patients in the early 1990s and the first report was published in 1996 (Baselga 1996). Since then, research has rapidly moved forward in the treatment of metastatic and early breast cancer with this drug, judging from the number of articles reporting results from randomised and observational trials in PubMed. See: Figure 1 for the results of the search strategy.

Figure 1. Study flow diagram



Search results from MEDLINE, EMBASE, CENTRAL, the CBCG's Specialised Register, BIOSIS databases and references in relevant papers, American Society of Clinical Oncology and San Antonio Breast Cancer Symposium proceedings, trial registers, and dossiers for drug approval, provided 3939 citations. After adjusting for duplicates, there were 3123 citations remaining. Of these, we discarded 3038 after reviewing the titles and abstracts because they clearly did not meet the inclusion criteria. We examined the full-text of the remaining 85 citations: 33 references did not meet the inclusion criteria and we excluded 17 references as we could not find either their abstracts or the full-text. Thirty-five publications (corresponding to eight trials) met the inclusion criteria and were included in this systematic review.

Included studies

See: Characteristics of included studies.

The eight included RCTs evaluated the efficacy of trastuzumab and other comparators in patients with early and locally advanced breast cancer (B31; BCIRG006; Buzdar; FinHer; HERA; N9831; NOAH; PACS-04). Seven RCTs (B31; Buzdar; FinHer; HERA; N9831; NOAH; PACS-04) were published in full in peer reviewed journals (some with meeting updates); at the time we submitted our review, data from the BCIRG006 trial were available only as meeting abstracts or presentations and we will evaluate a recent full-text publication in the following update of this review (Slamon 2011). For some trials, additional unpublished data were provided by the investigators or obtained from regulatory agency reports or trial registries. The BCIRG006 trial compared three arms, two experimental and one control. We removed one experimental arm from our analyses because the chemotherapy regimen used (i.e. only concurrent taxanes) was different from the one used in the control arm.

Five RCTs were multicentric (B31; FinHer; HERA; N9831; NOAH) while two RCTs (BCIRG006; PACS-04) were stated to be multicentric but the number and names of the centres and the number of patients enrolled in each centre were not clearly reported. The Buzdar trial was monocentric.

Characteristics of patients

In the eight RCTs, 11,991 women were randomised to the following treatment groups: 7,020 women to a trastuzumab-containing arm and 4,971 women to a treatment without trastuzumab. There was a wide age range (from 22 to 80 years) and the median age was 49 years. The trials recruited patient populations with varying risk profiles but all trials excluded patients with metastatic breast cancer. All included patients had local (axillary) node-positive breast cancer or high risk node-negative disease on the basis of the size of the primary tumour and were HER2-positive. Four trials included patients both with (node-positive) and without (node-negative) pathological axillary lymph nodes (BCIRG006; FinHer; HERA; N9831). To be eligible, node-negative patients in these studies had to have a primary tumour bigger than 2 cm (FinHer and N9831) or 1 cm (HERA) in diameter. The BCIRG006 trial did not specify the criteria for defining patients with node-negative disease at high risk. The B31 and PACS-04 trials included patients with positive axillary metastases. The two neoadjuvant trials, Buzdar and NOAH, included patients with T3N1, T4, any T plus N2 or N3, or any T plus involvement of ipsilateral supraclavicular nodes (NOAH), or patients with clinical stage II-IIIA (Buzdar).

Surgical resection of the primary tumour was required in all but two trials (Buzdar; NOAH) which considered a neoadjuvant therapeutic setting.

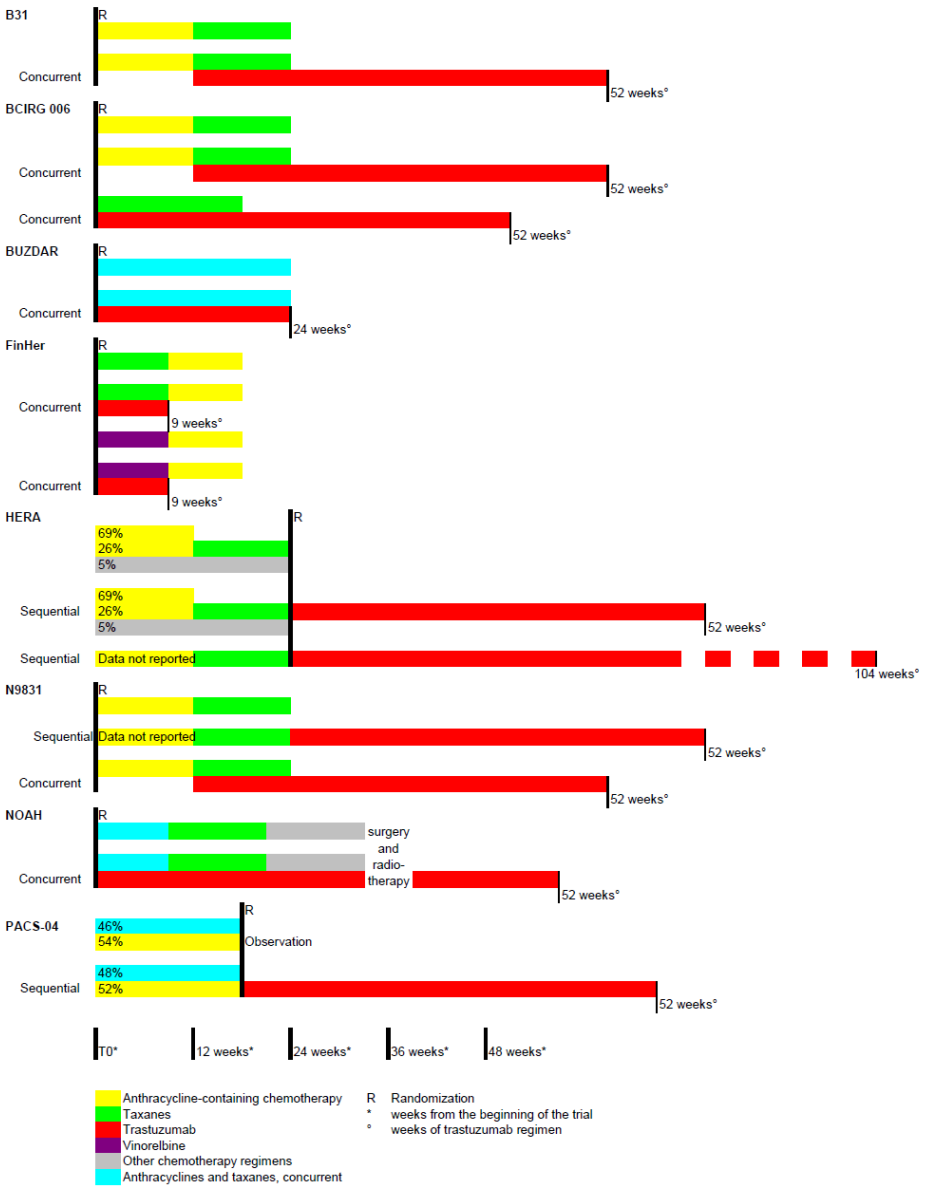
All the trials required normal heart function for inclusion and five trials (B31; Buzdar; HERA; N9831; PACS-04) specified the LVEF required for beginning trastuzumab therapy. Patients with cardiovascular disease of any grade were excluded.

Both premenopausal and postmenopausal women were eligible in all trials.

Interventions used in the trials

See: Figure 2.

Figure 2. Summary of trials characteristics with focus on concurrent or sequential trastuzumab.



Six trials evaluated trastuzumab as an adjuvant therapy (surgery followed by chemotherapy plus or minus trastuzumab) while the Buzdar and NOAH trials enrolled patients before surgery and gave trastuzumab on a neoadjuvant basis, together or without chemotherapy.

Seven trials (B31; BCIRG006; Buzdar; FinHer; N9831; NOAH; PACS-04) used an anthracycline (with or without a taxane) in both the trastuzumab and non-trastuzumab-containing arms. In the NOAH trial, the control therapeutic scheme contained cyclophosphamide, methotrexate, and fluorouracil. Two trials (BCIRG006; HERA) gave trastuzumab with different chemotherapy regimens. In the BCIRG006 trial, one of the three arms was excluded as trastuzumab was given with docetaxel and carboplatin (TCH arm), which was completely different from the control arm (i.e. doxorubicin plus cyclophosphamide followed by docetaxel). Although we recognise that the TCH arm has been adopted by many clinicians, our protocol considered trastuzumab as the only difference between arms. The HERA trial tested trastuzumab's efficacy in patients who had received at least four cycles of an approved (neo-) adjuvant chemotherapy regimen. This could include anthracyclines with or without taxanes, a non-anthracycline regimen that included cyclophosphamide, methotrexate and 5-fluorouracil, or any regimen approved by the trial committee. In the FinHer trial, the anthracycline was given after a regimen with vinorelbine or docetaxel.

The study design of the FinHer and PACS-04 trials involved two randomisations. The FinHer study was designed to compare vinorelbine with docetaxel, and PACS-04 evaluated the efficacy of fluorouracil plus epirubicin plus cyclophosphamide compared with epirubicin and docetaxel. In both of these trials, we considered the data concerning the second randomisation, i.e. the one focusing on trastuzumab, for the purpose of this review.

Trastuzumab and the other chemotherapies were given sequentially in two trials (HERA; PACS-04) and concurrently in the other six trials (B31; BCIRG006; Buzdar; FinHer; N9831; NOAH). The N9831 trial contained three arms: two treatment groups and one control group. Trastuzumab was administered sequentially in one arm, concurrently in the second arm, and not given in the third arm.

The National Cancer Institute and the Food and Drug Administration approved an unplanned joint analysis of two independent trials (B31 and N9831) as the experimental and control arms were considered similar. We were unable to find publications presenting the two results on efficacy separately. In this meta-analysis, we used data from Romond 2005 to assess efficacy in terms of OS and DFS. In the subgroup analysis pertaining to the effect of concurrent or sequential trastuzumab administration on adverse events, we halved the control arm of the N9831 trial to avoid the repeated use of participants in the pooled estimate.

We obtained the dose and duration of trastuzumab from the registered trial protocols and the treatment regimens varied amongst these trials. Six trials (B31; BCIRG006; HERA; N9831; NOAH; PACS-04) administered trastuzumab for one year with differing schedules: 6 mg/kg every three weeks (with a loading dose of 8 mg/kg) in the HERA, NOAH, and PACS-04 trials; and 2 mg/kg weekly doses (with a loading dose of 4 mg/kg) in the other three trials. In the Buzdar and FinHer trials, trastuzumab was prescribed for a shorter time, six months and nine weeks respectively, with 2 mg/kg weekly doses after the 4 mg/kg loading dose. In the BCIRG006 trial, trastuzumab was given for one year but the dosage was never reported. The trial reports did not specify the total doses of anthracyclines or taxanes administered, but they probably differed across the trials. Other adjuvant treatments included postoperative radiotherapy and endocrine therapy for women with hormone-receptor-positive disease (tamoxifen or aromatase inhibitors for postmenopausal women).

The median follow-up of patients ranged from 18 months (N9831) to 65 months (BCIRG006).

All the trials provided detailed safety data. Formal QoL data were reported only in one study (BCIRG006).

The included trials were partially or fully funded by the pharmaceutical industry; three trials (B31; FinHer; N9831) also received funding from public institutions.

Cardiotoxicity outcomes used in the trial

A list of cardiotoxicity outcomes and their definitions is presented in Additional Table 1.

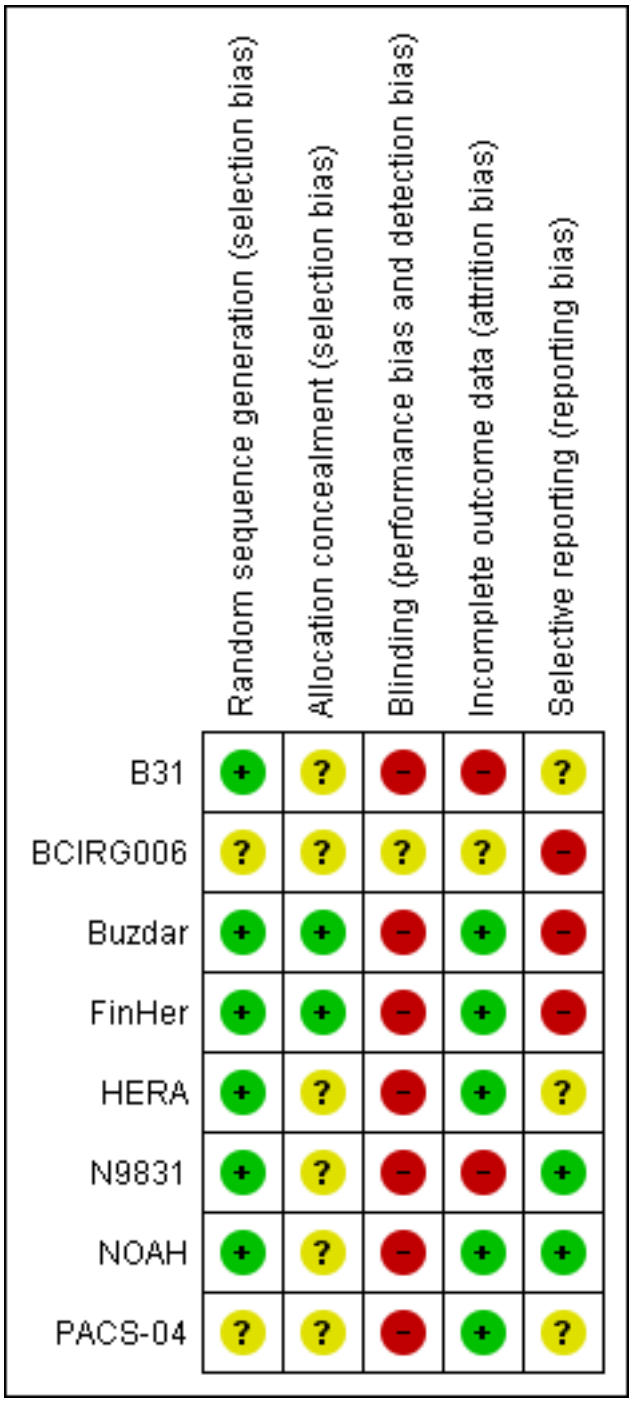
Excluded studies

We excluded four studies as ineligible for reasons reported in Characteristics of excluded studies.

Risk of bias in included studies

See: Figure 3, risk of bias summary table.

Figure 3. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.



Since the trials were large and conducted at multiple sites, it is likely that these trials had unbiased central randomisation procedures, protocol integrity and rigorous and reliable data registration, in order to satisfy regulatory authorities and human investigation committees. We could not directly assess methodological quality because details of the methods used (such as the mechanism of allocation concealment) were not always provided in the published reports or congress presentations. None of the studies used blinding to treatment allocation, a common practice in phase III oncological trials, because of the difficulty in concealing different infusion times, schedules and toxicities. This was unlikely to bias the results of the studies where OS was measured, as this outcome was not subject to observer or patient bias in interpretation.

Allocation

All the trials were described as randomised studies. Two trials (BCIRG006; PACS-04) did not give details about randomisation and we did not find additional information. We assessed the generation of the randomisation sequence as adequate for six trials (B31; Buzdar; FinHer; HERA; N9831; NOAH). We assessed allocation concealment as adequate in two trials (Buzdar; FinHer), however no information was reported in the remaining trials. The treatment groups were well balanced in most trials (B31; BCIRG006; HERA; N9831; NOAH). Where minor imbalances were reported (Buzdar; FinHer; PACS-04), we considered these as unlikely to bias the outcome of the trial.

Incomplete outcome data

Loss to follow-up was minimal (i.e. less than 6%) and accounted for in four trials (Buzdar; FinHer; HERA; NOAH). No information was reported about patients lost to follow-up in the BCIRG006 and PACS-04 trials. In the B31 and N9831 trials, efficacy data were reported for 85% and 58% of the patients, respectively; the trial authors stated that continued follow-up was pending at the time of publication.

Selective reporting

In the FinHer trial, distant disease-free survival (DDFS) became the primary endpoint in the final analysis instead of recurrence-free survival, which was considered the primary endpoint in the first interim analysis. DDFS was more closely associated with mortality than recurrence-free survival and this allowed for a longer follow-up period and the collection of more events. Although the authors provided a rationale for this amendment, from the available data we cannot rule out selective outcome reporting bias.

The study protocol of BCIRG006 was available but largely incomplete, and the study results were reported only as meeting abstracts or presentations, therefore it was likely that not all of the prespecified outcomes had been reported. In addition, the outcome reporting of Buzdar was largely incomplete. We were unable to judge the risk of selective reporting for the B31, HERA and PACS-04 trials. For these, we classified the risk of bias as unclear.

Other potential sources of bias

Three trials were stopped early because of an observed benefit (B31; N9831; Buzdar). Another trial (HERA) published the results of an interim analysis which strongly supported the efficacy of trastuzumab and allowed patients in the control arm to switch to the experimental arm. In three other trials (BCIRG006; FinHer; NOAH), some patients moved from the control arm to the trastuzumab arm. For our meta-analysis, we always used the data before the switch, adopting an ITT perspective.

We cannot rule out the possibility of publication bias in two trials (HERA; N9831). Data on efficacy from arm B (n = 981) in the N9831 trial, where trastuzumab was given sequentially, were never published in full. A second issue about the N9831 trial concerns the impact of the results of 152 patients who were assigned to the control group but were eventually excluded from the joint analysis without the trial authors providing an explanation for this decision. The results of the HERA trial, based on 1701 patients treated with trastuzumab over two years, have never been published. We cannot discount the possibility that the data are associated with partial efficacy or increased toxicity. Members of the steering committee of the study reported to us that the reason that these results have not been yet published is that the International Independent Data Monitoring Committee of the trial have recommended that the trial follow-up continue (Nicholas Wilcken, personal communication).

Effects of interventions

See: **Summary of findings for the main comparison** Trastuzumab-containing regimens for early breast cancer

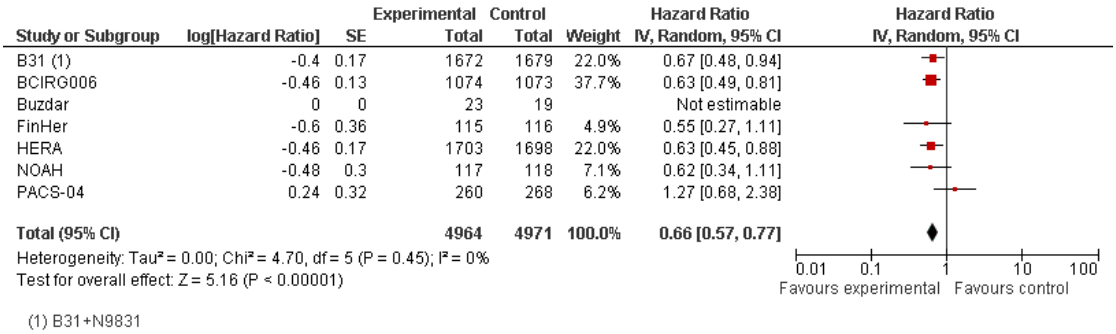
Efficacy of trastuzumab

Overall survival

We estimated OS from the eight included trials (B31; BCIRG006; Buzdar; FinHer; HERA; N9831; NOAH; PACS-04). We included a total of 9935 women in the analysis, with 655 reported deaths (6.6%). For the HERA trial, we considered the events and the HR before participants randomised to the control arm were switched to the intervention. In the HERA trial, the median follow-up can be expected to be less than 23 months, which was the median

follow-up reported by the trial authors and calculated considering the total follow-up without censoring patients who switched arms. In the Buzdar trial, no events were reported during a median follow-up of 36.1 months. The HR significantly favoured the trastuzumab-containing regimens over the non-trastuzumab control groups (HR 0.66; 95% CI 0.57 to 0.77, P < 0.00001). There was no heterogeneity among the studies (I^2 = 0%). The results are reported in Figure 4 and the Summary of findings for the main comparison.

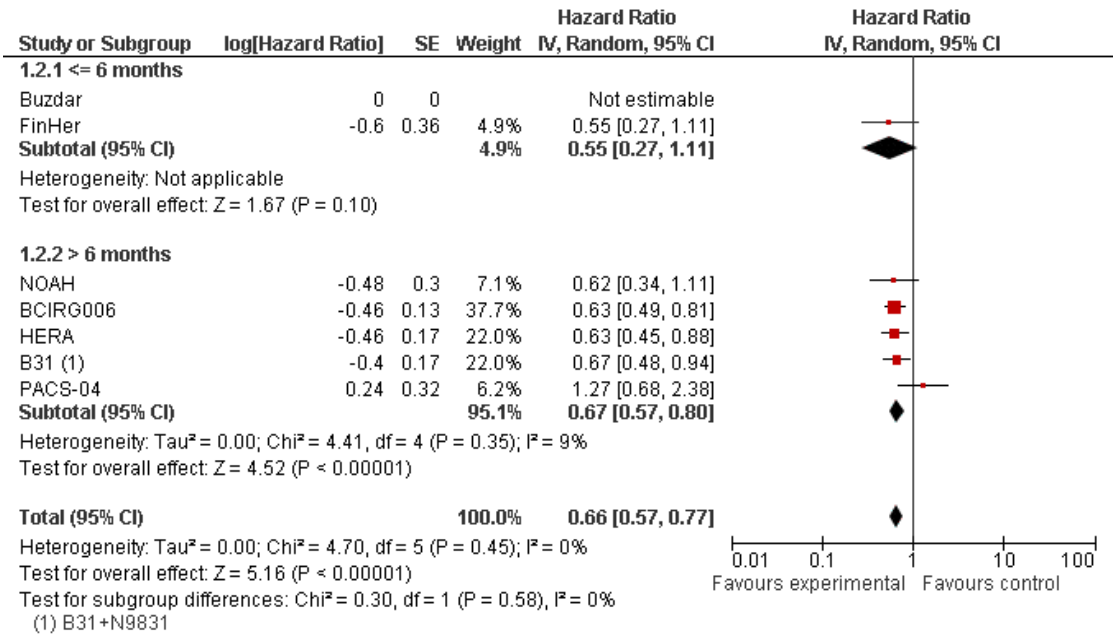
Figure 4. Overall survival: all studies.



Overall survival stratified by duration of trastuzumab treatment

Two trials (Buzdar; FinHer) which involved trastuzumab therapy for less than six months, included a total of 273 patients and 33 deaths (all recorded in FinHer). Six trials gave trastuzumab for more than six months (B31; BCIRG006; HERA; N9831; NOAH; PACS-04), and involved 9662 patients and 622 deaths. In the trials where trastuzumab was given for a longer period, the HR significantly favoured the trastuzumab-containing regimens (HR 0.67; 95% CI 0.57 to 0.80, P < 0.00001), while in the subgroup of trials that used trastuzumab for less than six months, efficacy failed to reach statistical significance (HR 0.55; 95% CI 0.27 to 1.11, P = 0.1). The test for differences between subgroups was not significant (P = 0.591). In these analyses, the lack of significant effect sizes should not be interpreted as implying an absence of effect because the subgroup analyses might have low power. The results are reported in Figure 5 and Table 2.

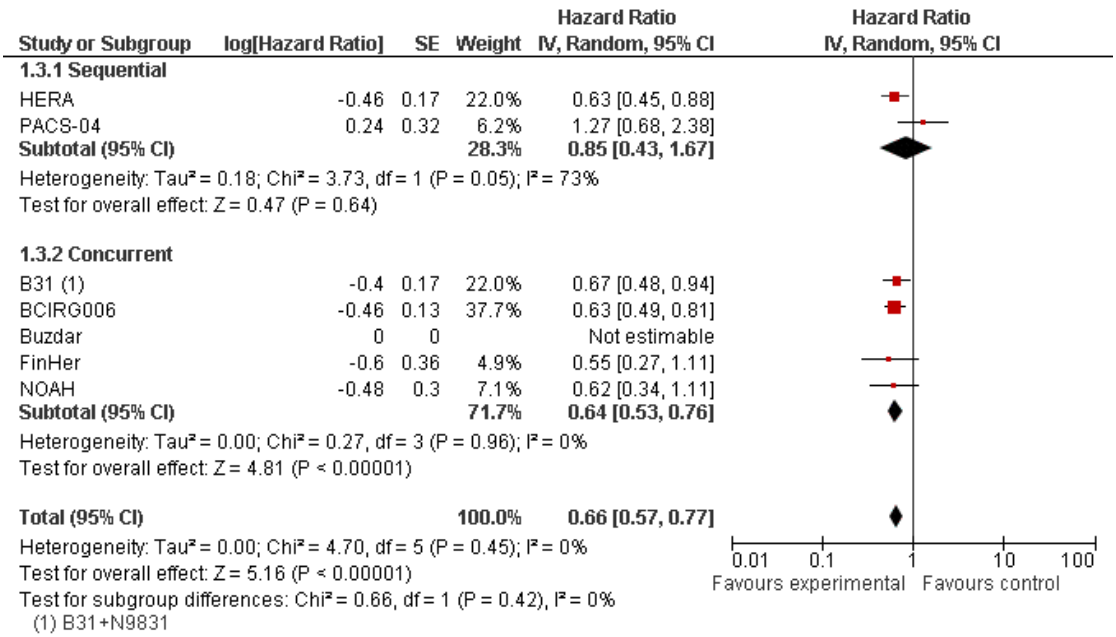
Figure 5. Overall survival stratified by duration of trastuzumab treatment.



Overall survival stratified by concurrent or sequential administration of trastuzumab

Two trials (HERA; PACS-04) gave trastuzumab sequentially to chemotherapy, involving 3929 patients and 189 deaths. The other six trials gave the drug concurrently (B31; BCIRG006; Buzdar; FinHer; N9831; NOAH), accounting for 6006 patients and 466 deaths. In the studies that gave trastuzumab and chemotherapy concurrently, the HR significantly favoured the trastuzumab-containing regimens (HR 0.64; 95% CI 0.53 to 0.76, P < 0.00001), while the other studies, where trastuzumab was administered after chemotherapy, failed to reach statistical significance (HR 0.85; 95% CI 0.43 to 1.67, P = 0.64). The test for differences between subgroups was not significant (P = 0.406). The results are reported in Figure 6 and Table 2. The N9831 trial directly evaluated sequential or concurrent administration. Although the results have still not been published in a peer reviewed journal, they were presented at the San Antonio Breast Cancer Symposium in 2009, showing that the two schedules were not significantly different (unadjusted HR 0.79; 95% CI not reported, P = 0.135).

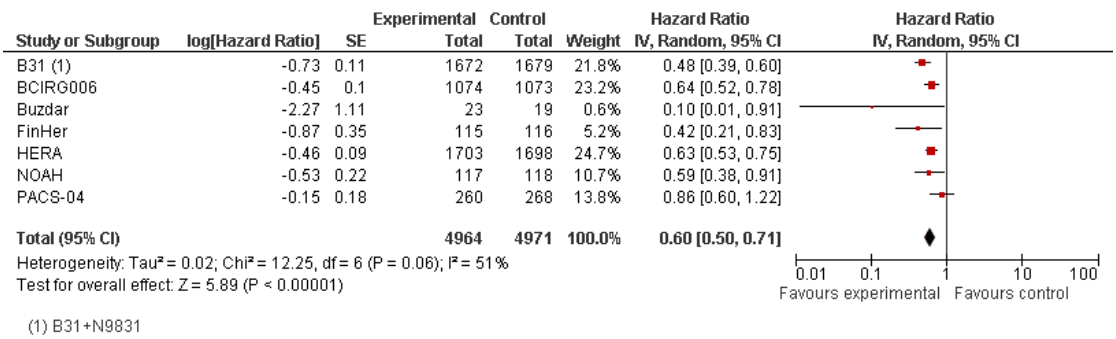
Figure 6. Overall survival stratified by concurrent or sequential administration of trastuzumab.



Disease-free survival

We obtained estimates of DFS from all eight trials (B31; BCIRG006; Buzdar; FinHer; HERA; N9831; NOAH; PACS-04). Data for the FinHer trial were extracted from the Joensuu 2006 reference. A total of 9935 women and 1604 events (16.1%) were analysed. In the Buzdar trial, the HR for DFS was estimated from the Kaplan-Meier curve (see Buzdar 2007). The overall HR for DFS significantly favoured the trastuzumab-containing regimens (HR 0.60; 95% CI 0.50 to 0.71, P < 0.00001). Heterogeneity across trials was moderate (I^2 = 51%). The results are reported in Figure 7 and the Summary of findings for the main comparison.

Figure 7. Disease-free survival: all studies.



Disease-free survival stratified by duration of trastuzumab treatment

In the trials in which trastuzumab was given for less than six months, involving 273 patients and 42 events, the HR significantly favoured the trastuzumab-containing regimens (HR 0.31; 95% CI 0.10 to 0.96, P = 0.04; Buzdar; FinHer). In the six trials that gave the drug for a longer time, accounting for 9662 patients and 1562 events, the HR also significantly favoured trastuzumab (HR 0.62; 95% CI 0.52 to 0.72, P < 0.00001; B31; BCIRG006; HERA; N9831; NOAH; PACS-04). The test for differences between subgroups was not significant (P = 0.150). The results are reported in Analysis 1.5 and Table 2.

Disease-free survival stratified by concurrent or sequential administration of trastuzumab

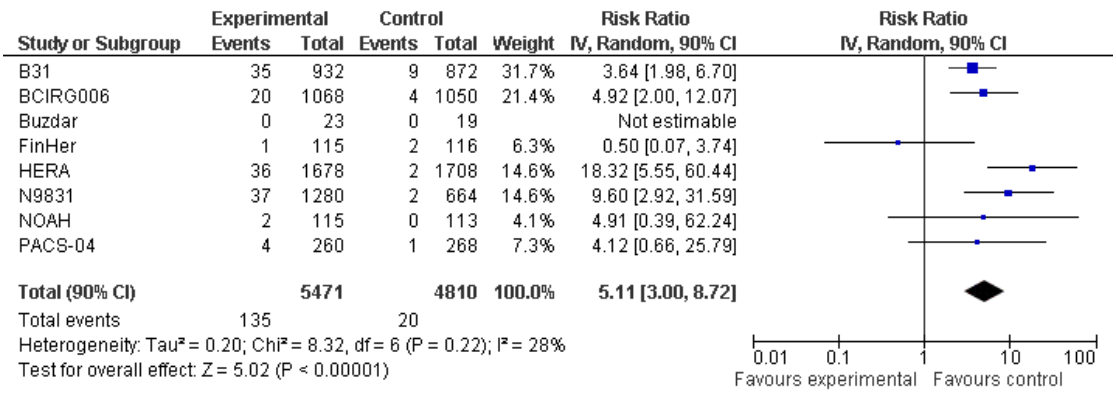
In both subgroups, comparing trastuzumab given sequentially or concurrently with chemotherapy and accounting for 3929 patients and 639 events, and 6006 patients and 965 events respectively, the HRs significantly favoured the trastuzumab-containing regimens (HR 0.71; 95% CI 0.53 to 0.95, P = 0.02; and HR 0.54; 95% CI 0.44 to 0.67, P < 0.00001, respectively). The test for differences between subgroups was not significant (P = 0.122). The results are reported in Analysis 1.6 and Table 2. In the N9831 trial, the only study that directly compared sequential and concurrent administrations, the comparison was not significant as the trial authors (Perez et al, San Antonio Breast Cancer Symposium 2009) set up a P value of statistical significance at 0.00116 (adjusted HR 0.75; 95% CI 0.60 to 0.94, P = 0.0134).

Safety of trastuzumab

Congestive heart failure

We extracted data on CHF from eight trials, totaling 10,281 patients with early breast cancer (B31; BCIRG006; Buzdar; FinHer; HERA; NOAH; N9831; PACS-04). Cardiac toxicity data for the BCIRG006 trial were extracted from the Slamon 2006 reference. There were 135 cases (2.5%) of CHF out of 5471 patients in the trastuzumab group and 20 cases (0.4%) out of 4810 in the control group. The overall result indicated a higher risk of CHF with trastuzumab (RR 5.11; 90% CI 3.00 to 8.72, P < 0.00001). Heterogeneity was minimal (I^2 = 28%). The results are reported in Figure 8 and the Summary of findings for the main comparison.

Figure 8. Congestive heart failure (CHF): all studies.



Congestive heart failure stratified by duration of trastuzumab treatment

In six trials, trastuzumab was administered for more than six months, totalling 10,008 patients and 152 events (B31; BCIRG006; HERA; N9831; NOAH; PACS-04). There was a significantly higher risk of CHF in regimens in which trastuzumab was given for more than six months than in regimens without trastuzumab (RR 5.39; 90% CI 3.56 to 8.17, P < 0.00001). A shorter treatment period did not appear to be associated with an

increase in the risk of CHF (RR 0.50; 90% CI 0.07 to 3.74, P = 0.57). However, this analysis was based only on one trial (FinHer) and three events as there were no reported events in the Buzdar trial. The test for differences between subgroups was statistically significant (P = 0.059). The results are reported in Analysis 2.2 and Table 3.

Congestive heart failure stratified by concurrent or sequential administration of trastuzumab

We further defined sequential subgroups on the basis of the time of randomisation: before or after chemotherapy. Two sequential trials (HERA; PACS-04) randomised patients after the completion of chemotherapy but the N9831 trial randomised patients before starting chemotherapy. Therefore the HERA and PACS-04 trials did not take into account cardiotoxicity during chemotherapy. The different randomisation times reflected the cardiotoxicity risk of the control group; the risk is extremely low in those trials which randomised patients later. If the trials with different randomisation times were grouped, we would find a subgroup difference for concurrent or sequential administration influenced by the different cardiotoxicity risk in the control arms. The N9831 trial monitored cardiotoxicity across chemotherapy and trastuzumab periods and reported a significantly higher risk of CHF (RR 8.42; 90% CI 1.56 to 45.45, P = 0.04), accounting for all patients and events in the subgroup (1042 and 19, respectively). In the other subgroups, the risk of CHF was significantly higher when trastuzumab was given sequentially with chemotherapy (RR 11.05; 90% CI 3.46 to 35.29, P < 0.0007) and concurrently (RR 3.90; 90% CI 2.42 to 6.28, P < 0.00001). The former analysis (i.e. sequential - randomisation after chemotherapy) was based on 3914 patients and 43 events, and the latter (i.e. concurrent) on 5325 patients and 93 events. The test for differences between subgroups was not significant (P = 0.2214). The results are reported in Analysis 2.3 and Table 3.

The only trial that directly compared trastuzumab given sequentially or concurrently with chemotherapy, N9831, reported a non-significant reduction in the risk of CHF (RR 0.76; 90% CI 0.45 to 1.30, P = 0.397).

Decline in left ventricular ejection fraction

We extracted data on LVEF decline from seven trials (BCIRG006; Buzdar; FinHer; HERA; NOAH; N9831; PACS-04). The definition of LVEF varied across the trials (see Additional Table 1). There were 466 cases (11.2%) of LVEF decline out of 4147 patients in the trastuzumab group and 215 cases (5.6%) out of 3792 patients in the control group. The results suggested that trastuzumab significantly increased the risk of LVEF decline (RR 1.83; 90% CI 1.36 to 2.47, P = 0.0008). As expected, there was substantial heterogeneity (I^2 = 71%). The results are reported in the Summary of findings for the main comparison.

Decline in left ventricular ejection fraction stratified by duration of administration of trastuzumab

Analysis of the effect of prolonged administration of trastuzumab, based on 7666 patients and 652 events, showed a significant increase in the risk of LVEF decline (RR 2.14; 90% CI 1.58 to 2.89, P < 0.0001). In the trials with shorter treatment regimens, there were no significant differences between groups (RR 0.89; 90% CI 0.51 to 1.57, P = 0.74). This latter analysis was based on two trials, including 273 patients and 29 events (Buzdar; FinHer). The test for differences between subgroups was significant (P = 0.060). The results are presented in Table 3.

Decline in left ventricular ejection fraction stratified by concurrent or sequential administration of trastuzumab

Similar to the CHF analysis, subgroups were further defined on the basis of the time of randomisation (see CHF stratified by concurrent or sequential administration of trastuzumab). The N9831 trial monitored cardiotoxicity across chemotherapy and trastuzumab periods, accounted for 791 patients and 41 events, and showed a non-significant increase in the risk of LVEF decline (RR 1.16; 90% CI 0.68 to 1.99, P = 0.65). In the other subgroups , there was a significantly higher risk of LVEF decline in the trastuzumab groups (sequential randomisation after chemotherapy RR 2.90; 90% CI 2.23 to 3.76, P < 0.00001; and concurrent RR 1.48; 90% CI 1.11 to 1.97, P = 0.03). The sequential with randomisation after chemotherapy analysis was based on 3914 patients and 283 events and the concurrent analysis on 3234 patients and 357 events. The test for differences between subgroups was significant (P = 0.0119). The results are presented in Table 3.

N9831, the only trial that directly compared trastuzumab and chemotherapy sequentially and concurrently, reported no difference in the rate of LVEF decline (RR 0.95; 90% CI 0.60 to 1.48, P = 0.843).

Other toxicities

Data for the BCIRG006 and FinHer trials were extracted from the references Slamon 2006 and Joensuu 2006, respectively. For the FinHer trial, it was assumed that the percentages of adverse events were similar for HER2-positive and HER2-negative patients.

Neutropenic fever

Four trials (BCIRG006; Buzdar; FinHer; NOAH) reported information on neutropenic fever. There were 146 cases (11.1%) out of 1321 patients in the trastuzumab group and 222 cases (10.7%) out of 2075 in the control group. Trastuzumab did not seem to be associated with an increase in the risk of neutropenic fever (RR 1.18; 90% CI 0.99 to 1.41, P = 0.13).

Neutropenic fever stratified by duration of trastuzumab treatment and by concurrent or sequential administration of trastuzumab

In both the shorter and longer trastuzumab treatment groups, neutropenic fever did not differ from controls (RR 1.14; 90% CI 0.83 to 1.58, P = 0.50; and RR 1.19; 90% CI 0.96 to 1.48, P = 0.17, respectively). In the first subgroup, 1050 patients and 151 events were reported and in the second subgroup 2346 patients and 217 events were reported.
There were no trials using sequential trastuzumab which reported information about neutropenic fever.

Anaemia

Two trials (BCIRG006; FinHer) reported information on anaemia. There were 33 cases (2.8%) out of 1183 patients in the trastuzumab group and 29 cases (1.6%) out of 1815 patients in the control group. The analysis did not show any evidence of a higher risk of anaemia in patients receiving trastuzumab (RR 1.24; 90% CI 0.81 to 1.88, P = 0.40).

Anaemia stratified by duration of trastuzumab treatment and by concurrent or sequential administration of trastuzumab

In the trials administering the drug for shorter (FinHer) and longer (BCIRG006) periods, the occurrence of anaemia did not differ between the groups (RR 0.94; 90% CI 0.08 to 11.28, P = 0.97; and RR 1.25; 90% CI 0.82 to 1.91, P = 0.39, respectively).
There were no trials using sequential trastuzumab which reported information on anaemia.

Neutropenia

Four trials (BCIRG006; Buzdar; FinHer; NOAH) reported information on neutropenia. There were 873 cases (66.1%) out of 1321 patients in the trastuzumab group and 1255 cases (64.5%) out of 1947 patients in the control group. The analysis did not show an increase in the risk of neutropenia in patients receiving trastuzumab (RR 1.10; 90% CI 1.00 to 1.22, P = 0.10).

Neutropenia stratified by duration of trastuzumab treatment and by concurrent or sequential administration of trastuzumab

In the longer trastuzumab treatment group (from the BCIRG006 and NOAH trials) involving 2346 patients and 1434 events, neutropenia was significantly more frequent than in the control group (RR 1.12; 90% CI 1.07 to 1.18, P = 0.0001), whereas in the shorter trastuzumab treatment group (from two trials), comprising 922 patients and 694 events, it was not (RR 1.21; 90% CI 0.85 to 1.72, P = 0.37). However, the test for differences between subgroups was not significant (P = 0.668).
There were no trials using sequential trastuzumab which reported information on neutropenia.

Brain metastases as the site of first relapse

Five trials (B31; FinHer; HERA; N9831; PACS-04) reported information on brain metastases as the first relapse event. The proportion of brain relapses out of all relapses ranges between 10% and 25% but this figure is indicative because the denominators used by trialists (i.e. relapses or patients) are unclear. In the trials reporting the outcome, there were 73 cases (2.3%) out of 3120 patients in the trastuzumab group and 50 cases (1.3%) out of 3761 patients in the control group. The risk of brain metastases was significantly higher in patients receiving trastuzumab (RR 1.75; 90% CI 1.29 to 2.38, P = 0.002). The results are reported in Analysis 4.1 and the Summary of findings for the main comparison.

Brain metastases stratified by duration of trastuzumab treatment

The four trials assessing a longer trastuzumab treatment, which involved 6650 patients and 115 events, showed a higher risk of brain metastases in the trastuzumab arm (RR 1.89; 90% CI 1.38 to 2.59, P = 0.0009; B31; HERA; N9831; PACS-04), whereas there was no increase in the only trial, comprising 231 patients and eight events, that administered trastuzumab for a shorter period (RR 0.61; 90% CI 0.19 to 1.97, P = 0.48; FinHer). The test for differences between subgroups was not significant (P = 0.125). The results are reported in Table 4.

Brain metastases stratified by concurrent or sequential administration of trastuzumab

Two trials, which enrolled 3299 patients and observed 67 events in which trastuzumab was given after chemotherapy, showed a higher risk of brain metastases in the trastuzumab group (RR 1.73; 90% CI 1.16 to 2.58, P = 0.02). No evidence of an increase in risk was found in the three trials that used the drugs concurrently (RR 1.71, 90% CI 0.91 to 3.21, P = 0.16). This result was based on 3582 patients and 56 events. The test for differences between subgroups was not significant (P = 0.927). The results are reported in Table 4.

Treatment-related deaths

Information about treatment-related deaths was reported only in the treatment arm of the joint analysis of the N9831 and B31 trials (i.e. three events).

Assessment of risk/benefit profile

The number of patients who need to be treated with trastuzumab are presented as an absolute difference per 1000 patients for OS and DFS at 36 months, and for CHF and LVEF decline. The results of the intervention and control groups are framed assuming increasing baseline risks in the control group. These absolute differences were obtained using the HR of OS and DFS estimated in the meta-analysis (HR 0.66; 95% CI 0.57 to 0.77; and HR 0.60; 95% CI 0.50 to 0.71, respectively). The absolute differences in safety were obtained using their RRs, estimated in the meta-analysis (RR 5.11; 90% CI 3.00 to 8.72; and RR 1.83; 90% CI 1.36 to 2.47). All results are reported in Summary of findings for the main comparison.

The benefit for OS varied from 33 to 133 more survivors per 1000 patients according to OS at 36 months, assuming survival ranged from 50% to 90%.

The benefit for DFS varied from 39 to 160 more survivors per 1000 patients according to DFS at 36 months, assuming DFS ranged from 50% to 90%.

The adverse event, CHF, varied from 21 to 206 more cases per 1000 patients according to the risk of CHF in the control group, assuming CHF ranged from 0.5% to 5%.

The adverse event, LVEF decline, varied from 24 to 241 cases according to the risk of LVEF decline in the control group, assuming LVEF ranged from 0.5% to 5%.

Subgroup analyses and investigation of heterogeneity

Trastuzumab efficacy according to allocation concealment

Two trials (FinHer; NOAH) reported adequate allocation concealment, whereas the other six trials (B31; BCIRG006; Buzdar; HERA; N9831; PACS-04) did not. The allocation concealment was not associated with the magnitude or direction of treatment effect, in terms of either OS (Analysis 5.1) or DFS (Analysis 5.2). The results are reported in Table 5.

Trastuzumab efficacy in patients stratified by prognostic factors

It was not possible to assess the efficacy of trastuzumab (considering OS as the outcome) and subgroups of patients classified according to their prognostic factors (i.e. lymph node status, hormone-receptor status, tumour size, and age) since the trial authors did not report mortality in such detail. We only considered DFS for this analysis, although not all trials reported the HRs of DFS stratified for subgroups. For the BCIRG006 trial, we extracted the HRs from an oral presentation by Slamon at the 2006 San Antonio Breast Cancer Symposium (see reference list BCIRG006).

The results suggested that the DFS did not vary in relation to lymph node status (negative or positive, RHR 0.74; 95% CI 0.51 to 1.08), hormone-receptor status (positive or negative, RHR 1.01; 95% CI 0.81 to 1.26), tumour size (> 2 cm or < 2 cm, RHR 1.01; 95% CI 0.77 to 1.34) and age (> 60 or < 60, RHR 1.14; 95% CI 0.63 to 2.09). The results are reported in Table 6, Table 7, Table 8 and Table 9 respectively.

Trastuzumab efficacy and trials stopped early for benefit

HERA was considered a trial that was stopped early since the results, based on an interim analysis strongly favouring trastuzumab, led to an early termination after one year of treatment with trastuzumab or control. The combination of the results from four trials which stopped early for benefit (B31; N9831; Buzdar; HERA) favoured trastuzumab in terms of either OS (HR 0.65; 95% CI 0.51 to 0.82) or DFS (HR 0.54; 95% CI 0.40 to 0.73). The results were similar when combining the other four trials which were not stopped early for benefit (BCIRG006; FinHer; NOAH; PACS-04): for OS, the HR was 0.70 (95% CI 0.51 to 0.95) and for DFS, the HR was 0.65 (95% CI 0.53 to 0.81). The differences between the two HRs (early termination or not) for OS and DFS were not significant (P = 0.837 and P = 0.289, respectively). The results are reported in Table 10.

DISCUSSION

Summary of main results

In view of the large effect, the large number of meta-analysed studies and patients, and the relative absence of substantial differences between studies, we believe that trastuzumab is associated with a beneficial effect for both OS and DFS in women with HER2-positive breast cancer with a moderate-to-high risk of recurrence. However, cardiac toxicities pose an appreciable clinical problem. For these toxicities, the definition of the outcome, congestive heart failure (CHF) was more homogeneous amongst trials than the left ventricular ejection fraction definition (LVEF decline); so the summary of CHF results can be considered more trustworthy. Overall mortality can be reduced by one-third but the risk of severe cardiac toxicity (i.e. CHF) is five times more likely. This means that if 1000 women at low risk were not treated with trastuzumab, five of them would be expected to experience severe cardiac toxicity and 900 would be expected to survive at three years, whereas if they were treated with trastuzumab the numbers would be 26 and 933, respectively. Therefore, careful attention is needed in patient selection, as the benefit of trastuzumab could be eroded by cardiac toxicity, particularly in low risk patients or those at increased cardiovascular risk. An assessment of the balance between benefit and harm of trastuzumab therapy is even more difficult as the importance of harmful and beneficial events can be perceived differently by patients and clinicians, along with the fact that some cardiac

toxicities might be reversible if the treatment is stopped immediately. In terms of haematological toxicities, there is no evidence of raised risk.

We explored the efficacy and safety of trastuzumab according to duration and concurrent or sequential administration in the subgroup analyses. These analyses have limitations, because they are observational by definition and they may lack statistical power to detect even an important clinical difference between groups. Misleading subgroup effects can result in withholding efficacious treatment from patients who would benefit, or prescribing ineffective or even potentially harmful treatments for patients who would fare better without the treatment. However, the efficacy of trastuzumab does not seem to vary in relation to the duration and concurrent or sequential administration. The incidence of cardiac toxicities seemed to be higher in regimens where trastuzumab was given for a longer period: the risk of severe CHF when trastuzumab was used for more than six months is estimated to be more than ten times than in those where trastuzumab was administered for less time, but the number of studies and patients in the shorter term subgroup was very limited. When the risks of cardiac toxicity in the longer trastuzumab schedules were compared to those with shorter duration, the test for subgroup differences reached statistical significance; heterogeneity across studies and subgroups was null or minimal. It seemed that differences may exist in cardiotoxicity between shorter and longer trastuzumab administration, and appeared to be large in terms of harm to the patient. These results need to be confirmed in randomised trials designed to test the optimal duration of adjuvant trastuzumab such as the Short-HER (Guarneri 2008), the SOLD, and the PHARE trials. These may either consolidate or negate the finding of a difference between short or long term treatment.

In patients receiving trastuzumab sequentially, the risks of LVEF decline seemed higher than in those patients receiving it concurrently. Although the subgroup differences were significant, these results should be interpreted with even more caution, since the higher risk in the subgroup of studies giving trastuzumab sequentially might be explained by different cardiotoxicity of the chemotherapy preceding trastuzumab. On the basis of these limitations, we believe that trastuzumab should be considered cardiotoxic irrespective of its sequential or concurrent administration. This interpretation is consistent with the very limited heterogeneity within subgroups. The potential difference needs to be further explored in a trial designed to randomise patients to sequential or concurrent schedules of trastuzumab, and by considering CHF as a primary outcome.

Overall completeness and applicability of evidence

The results of our meta-analysis could not be generalised to all women with early or locally advanced breast cancer. Firstly, the majority of women in the trials included in our review were younger (median age 49 years), tended to be healthier and had fewer comorbidities than women usually affected by the disease. This limitation especially concerns data on cardiotoxicity: all trials excluded patients with existing cardiovascular disorders or with suboptimal LVEF (< 55%). Therefore the results of this review may not be entirely generalised to clinical practice. Given the high risk of cardiotoxicity-related to the use of trastuzumab, oncologists should seriously consider that outside RCTs the number of patients experiencing these adverse events may be higher and the clinical consequences of the treatment even more severe. The higher risk of cardiotoxicity could erode the net benefit of trastuzumab, particularly in women with a low risk of recurrence, such as patients with small tumours and negative lymph nodes (i.e. size of less than 1 cm and lymph node-negative - T1a, bN0). These patients were largely excluded from all RCTs included in our review, further limiting the applicability of the evidence and the use of trastuzumab in this group.

Moreover, trastuzumab was associated with a longer DFS (composite outcome, defined as the time from randomisation to the date of recurrence of tumour or death from any cause) and probably with a lower risk of relapse overall. The patients treated with trastuzumab who relapse are more likely to have brain metastases as the site of first relapse than the controls, which were apparently unaffected by the duration or schedule of administration. There is debate on whether the higher incidence of CNS metastases is due to the higher aggressiveness of HER2-positive breast cancer, or to a better control of extra CNS disease by trastuzumab. This might be related to the limited penetration across the blood-brain-barrier of the drug and a general inefficacy in controlling CNS metastases or is a result of the improvements of the drug in systemic control and OS, that may lead to an unmasking of CNS disease recurrence that would otherwise remain clinically silent before the patient's death. The report of N9831 and B31 studies indicated that the overall incidence of CNS events after the first relapse was similar in the two study groups, thus supporting the view that the higher incidence of brain metastases in trastuzumab-treated patients reflects a greater control of systemic disease.

Quality of the evidence

All articles included in this systematic review were published in the past decade and could be classified as high quality trials, which probably reflects a generally increasing emphasis on higher expectations for study quality by researchers, regulatory agencies, and journal editors. The fact that three trials stopped early for benefit, raises concerns. Methodological studies have reported that RCTs stopped early showed implausible large treatment effects (Bassler 2010; Pocock 1999). Anyway, we assessed whether the RCTs that stopped early for benefit gave different results from those that did not. We did not find any statistical differences between these trials in terms of efficacy. Furthermore, in four trials selected patients were given (during the course of the study) the possibility to switch

from the control arm, in which they were initially allocated, to the trastuzumab arm. Patients were switched mostly for ethical considerations and when an interim analyses supported the hypothesis of trastuzumab efficacy. The consequences of this selective crossover gives problems in data analysis and interpretation of results (D'Amico 2011). It is worth considering the possible effect of the switch on efficacy and safety outcomes. In situations in which the intervention is effective but toxic, as in this review, the differences (deltas) between treatment and control, obtained by considering patients who have crossed over, tend to become smaller. After the crossover, the number of events in the control arm dropped, and the difference between arms was reduced. In general, the more effective the drug, the smaller is the difference after the crossover. The same applies to safety: the more toxic the drug, the smaller is the difference after the crossover. So the risk-benefit profile of trastuzumab might have been modified by the switch. For that reason we believe that reporting the efficacy results before the crossing over and the safety results after the crossing over may depict a biased risk-benefit profile.

Potential biases in the review process

While there is confidence about the benefit of trastuzumab, there is still uncertainty about its magnitude and duration over time. Trials that stopped early after favourable interim analyses could have partially influenced the results of this meta-analysis, inflating positive results. However, we did not find any strong evidence of that. It is also possible that publication bias in favour of significant findings may have led to an overestimation of the overall treatment effect: two completed trials (HERA and N9831) have never published data on 2782 patients, which is undoubtedly a considerable figure. The absence of the publication could also be related to a high frequency of cardiotoxic events, diminishing the net benefit of trastuzumab.

AUTHORS' CONCLUSIONS

Implications for practice

Despite some limitations of the trials examined, there is strong evidence that the use of trastuzumab for early and locally advanced breast cancer: a) improves both OS and DFS in HER2-positive women with moderate-to-high risk of recurrence; and b) increases the likelihood of few women experiencing cardiac toxicities. The fact that the relative risk of CHF is greater than the relative risk of LVEF decline may imply that trastuzumab is more likely to cause severe cardiac outcomes than mild ones. Another possible explanation is that the concomitant chemotherapy use may decline the LVEF in the control, therefore decreasing the difference between the groups. One year of trastuzumab is the standard care on the basis of scientific evidence. Although this systematic review does not provide definitive evidence that the risks of CHF rise with longer administration of trastuzumab, careful attention must still be paid to the duration and modality of administration of the drug. Because of the high prevalence of small tumours and node-negative disease in a more elderly patient population, women and clinicians should discuss the trade-offs between benefits and cardiac toxicities carefully when facing the decision on whether and how to start the treatment.

Implications for research

The next generation of studies is awaited to define the precise role of trastuzumab as adjuvant chemotherapy for HER2-positive early breast cancer.

The first compelling question is clinical and regards the different durations of treatment: a short term course (between nine and 24 weeks) or standard duration (over 24 weeks and usually 52 weeks)?

The second compelling question is methodological and concerns the impact of the offered crossover from the control to the trastuzumab group following positive results from interim trial analyses or from concomitant trials; it makes it difficult to complete the trial as planned. If women agree to cross over, data analysis is no longer straightforward and can bias the estimates of efficacy and safety, leaving the scientific community with greater uncertainty about the study results. As discussed above, the estimates of efficacy and safety outcomes obtained with ITT analyses tend to be biased towards the null hypothesis. In general, if a treatment is effective but toxic, the ITT analysis provides estimates of the chosen measure of associations, which are likely to be biased towards the hypothesis of no-difference for both outcomes. The direction and magnitude of the bias associated with the estimates of efficacy and safety outcomes obtained with censored analyses is highly dependent on the characteristics of patients who are offered and decide to cross over. If the treatment is effective and patients who cross over have a better prognosis, the direction of bias will go in the opposite direction of no-difference (D'Amico 2011).

We recommend publication of all randomised trial data evaluating trastuzumab as this might help clarify the net benefit and cardiac toxicities across all enrolled patients.

Finally it would be useful if independent researchers conducted an individual patient data meta-analysis, fully exploring the interactions between the effect size and prognostic patient characteristics in a multivariate model; and sequential and concurrent trastuzumab schedules were tested in a parallel randomised trial.

ACKNOWLEDGEMENTS

The authors are grateful to Cinzia Brambilla and Anna Compagnoni who assisted during the early stages of this systematic re-

view; Su Golder and Carol Lefebvre for valuable suggestions about bibliographic searches; Deirdre Price, Jessica Thomas and Andrew Herxheimer for valuable suggestions about methodological issues in analysing adverse events; Davina Ghersi, Sharon Parker, Nancy Owens and Nicholas Wilcken for their helpful assistance during all the phases of the preparation of the protocol; and Judith Baggott who helped with copy-editing this review.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

B31

Methods	Accrual time: February 2000 to February 2005. Multicentre, national (USA) Baseline comparability: balanced
Participants	2043 females enrolled; 1736 with follow-up at February 2005 included in the analysis Age: range from 22 to 80: 67.5% of patients were in the age range 40 to 59; 15% of patients were ≥ 60 years old • Diagnosis: adenocarcinoma of the breast spread to axillary-node • Inclusion criteria: complete resection of the primary tumour and axillary-node dissection. Adequate hematopoietic, hepatic and renal function, LVEF that met or exceeded the lower limit of normal. Tumours had to be strongly HER2-positive (immunohistochemistry score of 3+, or positive by FISH); positivity must have been confirmed at central or reference laboratories • Exclusion criteria: clinical or radiological evidence of metastatic disease, angina pectoris requiring antianginal medication, arrhythmia requiring medication, severe conduction abnormality, clinically significant valvular disease, cardiomegaly on chest radiography, left ventricular hypertrophy on echocardiography, poorly controlled hypertension, history of myocardial infarction, CHF, cardiomyopathy Note: 15% of patients had a tumour diameter larger than 4 cm; 14% had more than 10 nodes affected by the disease HER2-positive: 100%
Interventions	Adjuvant setting • Group 1 (randomised N = 1024; with follow-up N = 872): doxorubicin plus cyclophosphamide (60 mg/sm and 600 mg/sm every 21 days for four cycles) followed by paclitaxel (175 mg/sm, every 21 days, four cycles) • Group 2 (randomised N = 1019; with follow-up N = 864): doxorubicin plus cyclophosphamide (60 mg/sm and 600 mg/sm every 21 days, four cycles) followed by paclitaxel (175 mg/sm, every 21 days, four cycles) plus trastuzumab (first loading dose of 4 mg/kg given with the first dose of paclitaxel, 51 further weekly doses of 2 mg/kg) • Other adjuvant treatments: ○ radiotherapy in patients treated with lumpectomy (irradiation of the whole breast with an optional boost to the tumour bed and without irradiation of the internal mammary nodes), initiated after the completion of chemotherapy, without interruption of trastuzumab treatment; ○ tamoxifene (20 mg/day for 5 years) in oestrogen-receptor positive and progesterone-receptor positive patients, initiated on day 1 of the first cycle of doxorubicin and cyclophosphamide until an amendment on January 14, 2003, required hormonal therapy to be started after chemotherapy; ○ anastrozole in postmenopausal patients with oestrogen-receptor positive (ER+) or progesterone-receptor positive (PR+) tumours (permitted from January 14, 2003) It is not reported how many patients used these therapies in each arm of the trial

Outcomes	Primary: DFS Secondary: OS, time to distant recurrence, death from breast cancer, contralateral breast cancer, other second primary cancers, adverse events
Notes	Median follow-up for efficacy: 2.4 years Interim analysis: trial stopped early for benefit. Criteria: difference in terms of DFS with a P value less than 0.0001 Efficacy in the papers is reported only for joint analysis of B31 and N9831 trials: Group 1 from trial B31+ Group A from trial N9831 (control) vs Group 2 from trial B31+ Group C from trial N9831 (concomitant trastuzumab). From the two trials overall 4809 patients have been randomised. Follow-up data are available for 3351 patients (69.7%): 325 follow-up pending; 152 excluded for unclear reasons; 981 randomised to Group B and excluded Multicentre study: centres were all in US; the institutions involved in the trial enrolled at least 25 patients

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Treatment assignments were balanced according to nodal status, the planned hormonal therapy, the type of surgery, the intended radiotherapy and institution with the use of a biased coin minimisation algorithm
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	High risk	2043 enrolled; 22 declined protocol therapy (of which 13 with follow-up pending as of February 2005). Another 294 patients remaining with follow-up pending as of February 2005. Patients included in the analysis for the efficacy are those enrolled as of February 15, 2005 with at least one follow-up evaluation as of the date (i.e. 1736/2043 = 85% of the accrued)
Selective reporting (reporting bias)	Unclear risk	The study protocol is available but largely incomplete (http://clinicaltrials.gov/ct2/show/NCT00004067?term=b31&rank=1) The published report includes all expected efficacy outcomes but the car-

		diotoxicity outcome might have been reported incompletely (i.e. LVEF decline not reported)

BCIRG006

Methods	Accrual time: April 2001 to March 2004 Multicentre, international Baseline comparability: balanced
Participants	N = 3222 female Age: information about the age range of the patients not available; median age: 49 years • Diagnosis: axillary node-positive or high risk node-negative breast cancer with HER2-positive confirmed by central FISH • Inclusion criteria: definitive surgery of the breast cancer (either mastectomy with axillary lymph node involvement assessment, or breast conserving surgery with axillary lymph node involvement assessment); Karnofsky Performance status index > 80%; normal cardiac, hepatic and renal function, normal values of the haemochrome; negative pregnancy test; normal audiology assessment • Exclusion criteria: prior systemic anti-cancer therapy or radiation therapy for breast cancer or prior anthracycline therapy, for any malignancy; bilateral invasive breast cancer; pregnancy; pre-existing motor or sensory neurotoxicity; any T4 or N2 or known N3 or M1 breast cancer; cardiac diseases; other serious illness or medical condition (neurologic or psychiatric disorders, dementia or seizures, uncontrolled infection, active peptic ulcer, unstable diabetes mellitus, impaired hearing); past or current history of neoplasm other than breast carcinoma (except for: curatively treated non-melanoma skin cancer, in situ carcinoma of the cervix, other cancer curatively treated and with no evidence of disease for at least 10 years); current therapy with any hormonal agent such as Raloxifene, Tamoxifen, or other selective ER modulators, with corticosteroids, with ovarian hormonal replacement therapy; definite contraindications for the use of corticosteroids; and concurrent treatment with other experimental drugs or with any other anti-cancer therapy Note: 6% of patients had a tumour diameter larger than 5 cm; 9% had more than 10 nodes affected by the disease HER2-positive: 100%
Interventions	Adjuvant setting • Arm A (AC→T) (N = 1073): doxorubicin plus cyclophosphamide (60mg/sm and 600mg/sm, every three weeks, four cycles) followed by docetaxel (100mg/sm, every three weeks, 4 cycles) • Arm B (AC→TH) (N = 1074): doxorubicin plus cyclophosphamide (60mg/sm and 600mg/sm, every three weeks, four cycles) followed by docetaxel (100mg/sm, every three weeks, four cycles) plus trastuzumab (weekly, 52 cycles). Trastuzumab doses are not reported anywhere • Arm C (TCH) (N = 1075): docetaxel plus carboplatin (75mg/sm and 75 mg/sm, every three weeks, six cycles) plus trastuzumab (weekly, 52 cycles). Trastuzumab doses are not reported anywhere Other adjuvant therapies: hormonal therapy for five years after chemotherapy in patients

BCIRG006 (*Continued*)

	with hormone-receptor-positive tumours
Outcomes	Primary: DFS Secondary: OS; adverse events; and QoL
Notes	Median follow-up: 65 months Third interim analysis After the first interim analysis 17 patients (1.6%) in the observation group switched to trastuzumab Not reported if it is a multicentre study

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Patients were prospectively stratified by number of positive lymph nodes (0, 1 to 3 versus 4+) and hormone-receptor status
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Not reported
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Not reported
Selective reporting (reporting bias)	High risk	The study protocol is available but largely incomplete (http://clinicaltrials.gov/ct2/show/NCT00021255?term=bcirg006&rank=1). The results have been reported only as meeting abstracts or presentations. It is likely that not all the prespecified outcomes have been reported

Buzdar

Methods	Accrual time: June 2001 to May 2003 Single-centre, national (US) Baseline comparability: the median age was slightly higher for the patients treated with chemotherapy plus trastuzumab; one patient who had T3b disease was included in the chemotherapy plus trastuzumab arm; two patients in the chemotherapy plus trastuzumab arm had synchronous bilateral breast cancers
Participants	N = 42 female Age: range from 25 to 75 years; median age: 48 years in chemotherapy arm and 52 in chemotherapy plus trastuzumab arm

	• Diagnosis: invasive, but non-inflammatory, carcinoma of the breast with stage II to IIIA disease • Inclusion criteria: adequate bone marrow, liver and renal function. Each patient was reviewed in a multidisciplinary conference with designation of whether breast conservation was a reasonable surgical option before treatment was initiated. HER2-positivity had to be proven by an immunohistochemistry score 3 or FISH • Exclusion criteria: history of uncompensated CHF or a cardiac ejection fraction less than 45% Note: 93% (one patient had a IIIb stage disease; two patients had a bilateral breast cancer) HER2-positive: all tumours were HER2-positive by FISH or showed 3+ overexpression by immunohistochemistry
Interventions	Neoadjuvant setting • Arm chemotherapy alone (N = 19): paclitaxel (225 mg/sm, every three weeks, four cycles) followed by FEC [fluorouracil (500 mg/sm, every three weeks on days 1 and 4, four cycles), epirubicin and cyclophosphamide (75 mg/sm and 500 mg/sm, every three weeks on day 1, four cycles)] • Arm chemotherapy plus trastuzumab (N = 23): paclitaxel (225 mg/sm, every three weeks, four cycles) followed by FEC [fluorouracil (500 mg/sm, every three weeks on days 1 and 4, four cycles), epirubicin and cyclophosphamide (75 mg/sm and 500 mg/sm, every three weeks on day 1, four cycles)] plus trastuzumab (4 mg/kg loading dose and 2 mg/kg following doses, weekly, 24 cycles) beginning on day 1 of the first treatment cycle • Other treatments given: ○ after completion of 24 weeks of systemic neoadjuvant therapy, patients received local therapy; ○ after completion of local therapy, patients with ER-positive tumours received hormonal treatment planned for five years (Tamoxifen 20 mg/day or Anastrozole 1 mg/day if the patient was postmenopausal) Each patient was premedicated with either dexamethasone 20 mg orally, 12 and six hours before administration of paclitaxel, or dexamethasone 20 mg IV 30 minutes before chemotherapy. Patients received diphenhydramine 50 mg IV and cimetidine 300 mg IV 30 minutes before paclitaxel infusion
Outcomes	Primary: pCR defined as no evidence of residual invasive cancer in both the breast and axilla Secondary: clinical complete remission, partial response, minor response Outcomes of safety are reported in the results but not defined a priori. DFS is also evaluated but not defined as outcome a priori
Notes	Median follow-up: 36.1 months (range: 12.3 to 54.8 months) Interim analysis not scheduled: the trial was stopped early for benefit and the protocol was amended to discontinue the chemotherapy alone arm and to add an additional 22 patients to the chemotherapy and trastuzumab arm Trial performed in just one centre located in Texas (USA)
Risk of bias	

Buzdar (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	All patients were prospectively registered into a central research database. Patients were randomly assigned to treatment arms using a stratified blocked randomisation, with strata based on age (> 50 years or < 50 years) and stage of disease
Allocation concealment (selection bias)	Low risk	All patients were prospectively registered into a central research database
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	No patient was lost at follow-up
Selective reporting (reporting bias)	High risk	The study protocol is unavailable. The study reports fail to include results for the overall mortality

FinHer

Methods	Accrual time: October 2000 to September 2003 Multicentre, national (Finland) Baseline comparability: characteristics between trastuzumab and non-trastuzumab groups were not completely balanced: • tumours with diameter > 20 mm: were more common in the non-trastuzumab group • axillary nodal metastases: tended to be more frequent and more frequently involving > three axillary lymph nodes in the trastuzumab group • receptor for estrogens: positive status was more frequent in the trastuzumab group • receptor for progesterone: positive status was more frequent in the trastuzumab group
Participants	N = 1010 female. Number of patients HER2-positive: 232 female Age: range from 25 to 64 years; median age 51.4 in trastuzumab arms and 49.9 in non-trastuzumab arms • Diagnosis: early invasive breast cancer, with at least one positive axillary node or node-negative breast cancer with at least 20 mm in diameter • Inclusion criteria: less than 66 years of age, WHO performance status of 0 or 1, prior breast surgery with axillary-node dissection or sentinel-node biopsy for invasive breast cancer, and a negative test for PRs. HER2-positive for randomisation to receive or not receive trastuzumab • Exclusion criteria: distant metastases, pregnancy, severe hypertension, cardiac diseases, abnormal laboratory values for hepatic function, abnormal haemochrome

FinHer (*Continued*)

	values Note: 55% had a tumour diameter larger than two cms; 26% had more than three nodes affected by the disease HER2-positive: 100% among the patients randomised to receive or not receive trastuzumab
Interventions	Adjuvant setting. Randomisation within 12 weeks after surgery • Arm A (N = 58): docetaxel (100 mg/sm, every three weeks, three cycles) followed by FEC [fluorouracile, epirubicin and cyclophosphamide (600 mg/sm, 60 mg/sm, 600 mg/sm every three weeks, three cycles)] • Arm B (N = 54): docetaxel (100 mg/sm, every three weeks, three cycles) plus trastuzumab (4 mg/kg loading dose and 2 mg/kg following, weekly, nine cycles) followed by FEC [fluorouracile, epirubicin and cyclophosphamide (600 mg/sm, 60 mg/sm, 600 mg/sm every three weeks, three cycles)]. The first trastuzumab infusion was given on day 1 of the first docetaxel cycle • Arm C (N = 58): vinorelbine (25 mg/sm, weekly, nine cycles) followed by FEC [fluorouracile, epirubicin and cyclophosphamide (600 mg/sm, 60 mg/sm, 600 mg/sm every three weeks, three cycles)] • Arm D (N = 62): vinorelbine (25 mg/sm, weekly, nine cycles) plus trastuzumab (4 mg/kg loading dose and 2 mg/kg following, weekly, 9 cycles) followed by FEC [fluorouracile, epirubicin and cyclophosphamide (600 mg/sm, 60 mg/sm, 600 mg/sm every 3 weeks, 3 cycles)]. The first trastuzumab infusion was given on day 1 of the first vinorelbine cycle • Other adjuvant therapies: ○ radiotherapy (after the completion of chemotherapy, according to each institution's guideline); ○ tamoxifen 20 mg/day for five years in ER+ patients. (December 4, 2005 the protocol was amended to allow switching of tamoxifen to an aromatase inhibitor for postmenopausal women after two to three years of use of tamoxifen to complete the five-year administration of a hormonal agent and to allow administration of an aromatase inhibitor for a further two to three years after completion of five-year administration of tamoxifen) • Other therapies: dexamethasone 7.5 mg in patients assigned to docetaxel at the moment of docetaxel infusion
Outcomes	Primary: recurrence-free survival in the first interim analysis; DDFS in the final analysis (see notes) Secondary: OS, adverse effects, time to distant recurrence Outcomes for cardiac adverse effects not clearly defined
Notes	Study designed to compare vinorelbine or docetaxel. Median follow-up: 62 months Final analysis DDFS became the primary endpoint in the final analysis published in 2009 instead of recurrence-free survival, used as primary endpoint for the first interim analysis. In the paper of 2009 it reported: "DDFS was the primary analysis in the entire series and in the subgroup of HER2-positive disease (Statistical Plan, June 20, 2007). Patients diagnosed with local or regional cancer recurrence and who did not have distant recurrence were censored on the date of data collection closure (August 15, 2007). Contralateral breast cancer was not considered distant recurrence. DDFS was preferred to time to

FinHer (*Continued*)

	any recurrence as the primary endpoint, because it allowed a longer follow-up time and collection of more endpoints before the final analysis and distant recurrences are more closely associated with mortality than local ones" Trial performed in 17 centres; did not report the number of patients per centre

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Centrally permuted block randomisation used to randomly assign all participants to receive three cycles of either docetaxel or vinorelbine. Stratification according to HER2 status (positive or negative) and institution
Allocation concealment (selection bias)	Low risk	Computer-assisted blinding
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	No patient lost at follow-up
Selective reporting (reporting bias)	High risk	Distant-Disease-Free-Survival (DDFS) became the primary endpoint in the final analysis, instead of recurrence-free survival. Trial authors preferred DDFS to time to any recurrence because it allowed a longer follow-up time and the collection of more endpoints before the final analysis and distant recurrences are more closely associated with mortality than local ones

HERA

Methods	Accrual time: December 2001 to June 2005 Multicentre, international. Baseline comparability between trastuzumab and observation arm: balanced
Participants	N = 5102 females recruited Age range: not available; median age: 49; 16% of patients were $\geq$ 60 years • Diagnosis: node-positive or node-negative (if the pathological tumour size was larger than 1 cm), histologically confirmed invasive breast cancer • Inclusion criteria: complete excision of the cancer and HER2 over expression or HER2 amplification (immunohistochemistry score 3 or FISH positive) assessed in the participating institution and verified in the central laboratory of the trial. Patient must have received at least four cycles of an approved (neo-) adjuvant chemotherapy

	regimen. In all cases, the maximum cumulative allowable dose of Doxorubicin was 360 mg/m2 and of Epirubicin was 720 mg/m2. Adequate baseline hepatic, renal, and bone marrow function and use of adequate nonhormone-based contraceptive measures, if indicated, were required • Exclusion criteria: distant metastases; previous invasive breast carcinoma; neoplasm not involving the breast (except for curatively treated basal-cell or squamous-cell carcinoma of the skin or in situ of the cervix); clinical stage T4 tumours including inflammatory breast cancer or involvement of supraclavicular nodes; suspicious internal mammary nodes (unless subjected to radiotherapy); prior mediastinal irradiation (except for internal mammary node irradiation for the present breast cancer); stem-cell support for chemotherapy; LVEF of less than 55% after completion of all chemotherapy and radiotherapy; history of documented CHF; coronary artery disease with previous Q-wave myocardial infarction, angina pectoris requiring medication, uncontrolled hypertension, clinically significant valvular disease, unstable arrhythmias Note: 5% of patients had a tumour diameter larger than 5 cm; 28% had more than four nodes affected by the disease HER2-positive: 100%
Interventions	Trastuzumab is given in patients who completed a minimum of four courses of chemotherapy (administered as adjuvant treatment postoperatively or as neoadjuvant treatment preoperatively or as both neoadjuvant and adjuvant). 89% of the participants, before receiving trastuzumab, received adjuvant chemotherapy postoperatively; 5% of the women received neoadjuvant chemotherapy; 6% of the women received both neoadjuvant and adjuvant chemotherapy. (Prior chemotherapy could be anthracyclines alone (68% of patients in each group), anthracyclines plus taxanes (26% of patients in each group) or a regimen without anthracyclines, including (CMF) (6% of patients in each group)). Taxanes could be given concurrently or sequentially to anthracyclines • Observation group (N = 1698): observation alone • One-year trastuzumab group (N = 1703): trastuzumab, initial dose 8 mg/kg, maintenance dose 6 mg/kg every three weeks for one year • Two-years trastuzumab group (N = 1701): trastuzumab, initial dose 8 mg/kg, maintenance dose 6 mg/kg every three weeks for two years • Other adjuvant treatments: endocrine therapy, primarily tamoxifen, was given after chemotherapy to women with hormone-receptor-positive disease unless contraindicated; during the course of the trial, an amendment to the protocol allowed aromatase inhibitors to be used instead of, or in sequence with, tamoxifen
Outcomes	Primary: DFS Secondary: cardiac safety; OS; site of first DFS event; time to distant recurrence
Notes	Median follow-up: 23.5 months 358 patients (10.5%) were randomised to receive or not receive trastuzumab as neoadjuvant treatment • First interim efficacy analysis planned after 475 events. This number of events was recorded on March 29, 2005, after a median follow-up of one year; the database was locked on April 8, 2005 and results were released. At that moment a protocol amendment was made after recruitment had been completed (except for the last five patients) to allow women in the observation group the option of switching to trastuzumab, irrespective of the interval since randomisation. Women who opted to

	switch were also given the further choice of a secondary randomisation to one year or two years of treatment with trastuzumab. As of May 2006, 861 patients in the observation group had switched to trastuzumab. The median time elapsed from the time of switching was 2.6 months • Results from a second interim efficacy analysis for OS results for observation alone or treatment with trastuzumab for one year with a median follow-up of two years were published in January 2007. In addition to the main ITT analysis, an analysis that censored women at the time of switching to trastuzumab was also done to compensate for a potential effect of delayed administration of trastuzumab. 705 patients originally randomly assigned to observation alone were censored for DFS and OS at the date of switching treatment Results for the group of patients treated with trastuzumab for two years remain blinded; in the published papers it reported that it is because the comparison with the group treated for one year continues to mature Multicentric trial: 478 centres involved of which 35 enrolled more than 25 patients. Range of the number of patients enrolled per centre: 1 to 105

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	A minimisation procedure, according to the methods of Pocock and Simon, was used with stratification according to region of the world, age, nodal status, type of chemotherapy, and hormone-receptor status together with intention to use endocrine therapy
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	97 (5.7%) patients randomly assigned to observation alone and 58 (3.4%) patients randomly assigned to 1 year of treatment with trastuzumab were lost to follow-up. Not available data for the group of patients treated with trastuzumab for 2 years (1703 patients)
Selective reporting (reporting bias)	Unclear risk	The study protocol is available but largely incomplete (http://clinicaltrials.gov/ct2/show/NCT00045032?term=hera&rank=1) The published report includes all expected efficacy outcomes but the cardiotoxicity out-

		come might have been reported incompletely

N9831

Methods	Accrual time: May 2000 to November 2004 Multicentre, national (US) Baseline comparability: balanced
Participants	N = 2766 female enrolled. 1615 included in the analysis, i.e. those enrolled by November 1, 2004, with follow-up as of March 15, 2005 Age: range from 22 to 80; 67% of patients were 40 to 59 (67,5%); 17% of patients were 60 years or older • Diagnosis: adenocarcinoma of the breast initially with histologically proven node positivity and, as of May 2003, with node negativity but at high risk of positivity (tumours with a diameter of more than 2 cm regardless of hormone-receptor status or of more than 1 cm with hormone-receptor-negative disease) • Inclusion criteria: complete resection of the primary tumour and axillary-node dissection; adequate hematopoietic, hepatic and renal function. LVEF that met or exceeded the lower limit of normal. Tumours had to be strongly HER2-positive; HER2-positivity had to be confirmed at central or reference laboratories • Exclusion criteria: clinical or radiological evidence of metastatic disease, angina pectoris requiring anti-anginal medication, arrhythmia requiring medication, severe conduction abnormality, clinically significant valvular disease, cardiomegaly on chest radiography, poorly controlled hypertension, clinically significant pericardial effusion, history of myocardial infarction, CHF, cardiomyopathy Note: 13% of patients had a tumour diameter larger than 4 cm; 13% had more than ten nodes affected by the disease HER2-positive: 100%
Interventions	• Group A (randomised N = 819; with follow-up N = 807): doxorubicin plus cyclophosphamide (60 mg/sm and 600 mg/sm, every 21 days, for four cycles) followed by paclitaxel (80 mg/sm, weekly, for 12 doses) • Group B (randomised N = 981; group excluded from the analysis): the same chemotherapy regimen of the Group A followed by trastuzumab beginning with a loading dose of 4 mg/kg of body weight followed by 51 further weekly doses of 2 mg/kg • Group C (randomised N = 814; with follow-up N = 808): doxorubicin plus cyclophosphamide (60 mg/sm and 600 mg/sm, every 21 days, for four cycles) followed by paclitaxel (80 mg/sm, weekly, for 12 doses) plus trastuzumab (first dose of 4 mg/kg given with the first dose of paclitaxel and 51 further weekly doses of 2 mg/kg) • Other adjuvant treatments: ○ radiotherapy in patients treated with lumpectomy (irradiation of the whole breast with an optional boost to the tumour bed and without irradiation of the internal mammary nodes), initiated after the completion of chemotherapy, without interruption of trastuzumab treatment; ○ tamoxifene (20 mg/day for five years) in oestrogen-receptor positive and progesterone-receptor positive patients, initiated on day 1 of the first cycle of doxorubicin and cyclophosphamide until an amendment on January 14, 2003,

	required hormonal therapy to be started after chemotherapy; ○ anastrozole in postmenopausal patients with oestrogen-receptor positive (ER+) or progesterone-receptor positive (PR+) tumours (permitted from January 14, 2003) It is not reported how many patients used these therapies in each arm of the trial
Outcomes	Primary: DFS Secondary: OS; time to distant recurrence; death from breast cancer; contralateral breast cancer; other second primary cancers; adverse effects
Notes	Median follow-up for efficacy: 1.5 years Interim analysis: trial stopped early for benefit. Criteria: difference in terms of DFS with a P value less than 0.0001 Efficacy is reported in the papers only for joint analysis of B31 and N9831: Group 1+ Group A (control) versus Group 2+Group C (concomitant trastuzumab). Group B (sequential trastuzumab) of N9831 trial was excluded from joint analysis (reasons are not clearly reported). After the implementation of central HER2 testing in March 2002, 284 patients of N9831 trial were removed from study Overall from the two trials, 4809 patients have been randomised. Follow-up data are available for 3351 patients (69.7%): 325 follow-up pending; 152 excluded for unclear reasons; 981 randomised to Group B and excluded. After the implementation of central HER2 testing in March 2002, 284 patients were removed from study Multicenter study: centres were all in US; the institutions involved in the trial enrolled at least 25 patients

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Used a dynamic allocation procedure that balanced the marginal distributions of nodal status and hormone-receptor status between groups. After 11 January, 2002, N9831 patients were randomised conditionally on central confirmation of HER2-positivity
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	High risk	2766 enrolled. 18 declined protocol therapy (of which 14 with follow-up pending as of March 2005). Another 4 patients remaining with follow-up pending as of March 2005. 152 patients, assigned to Group A of the trial from February

		to September 2002 while Group C was closed to enrolment because of concerns regarding cardiotoxicity, were excluded from joint analysis. 981 patients were assigned to Group B and excluded from joint analysis. Patients considered in the trial and analysed include those enrolled by November 1, 2004 with follow-up as of March 15, 2005 (i.e. 1615/2766 = 58% of the patients enrolled). After the implementation of central HER2 testing in March 2002, 284 patients were removed from study
Selective reporting (reporting bias)	Low risk	The study protocol is available but largely incomplete (http://clinicaltrials.gov/ct2/show/NCT00005970?term=n9831&rank=2) The published reports include all expected efficacy and safety outcomes

NOAH

Methods	Accrual time: June 20, 2002 to December 12, 2005. Multicentre, international Baseline comparability: balanced
Participants	N = 235 HER2-positive females were enrolled and randomised Age: range not reported; 42.5% of patients were less than 50 years; 57.5% of patients were ≥ 50 years • Diagnosis: histologically proven locally advanced breast cancer, defined as T3N1 or T4 (skin or nipple invasion, peau d'orange, extension into chest wall or inflammatory carcinoma), or any T plus N2 or N3, or any T plus involvement of ipsilateral supraclavicular nodes • Inclusion criteria: all patients had to have tumour hormone-receptors assessed and to have at least one measurable lesion according to response evaluation criteria in solid tumours (with a minimum indicator lesion size of 20 mm when measured by palpation) or inflammatory carcinoma, adequate hematopoietic, hepatic and renal function, an Eastern Cooperative Oncology Group performance status 1 or lower, and a LVEF of 55% or higher; women of childbearing potential had to use contraception. Tumours had to be HER2-positive (defined as 3+ over expression by immunohistochemical testing or HER2 amplification by fluorescent in-situ hybridisation according to a central laboratory • Exclusion criteria: bilateral breast cancer; metastases (other than in ipsilateral supraclavicular nodes); previous treatment for invasive malignant disease; previous or concurrent malignant disease other than basal-cell carcinoma of the skin or in-situ cervical cancer; pregnancy or lactation; use of other investigational drugs within the past 30 days; other serious illness or medical disorders including cardiac failure (New York Heart Association class II or higher) Note: all included patients with locally advanced breast cancer HER2-positive: 100%

NOAH (*Continued*)

Interventions	Chemotherapy given just as neoadjuvant therapy; trastuzumab as neoadjuvant and adjuvant treatment • Group 1 (randomised N = 117) doxorubicin 60 mg/sm plus paclitaxel 150 mg/sm, every three weeks for three cycles, followed by paclitaxel 175 mg/sm administered every three weeks for four cycles followed by cyclophosphamide (600 mg/sm), methotrexate (40 mg/sm), and fluorouracil (600 mg/sm) given on days 1 and 8 every four weeks for three cycles • Group 2 (randomised N = 118): the same chemotherapy of the first group plus trastuzumab loading dose of 8 mg per kg, followed by ten cycles of 6 mg/kg every three weeks alongside chemotherapy Trastuzumab could be given every 4 weeks during cyclophosphamide, methotrexate, and fluorouracil chemotherapy. After surgery (see below), additional cycles of trastuzumab were given, starting before or during radiotherapy (at the investigator's discretion), to complete one year of trastuzumab treatment • Other adjuvant treatments: surgery followed by radiotherapy was scheduled after completion of chemotherapy in all patients; tamoxifen 20 mg per day for five years in patients with oestrogen or PR-positive tumours
Outcomes	• Primary outcome: event-free survival (defined as time from randomisation to disease recurrence or progression: local, regional, distant, or contralateral; or death from any cause) • Secondary outcomes: pathological complete response in breast tissue, total pathological complete response (in breast and axilla), overall clinical response rates, cardiac safety, survival
Notes	Median follow-up: 3.2 years After positive results of adjuvant trastuzumab trials became available, HER2-positive patients allocated to chemotherapy alone were offered one year of adjuvant trastuzumab postsurgery. 19 (16%) patients with HER2-positive disease assigned to chemotherapy alone crossed over to receive adjuvant trastuzumab Multicentric study: 27 centres involved in six different countries

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomisation done centrally with a computer program and minimisation technique, taking account of geographical area, disease stage, and hormone-receptor status
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label

NOAH (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Low risk	No patient was lost at follow-up
Selective reporting (reporting bias)	Low risk	The study protocol is available but largely incomplete (http://www.controlled-trials.com/ISRCTN86043495/). The published report include all expected efficacy and safety outcomes

PACS-04

Methods	Accrual time: February 2001 to August 2004 Multicentre: France and Belgium Baseline comparability between the two groups from the second randomisation: in the trastuzumab group there were more larger tumours (diameter > 20 mm) with less lymph nodes involved (between 1 and 3)
Participants	Total number of participants in the first randomisation (FEC100 or ED75): 3010 female. Number of participants who underwent the second randomisation (trastuzumab or observation): 528 Age (second randomisation): range 22 to 65 years; median age 48 years • Diagnosis: axillary node-positive, histologically confirmed, non-metastatic, unilateral breast adenocarcinoma, with axillary lymph node invasion (N1, N2, or N3) and HER2 over expression assessed by an immunohistochemistry score 3 or FISH positivity • Inclusion criteria: age between 18 and 65 years, breast cancer completely surgically removed, at least four weeks since prior chemotherapy, normally blood tests, normal lung functions, LVEF above 55% or between 50% and 55% if approved by cardiologists, normal blood tests • Exclusion criteria: women with T4 or greater disease, clinically or radiologically suspected metastases, deeply adherent or inflammatory disease, clinically or radiologically suspected contralateral lesion, prior breast cancer, LVEF of less than 50%, clinical signs of CHF Note: 54% had a tumour diameter larger than 2 cm; 42% had more than four nodes affected by the disease HER2-positive: 100%
Interventions	Adjuvant setting. Trastuzumab sequential to chemiotherapy and radiotherapy First randomisation [fluorouracil + epirubicine + cyclophosphamide (FE100C) vs epirubicin + docetaxel (ED75)]: • Arm FEC100 (N = 1515): fluorouracil plus epirubicin plus cyclophosphamide (FE100C: F and C 500 mg/m2, E 100 mg/m2), every three weeks for six cycles • Arm ED75 (N = 1495): epirubicin plus docetaxel (both 75 mg/m2), every three weeks for six cycles Second randomisation (trastuzumab vs observation): • Observation arm (N = 268): observation only • Trastuzumab arm (N = 260): one year of trastuzumab (8 mg/kg loading dose, 6 mg/kg subsequent doses) every three weeks for one year

PACS-04 (*Continued*)

	Other adjuvant treatments: radiotherapy (administered before starting trastuzumab, within four weeks after chemotherapy completion) in all patients who underwent breast-conserving surgery; endocrine therapy in patients with hormone-receptor-positive disease (tamoxifen 20 mg/day for five years and anastrozole or tamoxifen, at the discretion of investigator, for postmenopausal women)
Outcomes	• Primary endpoint: DFS • Secondary endpoints: adverse events; event-free survival OS Outcomes for evaluating cardiac adverse effect (not defined with precision): LVEF dropped below 45%; LVEF between 45% and 50% together with a relative decrease of 15% or more; heart failure; cardiac death
Notes	Primary objective of the trial was to evaluate the efficacy and tolerance of combined administration of docetaxel and epirubicin as compared to standard chemotherapy regimen with 5-fluorouracil, epirubicin and cyclophosphamide. The second objective was to evaluate the efficacy and tolerance of trastuzumab given for 1 year as sequential treatment after chemotherapy and radiotherapy. Sample size was determined according to the second objective. Efficacy data are not reported according the first allocated chemotherapy regimen; on the other hand cardiac safety effects are reported in this way Median follow-up: 47 months Median time between first and second randomisation was 2.6 months Of the 260 patients randomly assigned to receive trastuzumab 26 did not receive trastuzumab because of refusal (N = 17), occurrence of cardiac toxicity under previous chemotherapy (N = 5) and other reasons (N = 4) Study performed in 68 centres in France and Belgium, not specifically stated

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not reported
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding (performance bias and detection bias) All outcomes	High risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	Not reported
Selective reporting (reporting bias)	Unclear risk	The study protocol is available (http://clinicaltrials.gov/ct2/show/study/NCT00054587?term=NCT00054587&rank=1) but largely incomplete: description of the outcomes is not reported

CHF: congestive heart failure
CMF: cyclophosphamide combined with methotrexate and fluorouracil
DDFS: distant disease-free survival
DFS: disease-free survival
ER: oestrogen receptor
FEC: fluorouracil, epirubicin, cyclophosphamide
FISH: fluorescence in situ hybridisation
HER2: Human Epidermal growth factor Receptor 2
ITT: intention-to-treat
IV: intravenous
LVEF: left ventricular ejection fraction
OS: overall survival
pCR: pathologic complete remission rate
PR: progesterone receptor
QoL: quality of life
WHO: World Health Organization

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Belkacemi 2008	Trial not randomised, designed to evaluate the toxic effect of concurrent radiation therapy associated with two different trastuzumab administration regimens
E2198	All arms contain trastuzumab, even though at different doses
GeparQuattro trial	Trial designed to compare chemotherapy regimens other than trastuzumab. All HER2 patients receive trastuzumab, independently from randomisation arm
Hurley 2006	Trial not randomised

DATA AND ANALYSES

Comparison 1. Effect of trastuzumab

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Overall Survival (OS)- all studies	7	9935	Hazard Ratio (Random, 95% CI)	0.66 [0.57, 0.77]
2 OS stratified by duration of trastuzumab administration	7		Hazard Ratio (Random, 95% CI)	0.66 [0.57, 0.77]
2.1 <= 6 months	2		Hazard Ratio (Random, 95% CI)	0.55 [0.27, 1.11]
2.2 > 6 months	5		Hazard Ratio (Random, 95% CI)	0.67 [0.57, 0.80]
3 OS stratified by type of trastuzumab administration	7		Hazard Ratio (Random, 95% CI)	0.66 [0.57, 0.77]
3.1 Sequential	2		Hazard Ratio (Random, 95% CI)	0.85 [0.43, 1.67]
3.2 Concurrent	5		Hazard Ratio (Random, 95% CI)	0.64 [0.53, 0.76]
4 Disease Free Survival (DFS) - all studies	7	9935	Hazard Ratio (Random, 95% CI)	0.60 [0.50, 0.71]
5 DFS stratified by duration of trastuzumab administration	7		Hazard Ratio (Random, 95% CI)	0.60 [0.50, 0.71]
5.1 <= 6 months	2		Hazard Ratio (Random, 95% CI)	0.31 [0.10, 0.96]
5.2 > 6 months	5		Hazard Ratio (Random, 95% CI)	0.62 [0.52, 0.72]
6 DFS stratified by type of trastuzumab administration	7		Hazard Ratio (Random, 95% CI)	0.60 [0.50, 0.71]
6.1 Sequential	2		Hazard Ratio (Random, 95% CI)	0.71 [0.53, 0.95]
6.2 Concurrent	5		Hazard Ratio (Random, 95% CI)	0.54 [0.44, 0.67]

Comparison 2. Cardiac toxicity

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Congestive Heart Failure (CHF) - all studies	8	10281	Risk Ratio (IV, Random, 90% CI)	5.11 [3.00, 8.72]
2 CHF stratified by duration of trastuzumab administration	8	10281	Risk Ratio (IV, Random, 90% CI)	5.11 [3.00, 8.72]
2.1 <= 6 months	2	273	Risk Ratio (IV, Random, 90% CI)	0.50 [0.07, 3.74]
2.2 > 6 months	6	10008	Risk Ratio (IV, Random, 90% CI)	5.39 [3.56, 8.17]
3 CHF stratified by type of trastuzumab administration	8	10281	Risk Ratio (IV, Random, 90% CI)	5.12 [3.15, 8.32]
3.1 Sequential (randomisation before chemotherapy)	1	1042	Risk Ratio (IV, Random, 90% CI)	8.42 [1.56, 45.45]
3.2 Sequential (randomisation after chemotherapy)	2	3914	Risk Ratio (IV, Random, 90% CI)	11.05 [3.46, 35.29]
3.3 Concurrent	6	5325	Risk Ratio (IV, Random, 90% CI)	3.90 [2.42, 6.28]

4 Left Ventricular Ejection Fraction (LVEF) decline - all studies	7	7939	Risk Ratio (IV, Random, 90% CI)	1.83 [1.36, 2.47]
5 LVEF decline stratified by duration of trastuzumab administration	7	7939	Risk Ratio (IV, Random, 90% CI)	1.83 [1.36, 2.47]
5.1 <= 6 months	2	273	Risk Ratio (IV, Random, 90% CI)	0.89 [0.51, 1.57]
5.2 > 6 months	5	7666	Risk Ratio (IV, Random, 90% CI)	2.14 [1.58, 2.89]
6 LVEF decline stratified by type of trastuzumab administration	7	7939	Risk Ratio (IV, Random, 90% CI)	1.78 [1.35, 2.35]
6.1 Sequential (randomisation before chemotherapy)	1	791	Risk Ratio (IV, Random, 90% CI)	1.16 [0.68, 1.99]
6.2 Sequential (randomisation after chemotherapy)	2	3914	Risk Ratio (IV, Random, 90% CI)	2.90 [2.23, 3.76]
6.3 Concurrent	5	3234	Risk Ratio (IV, Random, 90% CI)	1.48 [1.11, 1.97]

Comparison 3. Other toxicities

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Neutropenic fever - all studies	4	3396	Risk Ratio (IV, Random, 90% CI)	1.18 [0.99, 1.41]
2 Neutropenic fever stratified by duration of trastuzumab administration	4	3396	Risk Ratio (IV, Random, 90% CI)	1.18 [0.99, 1.41]
2.1 <= 6 months	2	1050	Risk Ratio (IV, Random, 90% CI)	1.14 [0.83, 1.58]
2.2 > 6 months	2	2346	Risk Ratio (IV, Random, 90% CI)	1.19 [0.96, 1.48]
3 Anaemia - all studies	2	2998	Risk Ratio (IV, Random, 90% CI)	1.24 [0.81, 1.88]
4 Anaemia stratified by duration of trastuzumab administration	2	2998	Risk Ratio (IV, Random, 90% CI)	1.24 [0.81, 1.88]
4.1 <= 6 months	1	880	Risk Ratio (IV, Random, 90% CI)	0.94 [0.08, 11.28]
4.2 > 6 months	1	2118	Risk Ratio (IV, Random, 90% CI)	1.25 [0.82, 1.91]
5 Neutropenia - all studies	4	3268	Risk Ratio (IV, Random, 90% CI)	1.10 [1.00, 1.22]
6 Neutropenia stratified by duration of trastuzumab administration	4	3268	Risk Ratio (IV, Random, 90% CI)	1.10 [1.00, 1.22]
6.1 <= 6 months	2	922	Risk Ratio (IV, Random, 90% CI)	1.21 [0.85, 1.72]
6.2 > 6 months	2	2346	Risk Ratio (IV, Random, 90% CI)	1.12 [1.07, 1.18]

Comparison 4. Brain metastases as site of first relapse

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Brain metastases - all studies	5	6881	Risk Ratio (IV, Random, 90% CI)	1.75 [1.29, 2.38]
2 Brain metastases stratified by duration of trastuzumab administration	5	6881	Risk Ratio (IV, Random, 90% CI)	1.75 [1.29, 2.38]
2.1 <= 6 months	1	231	Risk Ratio (IV, Random, 90% CI)	0.61 [0.19, 1.97]
2.2 > 6 months	4	6650	Risk Ratio (IV, Random, 90% CI)	1.89 [1.38, 2.59]
3 Brain metastases stratified by type of trastuzumab administration	5	6881	Risk Ratio (IV, Random, 90% CI)	1.75 [1.29, 2.38]
3.1 Sequential	2	3299	Risk Ratio (IV, Random, 90% CI)	1.73 [1.16, 2.58]
3.2 Concurrent	3	3582	Risk Ratio (IV, Random, 90% CI)	1.71 [0.91, 3.21]

Comparison 5. Sensitivity analysis

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 OS - by allocation concealment	7	9935	Hazard Ratio (Random, 95% CI)	0.66 [0.57, 0.77]
1.1 Adequate	2	466	Hazard Ratio (Random, 95% CI)	0.59 [0.37, 0.93]
1.2 Inadequate	5	9469	Hazard Ratio (Random, 95% CI)	0.69 [0.56, 0.85]
2 DFS - by allocation concealment	7	9935	Hazard Ratio (Random, 95% CI)	0.60 [0.50, 0.71]
2.1 Adequate	2	466	Hazard Ratio (Random, 95% CI)	0.53 [0.37, 0.77]
2.2 Inadequate	5	9469	Hazard Ratio (Random, 95% CI)	0.61 [0.50, 0.75]

Analysis 1.1. Comparison 1 Effect of trastuzumab, Outcome 1 Overall Survival (OS)- all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 1 Effect of trastuzumab

Outcome: 1 Overall Survival (OS)- all studies

Study or subgroup	Experimental N	Control N	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
B31 (1)	1672	1679	-0.4 (0.17)		22.0 %	0.67 [0.48, 0.94]
BCIRG006	1074	1073	-0.46 (0.13)		37.7 %	0.63 [0.49, 0.81]
Buzdar	23	19	0 (0)			Not estimable
FinHer	115	116	-0.6 (0.36)		4.9 %	0.55 [0.27, 1.11]
HERA	1703	1698	-0.46 (0.17)		22.0 %	0.63 [0.45, 0.88]
NOAH	117	118	-0.48 (0.3)		7.1 %	0.62 [0.34, 1.11]
PACS-04	260	268	0.24 (0.32)		6.2 %	1.27 [0.68, 2.38]
Total (95% CI)	**4964**	**4971**			**100.0 %**	**0.66 [0.57, 0.77]**

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 4.70$, df = 5 (P = 0.45); $I^2 = 0.0\%$

Test for overall effect: Z = 5.16 (P < 0.00001)

Test for subgroup differences: Not applicable

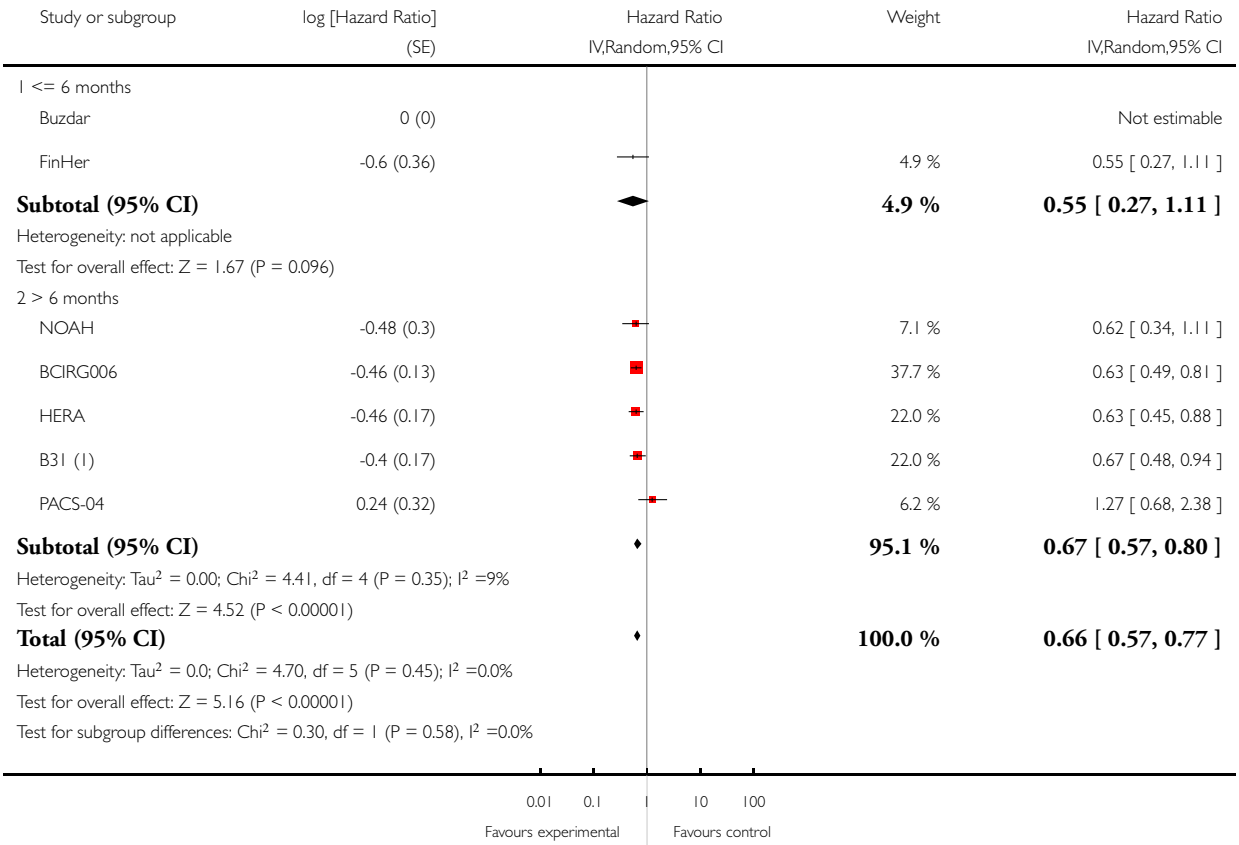
(1) B31+N9831

Analysis 1.2. Comparison 1 Effect of trastuzumab, Outcome 2 OS stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 1 Effect of trastuzumab

Outcome: 2 OS stratified by duration of trastuzumab administration



(1) B31+N9831

Analysis 1.3. Comparison 1 Effect of trastuzumab, Outcome 3 OS stratified by type of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 1 Effect of trastuzumab

Outcome: 3 OS stratified by type of trastuzumab administration

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
1 Sequential				
HERA	-0.46 (0.17)		22.0 %	0.63 [0.45, 0.88]
PACS-04	0.24 (0.32)		6.2 %	1.27 [0.68, 2.38]
Subtotal (95% CI)			**28.3 %**	**0.85 [0.43, 1.67]**
Heterogeneity: Tau^2 = 0.18; Chi^2 = 3.73, df = 1 (P = 0.05); I^2 =73%				
Test for overall effect: Z = 0.47 (P = 0.64)				
2 Concurrent				
B31 (1)	-0.4 (0.17)		22.0 %	0.67 [0.48, 0.94]
BCIRG006	-0.46 (0.13)		37.7 %	0.63 [0.49, 0.81]
Buzdar	0 (0)			Not estimable
FinHer	-0.6 (0.36)		4.9 %	0.55 [0.27, 1.11]
NOAH	-0.48 (0.3)		7.1 %	0.62 [0.34, 1.11]
Subtotal (95% CI)			**71.7 %**	**0.64 [0.53, 0.76]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.27, df = 3 (P = 0.96); I^2 =0.0%				
Test for overall effect: Z = 4.81 (P < 0.00001)				
Total (95% CI)			**100.0 %**	**0.66 [0.57, 0.77]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 4.70, df = 5 (P = 0.45); I^2 =0.0%				
Test for overall effect: Z = 5.16 (P < 0.00001)				
Test for subgroup differences: Chi^2 = 0.66, df = 1 (P = 0.42), I^2 =0.0%				

(1) B31+N9831

Analysis 1.4. Comparison 1 Effect of trastuzumab, Outcome 4 Disease Free Survival (DFS) - all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 1 Effect of trastuzumab

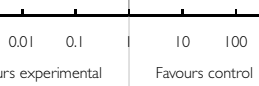
Outcome: 4 Disease Free Survival (DFS) - all studies

Study or subgroup	Experimental N	Control N	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
B31 (1)	1672	1679	-0.73 (0.11)		21.8 %	0.48 [0.39, 0.60]
BCIRG006	1074	1073	-0.45 (0.1)		23.2 %	0.64 [0.52, 0.78]
Buzdar	23	19	-2.27 (1.11)		0.6 %	0.10 [0.01, 0.91]
FinHer	115	116	-0.87 (0.35)		5.2 %	0.42 [0.21, 0.83]
HERA	1703	1698	-0.46 (0.09)		24.7 %	0.63 [0.53, 0.75]
NOAH	117	118	-0.53 (0.22)		10.7 %	0.59 [0.38, 0.91]
PACS-04	260	268	-0.15 (0.18)		13.8 %	0.86 [0.60, 1.22]
Total (95% CI)	**4964**	**4971**			**100.0 %**	**0.60 [0.50, 0.71]**

Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 12.25$, df = 6 (P = 0.06); $I^2 = 51\%$

Test for overall effect: Z = 5.89 (P < 0.00001)

Test for subgroup differences: Not applicable



(1) B31+N9831

Analysis 1.5. Comparison 1 Effect of trastuzumab, Outcome 5 DFS stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 1 Effect of trastuzumab

Outcome: 5 DFS stratified by duration of trastuzumab administration

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
1 <= 6 months				
Buzdar	-2.27 (1.11)		0.6 %	0.10 [0.01, 0.91]
FinHer	-0.87 (0.35)		5.2 %	0.42 [0.21, 0.83]
Subtotal (95% CI)			**5.8 %**	**0.31 [0.10, 0.96]**
Heterogeneity: Tau^2 = 0.30; Chi^2 = 1.45, df = 1 (P = 0.23); I^2 =31%				
Test for overall effect: Z = 2.03 (P = 0.042)				
2 > 6 months				
B31 (1)	-0.73 (0.11)		21.8 %	0.48 [0.39, 0.60]
BCIRG006	-0.45 (0.1)		23.2 %	0.64 [0.52, 0.78]
HERA	-0.46 (0.09)		24.7 %	0.63 [0.53, 0.75]
NOAH	-0.53 (0.22)		10.7 %	0.59 [0.38, 0.91]
PACS-04	-0.15 (0.18)		13.8 %	0.86 [0.60, 1.22]
Subtotal (95% CI)			**94.2 %**	**0.62 [0.52, 0.72]**
Heterogeneity: Tau^2 = 0.02; Chi^2 = 8.62, df = 4 (P = 0.07); I^2 =54%				
Test for overall effect: Z = 5.83 (P < 0.00001)				
Total (95% CI)			**100.0 %**	**0.60 [0.50, 0.71]**
Heterogeneity: Tau^2 = 0.02; Chi^2 = 12.25, df = 6 (P = 0.06); I^2 =51%				
Test for overall effect: Z = 5.89 (P < 0.00001)				
Test for subgroup differences: Chi^2 = 1.40, df = 1 (P = 0.24), I^2 =28%				

(1) B31+N9831

Analysis 1.6. Comparison 1 Effect of trastuzumab, Outcome 6 DFS stratified by type of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 1 Effect of trastuzumab

Outcome: 6 DFS stratified by type of trastuzumab administration

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
1 Sequential				
HERA	-0.46 (0.09)		24.7 %	0.63 [0.53, 0.75]
PACS-04	-0.15 (0.18)		13.8 %	0.86 [0.60, 1.22]
Subtotal (95% CI)			**38.4 %**	**0.71 [0.53, 0.95]**
Heterogeneity: Tau^2 = 0.03; Chi^2 = 2.37, df = 1 (P = 0.12); I^2 =58%				
Test for overall effect: Z = 2.30 (P = 0.022)				
2 Concurrent				
B31 (1)	-0.73 (0.11)		21.8 %	0.48 [0.39, 0.60]
BCIRG006	-0.45 (0.1)		23.2 %	0.64 [0.52, 0.78]
Buzdar	-2.27 (1.11)		0.6 %	0.10 [0.01, 0.91]
FinHer	-0.87 (0.35)		5.2 %	0.42 [0.21, 0.83]
NOAH	-0.53 (0.22)		10.7 %	0.59 [0.38, 0.91]
Subtotal (95% CI)			**61.6 %**	**0.54 [0.44, 0.67]**
Heterogeneity: Tau^2 = 0.02; Chi^2 = 6.58, df = 4 (P = 0.16); I^2 =39%				
Test for overall effect: Z = 5.74 (P < 0.00001)				
Total (95% CI)			**100.0 %**	**0.60 [0.50, 0.71]**
Heterogeneity: Tau^2 = 0.02; Chi^2 = 12.25, df = 6 (P = 0.06); I^2 =51%				
Test for overall effect: Z = 5.89 (P < 0.00001)				
Test for subgroup differences: Chi^2 = 2.15, df = 1 (P = 0.14), I^2 =53%				

(1) B31+N9831

Analysis 2.1. Comparison 2 Cardiac toxicity, Outcome 1 Congestive Heart Failure (CHF) - all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 2 Cardiac toxicity

Outcome: 1 Congestive Heart Failure (CHF) - all studies

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
B31	35/932	9/872		31.7 %	3.64 [1.98, 6.70]
BCIRG006	20/1068	4/1050		21.4 %	4.92 [2.00, 12.07]
Buzdar	0/23	0/19			Not estimable
FinHer	1/115	2/116		6.3 %	0.50 [0.07, 3.74]
HERA	36/1678	2/1708		14.6 %	18.32 [5.55, 60.44]
N9831	37/1280	2/664		14.6 %	9.60 [2.92, 31.59]
NOAH	2/115	0/113		4.1 %	4.91 [0.39, 62.24]
PACS-04	4/260	1/268		7.3 %	4.12 [0.66, 25.79]
Total (0.0% CI)	**5471**	**4810**		**100.0 %**	**5.11 [3.00, 8.72]**

Total events: 135 (Experimental), 20 (Control)

Heterogeneity: $Tau^2 = 0.20$; $Chi^2 = 8.32$, df = 6 (P = 0.22); $I^2 = 28\%$

Test for overall effect: Z = 5.02 (P < 0.00001)

Test for subgroup differences: Not applicable

Analysis 2.2. Comparison 2 Cardiac toxicity, Outcome 2 CHF stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 2 Cardiac toxicity

Outcome: 2 CHF stratified by duration of trastuzumab administration

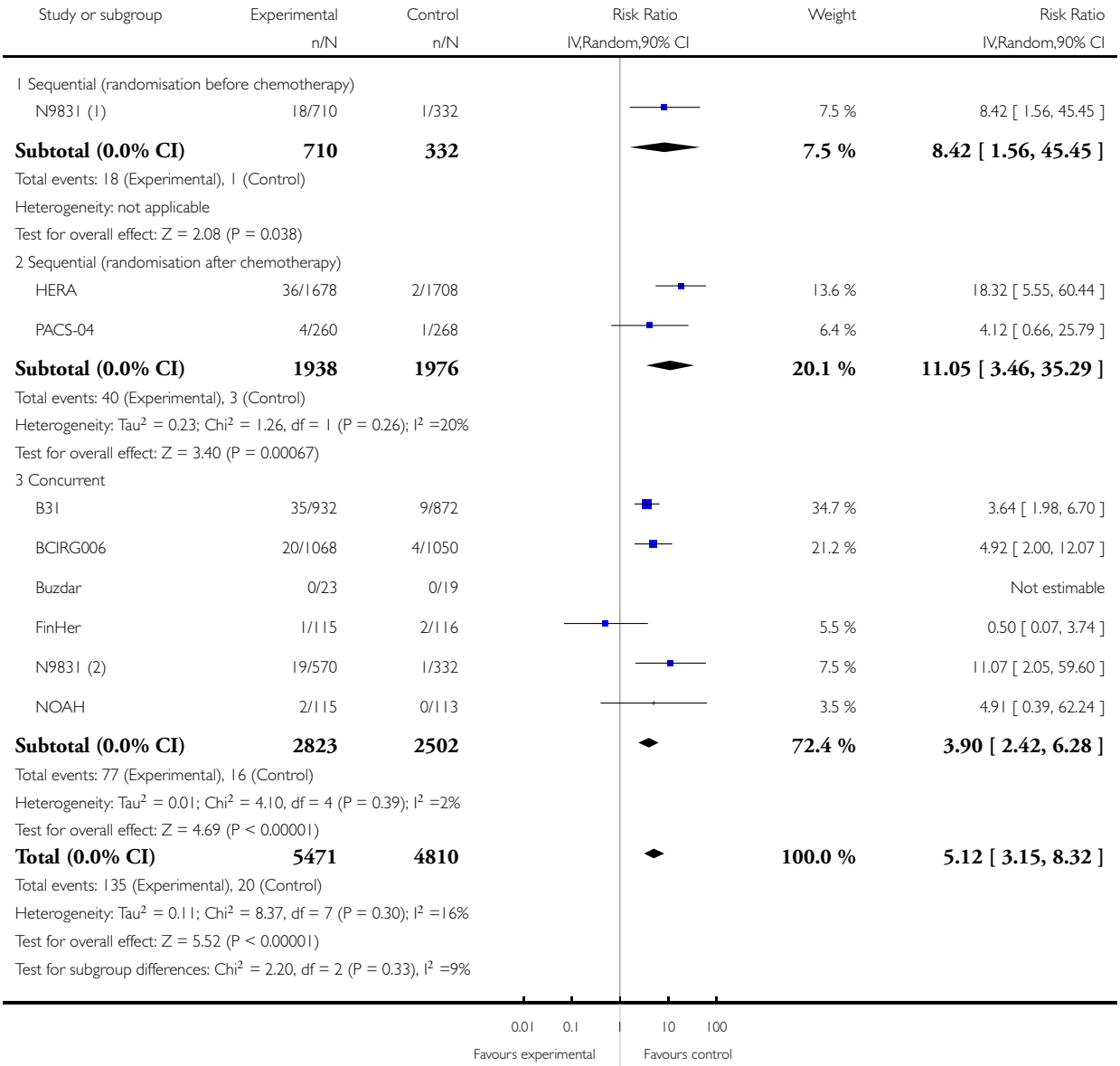
Study or subgroup	Experimental n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
1 <= 6 months					
Buzdar	0/23	0/19			Not estimable
FinHer	1/115	2/116		6.3 %	0.50 [0.07, 3.74]
Subtotal (0.0% CI)	**138**	**135**		**6.3 %**	**0.50 [0.07, 3.74]**
Total events: 1 (Experimental), 2 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 0.56 (P = 0.57)					
2 > 6 months					
B31	35/932	9/872		31.7 %	3.64 [1.98, 6.70]
BCIRG006	20/1068	4/1050		21.4 %	4.92 [2.00, 12.07]
HERA	36/1678	2/1708		14.6 %	18.32 [5.55, 60.44]
N9831	37/1280	2/664		14.6 %	9.60 [2.92, 31.59]
NOAH	2/115	0/113		4.1 %	4.91 [0.39, 62.24]
PACS-04	4/260	1/268		7.3 %	4.12 [0.66, 25.79]
Subtotal (0.0% CI)	**5333**	**4675**		**93.7 %**	**5.39 [3.56, 8.17]**
Total events: 134 (Experimental), 18 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 4.69, df = 5 (P = 0.45); I^2 =0.0%					
Test for overall effect: Z = 6.67 (P < 0.00001)					
Total (0.0% CI)	**5471**	**4810**		**100.0 %**	**5.11 [3.00, 8.72]**
Total events: 135 (Experimental), 20 (Control)					
Heterogeneity: Tau^2 = 0.20; Chi^2 = 8.32, df = 6 (P = 0.22); I^2 =28%					
Test for overall effect: Z = 5.02 (P < 0.00001)					
Test for subgroup differences: Chi^2 = 3.63, df = 1 (P = 0.06), I^2 =72%					

Analysis 2.3. Comparison 2 Cardiac toxicity, Outcome 3 CHF stratified by type of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 2 Cardiac toxicity

Outcome: 3 CHF stratified by type of trastuzumab administration



(1) arm B vs arm A *

(2) arm C vs arm A *

Analysis 2.4. Comparison 2 Cardiac toxicity, Outcome 4 Left Ventricular Ejection Fraction (LVEF) decline - all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 2 Cardiac toxicity

Outcome: 4 Left Ventricular Ejection Fraction (LVEF) decline - all studies

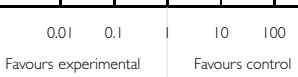
Study or subgroup	Favours experimental n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
BCIRG006	189/1042	102/1014		22.9 %	1.80 [1.49, 2.18]
Buzdar	7/23	5/19		8.7 %	1.16 [0.51, 2.62]
FinHer	7/115	10/116		9.2 %	0.71 [0.32, 1.54]
HERA	118/1678	35/1708		19.8 %	3.43 [2.51, 4.69]
N9831	51/914	25/554		17.6 %	1.24 [0.84, 1.83]
NOAH	2/115	0/113		1.3 %	4.91 [0.39, 62.24]
PACS-04	92/260	38/268		20.5 %	2.50 [1.88, 3.31]
Total (0.0% CI)	**4147**	**3792**		**100.0 %**	**1.83 [1.36, 2.47]**

Total events: 466 (Favours experimental), 215 (Control)

Heterogeneity: $Tau^2 = 0.13$; $Chi^2 = 21.04$, df = 6 (P = 0.002); $I^2 = 71\%$

Test for overall effect: Z = 3.34 (P = 0.00085)

Test for subgroup differences: Not applicable



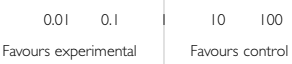
Analysis 2.5. Comparison 2 Cardiac toxicity, Outcome 5 LVEF decline stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 2 Cardiac toxicity

Outcome: 5 LVEF decline stratified by duration of trastuzumab administration

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
1 <= 6 months					
Buzdar	7/23	5/19		8.7 %	1.16 [0.51, 2.62]
FinHer	7/115	10/116		9.2 %	0.71 [0.32, 1.54]
Subtotal (0.0% CI)	**138**	**135**		**17.9 %**	**0.89 [0.51, 1.57]**
Total events: 14 (Experimental), 15 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.52, df = 1 (P = 0.47); I^2 =0.0%					
Test for overall effect: Z = 0.33 (P = 0.74)					
2 > 6 months					
BCIRG006	189/1042	102/1014		22.9 %	1.80 [1.49, 2.18]
HERA	118/1678	35/1708		19.8 %	3.43 [2.51, 4.69]
N9831	51/914	25/554		17.6 %	1.24 [0.84, 1.83]
NOAH	2/115	0/113		1.3 %	4.91 [0.39, 62.24]
PACS-04	92/260	38/268		20.5 %	2.50 [1.88, 3.31]
Subtotal (0.0% CI)	**4009**	**3657**		**82.1 %**	**2.14 [1.58, 2.89]**
Total events: 452 (Experimental), 200 (Control)					
Heterogeneity: Tau^2 = 0.10; Chi^2 = 14.74, df = 4 (P = 0.01); I^2 =73%					
Test for overall effect: Z = 4.17 (P = 0.000031)					
Total (0.0% CI)	**4147**	**3792**		**100.0 %**	**1.83 [1.36, 2.47]**
Total events: 466 (Experimental), 215 (Control)					
Heterogeneity: Tau^2 = 0.13; Chi^2 = 21.04, df = 6 (P = 0.002); I^2 =71%					
Test for overall effect: Z = 3.34 (P = 0.00085)					
Test for subgroup differences: Chi^2 = 5.04, df = 1 (P = 0.02), I^2 =80%					

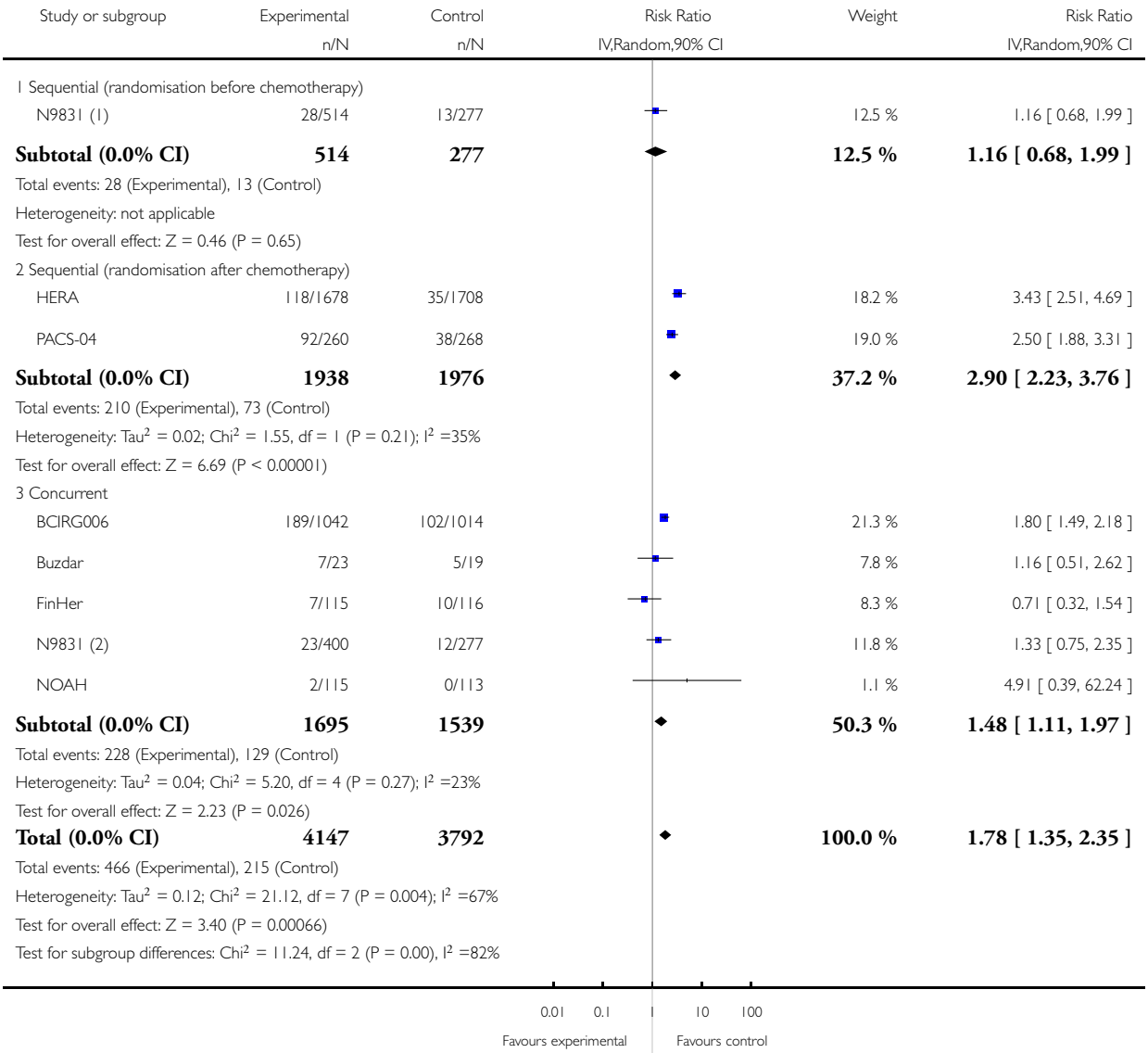


Analysis 2.6. Comparison 2 Cardiac toxicity, Outcome 6 LVEF decline stratified by type of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 2 Cardiac toxicity

Outcome: 6 LVEF decline stratified by type of trastuzumab administration



(1) arm B vs arm A *

(2) arm C vs arm A *

Analysis 3.1. Comparison 3 Other toxicities, Outcome 1 Neutropenic fever - all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 3 Other toxicities

Outcome: 1 Neutropenic fever - all studies

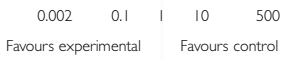
Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
BCIRG006	117/1068	96/1050		68.5 %	1.20 [0.97, 1.49]
Buzdar	8/23	8/19		7.6 %	0.83 [0.43, 1.57]
FinHer (1)	19/115	116/893		22.7 %	1.27 [0.88, 1.85]
NOAH	2/115	2/113		1.2 %	0.98 [0.19, 5.02]
Total (0.0% CI)	**1321**	**2075**		**100.0 %**	**1.18 [0.99, 1.41]**

Total events: 146 (Trastuzumab), 222 (Control)

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.98$, df = 3 (P = 0.81); $I^2 = 0.0\%$

Test for overall effect: Z = 1.51 (P = 0.13)

Test for subgroup differences: Not applicable



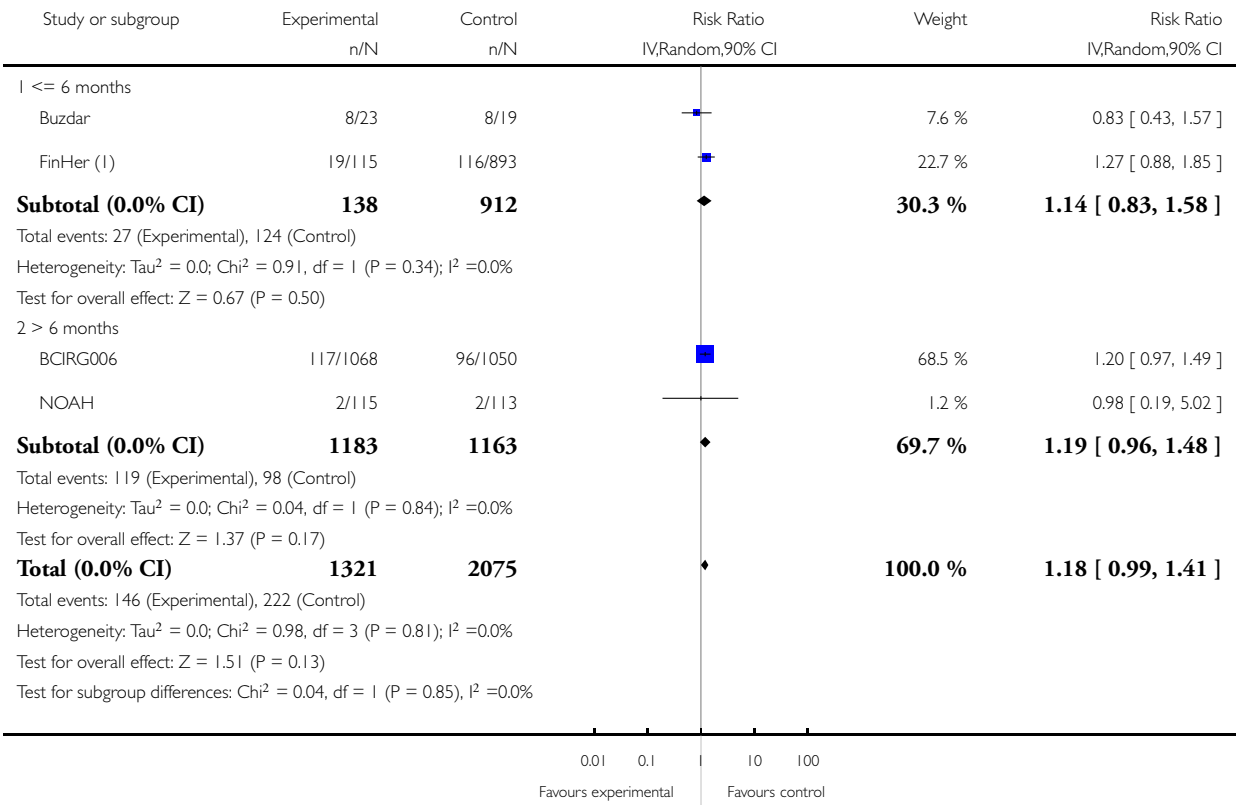
(1) FinHer control arm includes both HER2 positive and negative patients

Analysis 3.2. Comparison 3 Other toxicities, Outcome 2 Neutropenic fever stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 3 Other toxicities

Outcome: 2 Neutropenic fever stratified by duration of trastuzumab administration



(1) FinHer control arm includes both HER2 positive and negative patients

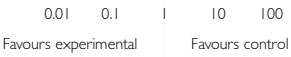
Analysis 3.3. Comparison 3 Other toxicities, Outcome 3 Anaemia - all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 3 Other toxicities

Outcome: 3 Anaemia - all studies

Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
BCIRG006	33/1068	26/1050		97.1 %	1.25 [0.82, 1.91]
FinHer (1)	0/115	3/765		2.9 %	0.94 [0.08, 11.28]
Total (0.0% CI)	**1183**	**1815**		**100.0 %**	**1.24 [0.81, 1.88]**
Total events: 33 (Trastuzumab), 29 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.03$, df = 1 (P = 0.85); $I^2 = 0.0\%$					
Test for overall effect: Z = 0.84 (P = 0.40)					
Test for subgroup differences: Not applicable					



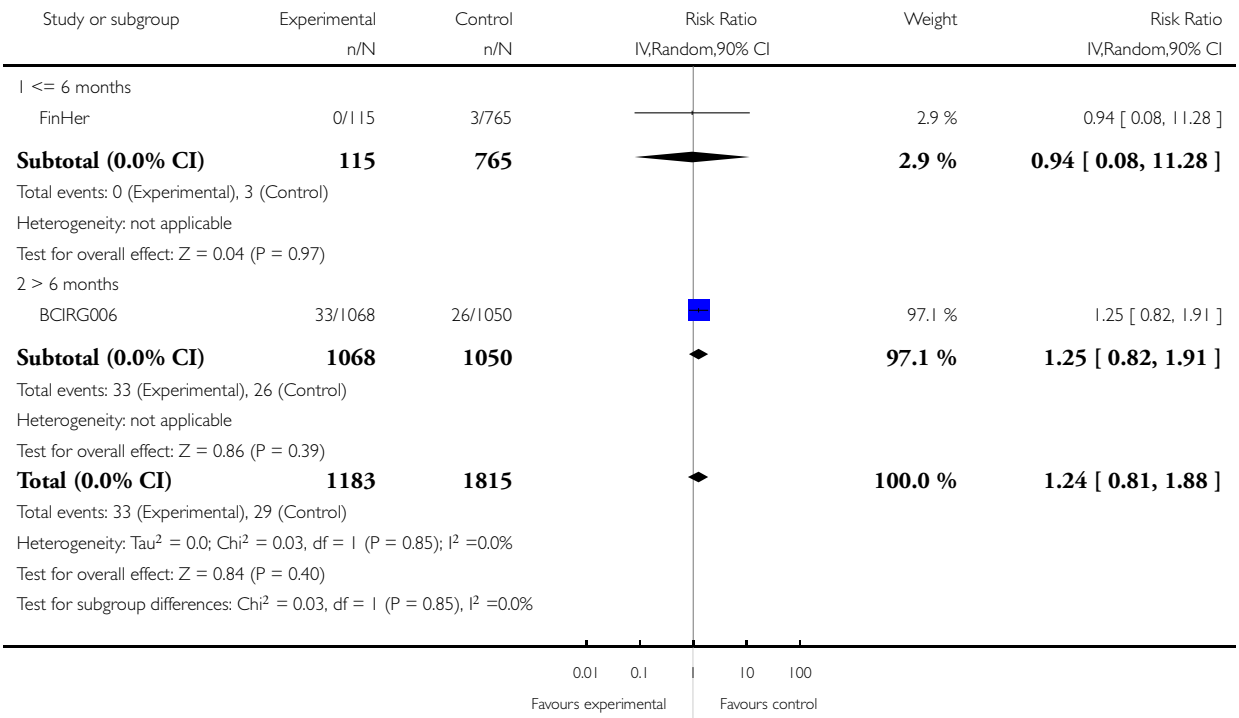
(1) FinHer control arm includes both HER2 positive and negative patients

Analysis 3.4. Comparison 3 Other toxicities, Outcome 4 Anaemia stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 3 Other toxicities

Outcome: 4 Anaemia stratified by duration of trastuzumab administration



Analysis 3.5. Comparison 3 Other toxicities, Outcome 5 Neutropenia - all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 3 Other toxicities

Outcome: 5 Neutropenia - all studies

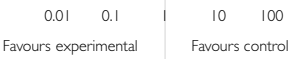
Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
BCIRG006	761/1068	665/1050		52.3 %	1.13 [1.07, 1.18]
Buzdar	21/23	11/19		7.4 %	1.58 [1.12, 2.21]
FinHer (1)	88/115	574/765		39.7 %	1.02 [0.93, 1.12]
NOAH	3/115	5/113		0.7 %	0.59 [0.18, 1.92]
Total (0.0% CI)	**1321**	**1947**		**100.0 %**	**1.10 [1.00, 1.22]**

Total events: 873 (Trastuzumab), 1255 (Control)

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 6.17$, df = 3 (P = 0.10); $I^2 = 51\%$

Test for overall effect: Z = 1.66 (P = 0.096)

Test for subgroup differences: Not applicable



(1) FinHer control arm includes both HER2 positive and negative patients

Analysis 3.6. Comparison 3 Other toxicities, Outcome 6 Neutropenia stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 3 Other toxicities

Outcome: 6 Neutropenia stratified by duration of trastuzumab administration

Study or subgroup	Experimental n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
1 <= 6 months					
Buzdar	21/23	11/19		7.4 %	1.58 [1.12, 2.21]
FinHer (1)	88/115	574/765		39.7 %	1.02 [0.93, 1.12]
Subtotal (0.0% CI)	**138**	**784**		**47.0 %**	**1.21 [0.85, 1.72]**
Total events: 109 (Experimental), 585 (Control)					
Heterogeneity: Tau^2 = 0.07; Chi^2 = 4.17, df = 1 (P = 0.04); I^2 =76%					
Test for overall effect: Z = 0.90 (P = 0.37)					
2 > 6 months					
BCIRG006	761/1068	665/1050		52.3 %	1.13 [1.07, 1.18]
NOAH	3/115	5/113		0.7 %	0.59 [0.18, 1.92]
Subtotal (0.0% CI)	**1183**	**1163**		**53.0 %**	**1.12 [1.07, 1.18]**
Total events: 764 (Experimental), 670 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.81, df = 1 (P = 0.37); I^2 =0.0%					
Test for overall effect: Z = 3.83 (P = 0.00013)					
Total (0.0% CI)	**1321**	**1947**		**100.0 %**	**1.10 [1.00, 1.22]**
Total events: 873 (Experimental), 1255 (Control)					
Heterogeneity: Tau^2 = 0.01; Chi^2 = 6.17, df = 3 (P = 0.10); I^2 =51%					
Test for overall effect: Z = 1.66 (P = 0.096)					
Test for subgroup differences: Chi^2 = 0.12, df = 1 (P = 0.72), I^2 =0.0%					

(1) FinHer control arm includes both HER2 positive and negative patients

Analysis 4.1. Comparison 4 Brain metastases as site of first relapse, Outcome 1 Brain metastases - all studies.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 4 Brain metastases as site of first relapse

Outcome: 1 Brain metastases - all studies

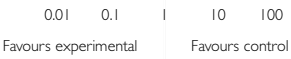
Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
B31	21/864	11/872		25.1 %	1.93 [1.05, 3.54]
FinHer	3/115	5/116		6.6 %	0.61 [0.19, 1.97]
HERA	26/1073	22/1698		41.5 %	1.87 [1.17, 3.00]
N9831	12/808	4/807		10.3 %	3.00 [1.16, 7.72]
PACS-04	11/260	8/268		16.4 %	1.42 [0.67, 3.00]
Total (0.0% CI)	**3120**	**3761**		**100.0 %**	**1.75 [1.29, 2.38]**

Total events: 73 (Trastuzumab), 50 (Control)

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 3.39$, df = 4 (P = 0.49); $I^2 = 0.0\%$

Test for overall effect: Z = 3.04 (P = 0.0024)

Test for subgroup differences: Not applicable

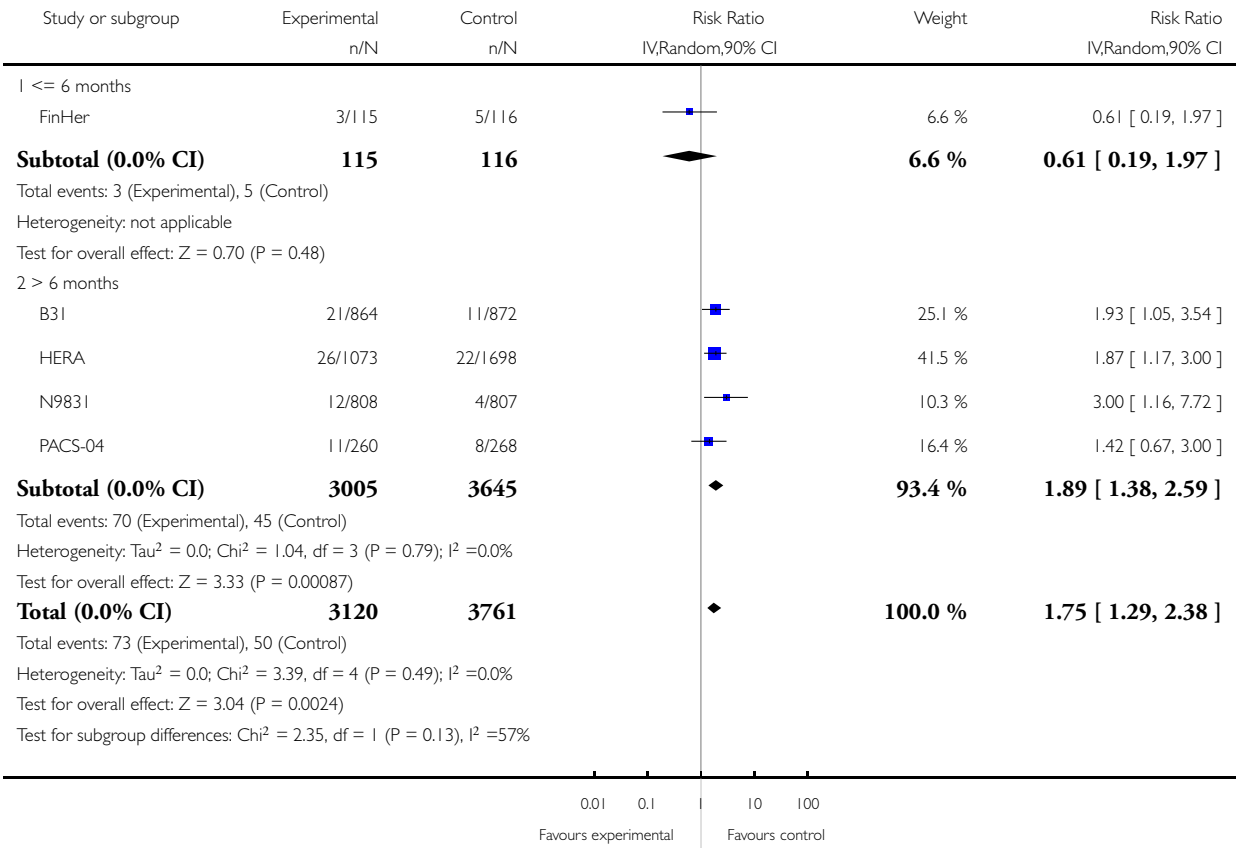


Analysis 4.2. Comparison 4 Brain metastases as site of first relapse, Outcome 2 Brain metastases stratified by duration of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 4 Brain metastases as site of first relapse

Outcome: 2 Brain metastases stratified by duration of trastuzumab administration

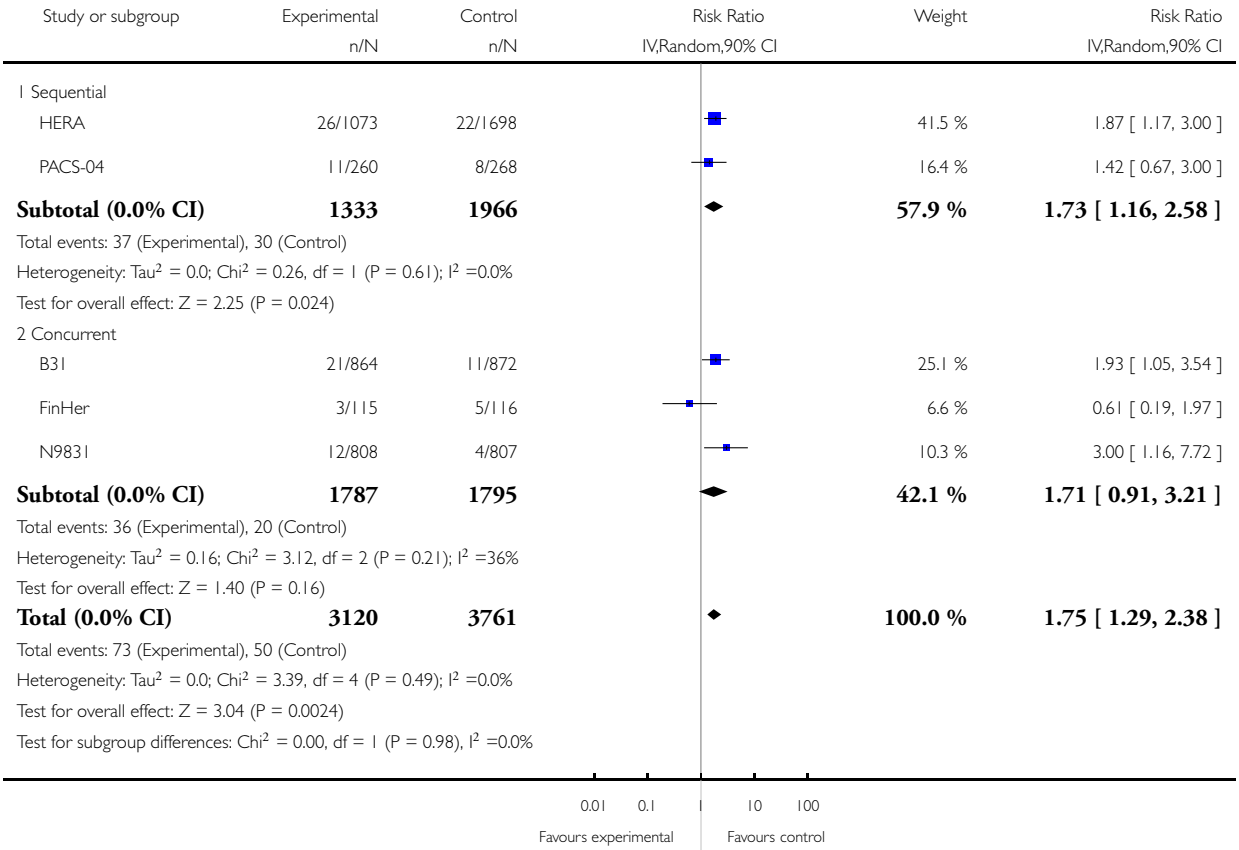


Analysis 4.3. Comparison 4 Brain metastases as site of first relapse, Outcome 3 Brain metastases stratified by type of trastuzumab administration.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 4 Brain metastases as site of first relapse

Outcome: 3 Brain metastases stratified by type of trastuzumab administration



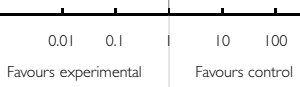
Analysis 5.1. Comparison 5 Sensitivity analysis, Outcome 1 OS - by allocation concealment.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 5 Sensitivity analysis

Outcome: 1 OS - by allocation concealment

Study or subgroup	Experimental N	Control N	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
1 Adequate						
FinHer	115	116	-0.6 (0.36)		4.9 %	0.55 [0.27, 1.11]
NOAH	117	118	-0.48 (0.3)		7.1 %	0.62 [0.34, 1.11]
Subtotal (95% CI)	**232**	**234**			**12.0 %**	**0.59 [0.37, 0.93]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.07, df = 1 (P = 0.80); I^2 =0.0%						
Test for overall effect: Z = 2.30 (P = 0.022)						
2 Inadequate						
B31 (1)	1672	1679	-0.4 (0.17)		22.0 %	0.67 [0.48, 0.94]
BCIRG006	1074	1073	-0.46 (0.13)		37.7 %	0.63 [0.49, 0.81]
Buzdar	23	19	0 (0)			Not estimable
HERA	1703	1698	-0.46 (0.17)		22.0 %	0.63 [0.45, 0.88]
PACS-04	260	268	0.24 (0.32)		6.2 %	1.27 [0.68, 2.38]
Subtotal (95% CI)	**4732**	**4737**			**88.0 %**	**0.69 [0.56, 0.85]**
Heterogeneity: Tau^2 = 0.01; Chi^2 = 4.33, df = 3 (P = 0.23); I^2 =31%						
Test for overall effect: Z = 3.51 (P = 0.00045)						
Total (95% CI)	**4964**	**4971**			**100.0 %**	**0.66 [0.57, 0.77]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 4.70, df = 5 (P = 0.45); I^2 =0.0%						
Test for overall effect: Z = 5.16 (P < 0.00001)						
Test for subgroup differences: Chi^2 = 0.37, df = 1 (P = 0.54), I^2 =0.0%						



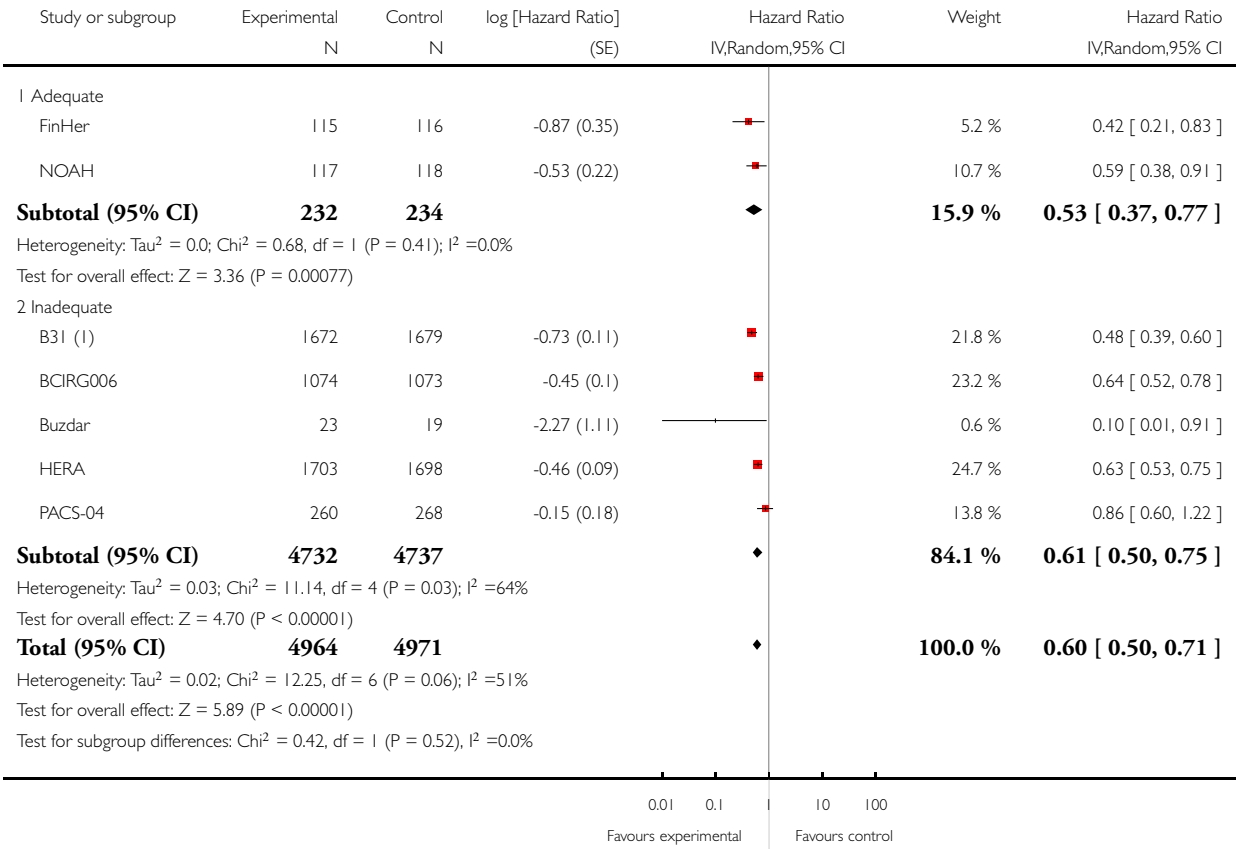
(1) B31+N9831

Analysis 5.2. Comparison 5 Sensitivity analysis, Outcome 2 DFS - by allocation concealment.

Review: Trastuzumab containing regimens for early breast cancer

Comparison: 5 Sensitivity analysis

Outcome: 2 DFS - by allocation concealment



(1) B31+N9831

ADDITIONAL TABLES

Table 1. Definitions of the outcomes of cardiac toxicity

Trial	Outomes of cardiac toxicity as named in the trials	Explanation of the outcomes of cardiac toxicity
B31	CHF	NYHA class III or IV CHF
	Possible/probable cardiac death	Not reported

Table 1. Definitions of the outcomes of cardiac toxicity (*Continued*)

BCIRG006	Cardiac-related death	Not reported
	CHF	Grade 3/4, not further specified
	Cardiac ischaemia/infarction	Grade 3/4, not further specified
	Arrythmias	Grade 3/4, not further specified
	LVEF decrease	> 10% relative LVEF decline
Buzdar	CHF	Not reported
	LVEF decrease	Decrease of LVEF > 10% from the baseline
FinHer	Cardiac infarction	Not reported
	CHF	Not reported
	LVEF decrease	Any change in LVEF values between pre- and post-treatment or decrease in LVEF > 20% less than pre-treatment value or decrease in LVEF >10% resulting in a LVEF < 50%
HERA	Death from cardiac causes	Death due to CHF, myocardial infarction, documented primary arrhythmia or sudden unexpected death after a definite or probable cardiac event without a documented alternative cause within 24 hours of a cardiac event
	Symptomatic CHF	CHF considered symptomatic by a cardiologist and a decrease in LVEF of at least 10% from baseline to an LVEF of less than 50% at any time; it includes severe CHF
	Severe CHF	NYHA class III or IV CHF and a decrease in LVEF of at least 10% from baseline to an LVEF of less than 50% at any time
	Significant LVEF drop	Decrease in LVEF of 10% or more from baseline to a level below 50%
	Confirmed significant LVEF drop	Asymptomatic (NYHA class I) or mildly symptomatic (NYHA class II) significant drop in LVEF also confirmed on repeat LVEF assessment about 3 weeks after the first documented drop, or identified by the cardiac advisory board review
NOAH	Cardiac events	Not defined
	LVEF decrease	Asymptomatic (grade II decrease)
N9831	LVEF decrease	Any change in LVEF from registration level
	CHF	NYHA class III or IV

Table 1. Definitions of the outcomes of cardiac toxicity (*Continued*)

	Possible/probable cardiac death	Sudden death without any documented cause
	Definite cardiac death	Death due to CHF, myocardial infarction or primary arrhyhtmia
PACS-04	Severe LVEF decline	Decrease in LVEF to a level below 45% or decrease in LVEF to a level between 45% and 50% with a relative decrease of LVEF of 15% or more
	Moderate LVEF decline between 45% and 49%	Decrease in LVEF to a level between 45% and 49%
	Mild LVEF decline between 50% and 55%	Decrease in LVEF to a level between 50% and 55%
	Heart failure	Not reported
	Cardiac death	Not reported

CHF: congestive heart failure
LVEF: left ventricular ejection fraction
IV: intravenous
NYHA: New York Heart Association

Table 2. Meta-regression: OS and DFS stratified by duration and type of administration

	OS			DFS		
	N° studies	**HR (95% CI)**	**P***	**N° studies**	**HR (95% CI)**	**P***
Duration of administration						
<= 6 months	2	0.55 (0.27 to 1.11)	0.591	2	0.31 (0.10 to 0.96)	0.150
> 6 months	6	0.67 (0.57 to 0.80)		6	0.62 (0.52 to 0.72)	
Type of administration						
Sequential	2	0.85 (0.43 to 1.67)	0.406	2	0.71 (0.53 to 0.95)	0.122
Concurrent	6	0.64 (0.53 to 0.76)		6	0.54 (0.44 to 0.67)	

* P values referring to beta parameter of meta-regression model
CI: confidence interval

DFS: disease-free survival
HR: hazard ratio
OS: overall survival

Table 3. Meta-regression: CHF and LVEF decline stratified by duration and type of administration

	CHF			LVEF decline		
	N° studies	**RR (90% CI)**	**P***	**N° studies**	**RR (90% CI)**	**P***
Duration of administration						
<= 6 months	2	0.50 (0.07 to 3.74)	0.059	2	0.89 (0.51 to 1.57)	0.060
> 6 months	6	5.39 (3.56 to 8.17)		6	2.14 (1.58 to 2.89)	
Type of administration						
Sequential (randomisation before chemotherapy)	1	8.42 (1.56 to 45.45)	0.2214	1	1.16 (0.68 to 1.99)	0.0119
Sequential (randomisation after chemotherapy)	2	11.05 (3.46 to 35.29)		2	2.90 (2.23 to 3.76)	
Concurrent	6	3.90 (2.42 to 6.28)		6	1.48 (1.11 to 1.97)	

* P values referring to beta parameter of meta-regression model
CHF: congestive heart failure
CI: confidence interval
LVEF: left ventricular ejection fraction
RR: risk ratio

Table 4. Meta-regression: brain metastases as site of first relapse stratified by duration and type of administration

	Brain Metastases		
	N° studies	**RR (90% CI)**	**P***
Duration of administration			
<= 6 months	1	0.61 (0.19 to 1.97)	0.125

Table 4. Meta-regression: brain metastases as site of first relapse stratified by duration and type of administration (*Continued*)

> 6 months	4	1.89 (1.38 to 2.59)	
Type of administration			
Sequential	2	1.73 (1.16 to 2.58)	0.927
Concurrent	3	1.71 (0.91 to 3.21)	

* P values referring to beta parameter of meta-regression model
CI: confidence interval
RR: risk ratio

Table 5. Meta-regression: assessment of risk of bias - allocation concealment on OS and DFS

	OS			DFS		
	N° studies	**HR (95%CI)**	**P***	**N° studies**	**HR (95%CI)**	**P***
Allocation concealment						
Adequate	2	0.59 (0.37 to 0.93)	0.586	2	0.53 (0.37 to 0.77)	0.525
Not adequate	6	0.69 (0.56 to 0.85)		6	0.61 (0.50 to 0.75)	

* P values referring to beta parameter of meta-regression model
CI: confidence interval
DFS: disease-free survival
HR: hazard ratio
OS: overall survival

Table 6. Assessment of consistency of the treatment effect with respect to lymph nodal status

	Lymph nodal status		
	Negative HR (95% CI)	**Positive HR (95% CI)**	**RHR (95% CI)**
BCIRG006	0.32 (0.17 to 0.62)	0.68 (0.52 to 0.86)	0.47 (0.23 to 0.94)
B31+N9831	0.47 (0.04 to 5.22)	0.48 (0.39 to 0.59)*	0.98 (0.09 to 11.23)
HERA	0.59 (0.39 to 0.91)	0.63 (0.51 to 0.78)*	0.94 (0.58 to 1.52)

Table 6. Assessment of consistency of the treatment effect with respect to lymph nodal status (*Continued*)

NOAH	0.35 (0.09 to 1.29)	0.62 (0.39 to 0.98)	0.57 (0.14 to 2.32)
Summary			0.74 (0.51 to 1.08)

Q = 2.74 (dof = 3); P = 0.434; I^2 = 0
* The hazard ratios (HRs) were obtained by pooling the HRs reported by the authors at different cut-points
CI: confidence interval
RHR: ratios of HR

Table 7. Assessment of consistency of the treatment effect with respect to hormone-receptor status

	Hormone-receptor status		
	Positive HR (95% CI)	**Negative HR (95% CI)**	**RHR (95% CI)**
BCIRG006	0.58 (0.39 to 0.80)	0.64 (0.57 to 0.88)	0.91 (0.60 to 1.37)
B31+N9831	0.44 (0.32 to 0.61)*	0.51 (0.39 to 0.67)	0.86 (0.57 to 1.31)
HERA	0.70 (0.53 to 0.94)*	0.63 (0.50 to 0.78)	1.11 (0.77 to 1.60)
NOAH	0.87 (0.43 to 1.74)	0.46 (0.27 to 0.80)	1.89 (0.77 to 4.62)
Summary			1.01 (0.81 to 1.26)

Q = 2.97 (dof = 3); P = 0.397; I^2 = 0
* The hazard ratios (HRs) were obtained by pooling the HRs reported by the authors at different cut-points
CI: confidence interval
RHR: ratios of HR

Table 8. Assessment of consistency of the treatment effect with respect to tumour size

	Tumour size		
	> 2 cm HR (95% CI)	**< 2 cm HR (95% CI)**	**RHR (95% CI)**
BCIRG006	0.60 (0.43 to 0.88)	0.63 (0.40 to 1.00)	0.96 (0.54 to 1.69)
B31+N9831	0.50 (0.39 to 0.64)*	0.42 (0.27 to 0.64)	1.19 (0.72 to 1.96)
HERA	0.61 (0.49 to 0.77)*	0.65 (0.47 to 0.90)	0.94 (0.62 to 1.41)
Summary			1.01 (0.77 to 1.34)

Q = 0.58 (dof = 2); P = 0.749; I^2 = 0
* The hazard ratios (HRs) were obtained by pooling the HRs reported by the authors at different cut-points
CI: confidence interval
RHR: ratios of HR

Table 9. Assessment of consistency of the treatment effect with respect to age

	Age		
	> 60 HR (95% CI)	**< 60 HR (95% CI)**	**RHR (95% CI)**
B31+N9831	0.41 (0.24 to 0.68)	0.50 (0.40 to 0.63)*	0.82 (0.46 to 1.46)
HERA	0.91 (0.59 to 1.41)	0.60 (0.50 to 0.72)*	1.52 (0.95 to 2.42)
Summary			1.14 (0.63 to 2.09)

Q = 2.63 (dof = 1); P = 0.105; I^2 = 62%
* The hazard ratios (HRs) were obtained by pooling the HRs reported by the authors at different cut-points.
CI: confidence interval
RHR: ratios of HR

Table 10. Meta-regression: assessment of the impact of RCTs stopped early on the results

	OS			**DFS**		
	N° studies	**HR (95% CI)**	**P***	**N° studies**	**HR (95% CI)**	**P***
RCT stopped early	4	0.65 (0.51 to 0.82)	0.837	4	0.54 (0.40 to 0.73)	0.289
RCT not stopped early	4	0.70 (0.51 to 0.95)		4	0.65 (0.53 to 0.81)	

* P values referring to beta parameter of meta-regression model
CI: confidence interval
DFS: disease-free survival
HR: hazard ratio
OS: overall survival

APPENDICES

Appendix 1. MEDLINE (via OvidSP)

1. Breast Neoplasms/
2. (breast or mammary).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
3. (cancer* or tumour* or tumor* or neoplasm* or metastas* or carcinoma).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
4. 2 and 3
5. 1 or 4
6. (Trastuzumab or Herceptin).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
7. Antineoplastic Agents/ae, ct, to [Adverse Effects, Contraindications, Toxicity]
8. Drug Hypersensitivity/
9. Drug Toxicity/
10. Drug Tolerance/
11. Causality/
12. Risk/
13. Product Surveillance, Postmarketing/
14. 7 or 8 or 9 or 10 or 11 or 12 or 13
15. (safe* or adr or adrs or tolerabilit* or toxicit* or "undesirable effect*" or "adverse reaction*" or hypersensitivit*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
16. (toxic effect* or complication* or causalit* or risk*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
17. (side or adverse).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
18. (effect* or event* or outcome*).mp. [mp=title, original title, abstract, name of substance word, subject heading word, unique identifier]
19. 17 and 18
20. postmarketing.mp.
21. post marketing.mp.
22. 20 or 21
23. 15 or 16 or 19 or 22
24. 14 or 23
25. 5 and 6 and 24
26. limit 25 to yr="1996 - 2010"

Appendix 2. EMBASE via Embase.com

1. 'breast cancer'/exp AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
2. breast:ab,ti OR mammary:ab,ti OR mammaries:ab,ti AND (cancer*:ab,ti OR tumour*:ab,ti OR tumor*:ab,ti OR neoplasm*:ab,ti OR metastas*:ab,ti OR carcinoma*:ab,ti) AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
3. #1 OR #2 AND [embase]/lim AND [1906-2009]/py
4. 'trastuzumab'/exp AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
5. trastuzumab:ab,ti OR herceptin:ab,ti AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
6. #4 OR #5 AND [embase]/lim AND [1996-2009]/py
7. 'antineoplastic agent'/exp/dd˙ae,dd˙to AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
8. 'drug hypersensitivity'/exp AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
9. 'drug toxicity'/exp AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
10. 'drug tolerance'/exp AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
11. 'risk'/exp AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
12. 'postmarketing surveillance'/exp AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
13. safe*:ab,ti OR adr:ab,ti OR adrs:ab,ti OR tolerability*.:ab,ti OR toxicit*:ab,ti OR undesirable:ab,ti AND effect*:ab,ti OR adverse:ab,ti AND reaction*:ab,ti OR hypersensitivit*:ab,ti OR toxic:ab,ti AND effect*:ab,ti OR complication*:ab,ti OR causalit*:

ab,ti OR risk:ab,ti OR postmarketing:ab,ti OR post:ab,ti AND marketing:ab,ti AND [humans]/lim AND [embase]/lim AND [1996-2009]/py

14. side:ab,ti OR adverse:ab,ti AND (effect*:ab,ti OR event*:ab,ti OR outcome*:ab,ti) AND [humans]/lim AND [embase]/lim AND [1996-2009]/py
15. #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 AND [embase]/lim AND [1996-2009]/py
16. #3 AND #6 AND #15 AND [embase]/lim AND [1996-2009]/py.

Appendix 3. CENTRAL

1. MeSH descriptor Breast Neoplasms
2. MeSH descriptor Adverse Drug Reaction Reporting Systems OR MeSH descriptor Drug Toxicity
3. trastuzumab OR herceptin
4. (#2 AND #3)
5. (#1 AND #4)
6. breast AND (cancer* OR tumour* OR tumor* OR neoplasm*)
7. (#6 AND #3)
8. adverse effect* OR side effect* OR toxic effect* OR adverse event* OR adverse drug reaction*
9. (#7 AND #8)
10. (#9 OR #5)
11. (#10), from 1996 to 2009

Appendix 4. BIOSIS (via ISI Web of Knowledge)

1. TS=(breast cancer*) *Databases=PREVIEWS Timespan=1996-2009*
2. TS=(breast neoplasm*) *Databases=PREVIEWS Timespan=1996-2009*
3. TS=(breast tumour*) *Databases=PREVIEWS Timespan=1996-2009*
4. TS=(breast tumor*) *Databases=PREVIEWS Timespan=1996-2009*
5. #4 OR #3 OR #2 OR #1 *Databases=PREVIEWS Timespan=1996-2009*
6. TS=(trastuzumab) AND MC=(Oncology) AND CC=(Toxicology - Pharmacology) *Databases=PREVIEWS Timespan=1996-2009*
7. TS=(herceptin) AND MC=(Oncology) AND CC=(Toxicology - Pharmacology) *Databases=PREVIEWS Timespan=1996-2009*
8. #7 OR #6 *Databases=PREVIEWS Timespan=1996-2009*
9. #8 AND #5 *Databases=PREVIEWS Timespan=1996-2009*

WHAT'S NEW

Last assessed as up-to-date: 1 February 2010.

Date	Event	Description
23 February 2012	Feedback has been incorporated	The current version of this review includes data up until 2010. An update of this review will be conducted shortly to incorporate evidence from the BCIRG-006 trial

HISTORY

Protocol first published: Issue 4, 2006

Review first published: Issue 4, 2012

CONTRIBUTIONS OF AUTHORS

Study concept: RD, LM.

Study protocol: RD, LM.

Search Strategy: VP.

Selection of studies: SB, LM, EP, LT.

Acquisition of data: SB, EP, LT.

Risk of bias assessment: SB, RD, LM, EP, LT.

Analysis of data: SB, RD, LM, EP.

Drafting of the manuscript: RD, LM, LT.

Interpretation and critical revision of the manuscript for important intellectual content: SB, RD, VG, LM, LT, EP, VP.

DECLARATIONS OF INTEREST

None known.

SOURCES OF SUPPORT

Internal sources

- Mario Negri Institute for Pharmacological Research, Milan, Italy.
- Italian Cochrane Centre, Milan, Italy.
- University of Modena and Reggio-Emilia, Modena, Italy.
- University of Milan, Italy.

External sources

- Italian Medicines Agency (AIFA - Agenzia Italiana del Farmaco) - CUP H95E07000130005, Italy.

INDEX TERMS

Medical Subject Headings (MeSH)

*Receptor, erbB-2; Antibodies, Monoclonal, Humanized [adverse effects; *therapeutic use]; Antineoplastic Agents [adverse effects; *therapeutic use]; Antineoplastic Combined Chemotherapy Protocols [*therapeutic use]; Breast Neoplasms [chemistry; *drug therapy; mortality; pathology]; Disease-Free Survival; Heart Failure [chemically induced]; Neoplasm Recurrence, Local [prevention & control]; Stroke Volume [drug effects; physiology]; Time Factors; Ventricular Dysfunction, Left [chemically induced]

MeSH check words

Female; Humans