Trastuzumab-containing regimens for metastatic breast cancer (Review)

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

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[Intervention Review]

Trastuzumab-containing regimens for metastatic breast cancer

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ABSTRACT

Background

Patients with breast cancer are classified as having cells that over-express the human epidermal growth factor receptor 2 (known as HER2-positive) or not (HER2-negative). Typically, patients with HER2-positive disease have a worse prognosis. Trastuzumab is a selective treatment that targets the HER2 pathway. The available evidence supporting trastuzumab regimens mostly relies upon surrogate endpoints and, although the efficacy results seem to support its use, other uncertainties have been raised about its net benefit in relation to transient cardiac toxicity and a long-term increased risk of metastasis to the central nervous system.

Objectives

To assess the evidence on the efficacy and safety of therapy with trastuzumab (overall) and in relation to the type of co-administered regimen and the line of treatment, i.e. first-line or beyond progression, in women with HER2-positive metastatic breast cancer.

Search methods

We searched the Cochrane Breast Cancer Group's (CBCG) Specialised Register and used the search strategy developed by the CBCG to search for randomised controlled trials (RCTs) in CENTRAL (2013, Issue 1), MEDLINE, EMBASE, BIOSIS, the WHO International Clinical Trials Registry Platform (ICTRP) search portal and ClinicalTrials.gov (up to 17 January 2013).

Selection criteria

RCTs comparing the efficacy and safety of trastuzumab alone or in combination with chemotherapy, hormonal therapy or targeted agents in women with HER2-positive metastatic breast cancer.

Data collection and analysis

We collected data from published trials. We used hazard ratios (HRs) for time-to-event outcomes and risk ratio (RRs) for binary outcomes. Subgroup analyses included type of regimen (taxane-containing, anthracycline-containing, aromatase inhibitor-containing or other) and treatment line (first-line, beyond progression).

Main results

The review found seven trials, involving 1497 patients, which met the criteria to be included. The trials were generally of moderate methodological quality; two studies have not published their results on overall survival so the presence of selective outcome reporting bias cannot be ruled out. None of the studies used blinding to treatment allocation, though this is unlikely to have biased the results for overall survival. Studies varied in terms of co-administered regimen and in terms of treatment line. In four studies, trastuzumab was administered with a chemotherapy, such as a taxane-containing, anthracycline-containing or capecitabine-containing regimen. Two studies considered postmenopausal women and administered trastuzumab with hormone-blocking medications, such as an aromatase inhibitor. One study administered trastuzumab in addition to lapatinib. Five studies out of seven included women treated with trastuzumab administered until progression as first-line treatment and two studies considered trastuzumab beyond progression. The combined HRs for overall survival and progression-free survival favoured the trastuzumab-containing regimens (HR 0.82, 95% confidence interval (CI) 0.71 to 0.94, P = 0.004; and HR 0.61, 95% CI 0.54 to 0.70, P < 0.00001, respectively; moderate-quality evidence). Trastuzumab increased the risk of congestive heart failure (RR 3.49, 90% CI 1.88 to 6.47, P = 0.0009; moderate-quality evidence) and left ventricular ejection fraction (LVEF) decline (RR 2.65, 90% CI 1.48 to 4.74, P = 0.006). For haematological toxicities, such as neutropenic fever and anaemia, there was no clear evidence that risks differed between groups, while trastuzumab seemed to raise the risk of neutropenia. The overall survival improvement was maintained when considering patients treated as first-line or patients receiving taxane-based regimens. The progression-free survival improvement was maintained when considering patients receiving taxane-based regimens, and patients treated as first-line or subsequent lines. Few data were collected on central nervous system progression. Similarly, few studies reported on quality of life and treatment-related deaths.

Authors' conclusions

Trastuzumab improved overall survival and progression-free survival in HER2-positive women with metastatic breast cancer, but it also increased the risk of cardiac toxicities, such as congestive heart failure and LVEF decline. The available subgroup analyses are limited by the small number of studies. Studies that administered trastuzumab as first-line treatment, or along with a taxane-based regimen, improved mortality outcomes. The evidence to support the use of trastuzumab beyond progression is limited. The recruitment in three out of seven studies was stopped early and in three trials more than 50% of patients in the control groups were permitted to switch to the trastuzumab arms at progression, making it more difficult to understand the real net benefit of trastuzumab.

Trastuzumab is generally used for women with HER2-positive early breast cancer in clinical practice, while women enrolled in most of the trials in the metastatic setting were naive to trastuzumab. The effectiveness of trastuzumab for women relapsing after adjuvant trastuzumab is therefore still an open issue, although it is likely that the majority are being offered it again.

PLAIN LANGUAGE SUMMARY

Efficacy and safety of trastuzumab in metastatic breast cancer

Tumours characterised by the presence of the HER2 protein are found in about one in five women with metastatic breast cancer. These tend to be more aggressive and the prognosis and choice of treatment are affected. Trastuzumab (Herceptin®) is a targeted biological drug (a monoclonal antibody) that attaches to the HER2 protein, blocking the growth of malignant cells.

We included seven trials with 1497 women who had HER2-positive metastatic breast cancer in this review. They were assigned by chance to receive trastuzumab with or without chemotherapy (taxane, anthracycline or capecitabine in four studies), hormonal therapy (aromatase inhibitors including letrozole or anastrozole in two studies) or targeted therapy (lapatinib in one study). Women treated with trastuzumab were followed up until disease progression in five studies and beyond disease progression in two studies. The length of trastuzumab administration varied between 8.7 and 30 months, and follow-up averaged two years after starting trastuzumab.

All studies found that trastuzumab extends time to disease progression, with gains varying between two and 11 months, and in five studies it extended time to death by between five and eight months. However, some patients develop severe heart toxicity (congestive heart failure) during treatment. While trastuzumab reduces breast cancer mortality by one-fifth, the risk of heart toxicity is between

three and four times more likely. If 1000 women were given standard therapy alone (with no trastuzumab) about 300 would survive and 10 would have heart toxicities. With the addition of trastuzumab to this treatment, an additional 73 would have their lives prolonged, and an additional 25 would have severe heart toxicity. Omitting the anthracycline-trastuzumab arms (which would not be regarded as standard of care) 21 patients would have severe heart toxicity (11 more than the chemotherapy alone group). These heart toxicities are often reversible if the treatment is stopped once heart disease is discovered. Women with advanced disease might choose to accept this risk. On balance, this review shows that with trastuzumab the time to disease progression and survival benefits outweigh the risk of heart harm.

Trastuzumab does not increase the risk of haematological toxicities, such as neutropenic fever and anaemia; however, it seems to raise the risk of neutropenia. There were insufficient data on the impact of trastuzumab on quality of life, treatment-related deaths and brain metastases to reach a conclusion for these outcomes.

We rated the overall quality of the evidence as moderate, with the main weaknesses being the fact that all studies included were open-label (not blinded), which may have affected the outcome assessments for time to disease progression and toxicities, and that two studies have not published their results for mortality. Furthermore, the recruitment in three out of seven studies was stopped early and in three trials more than 50% of patients in the control groups were permitted to switch to the trastuzumab arms at disease progression, making it more difficult to understand the real net benefit of trastuzumab on mortality. The evidence to support the use of trastuzumab beyond disease progression is limited.

It is important to highlight that, although trastuzumab is used for women with HER2-positive early breast cancer, the women enrolled in these metastatic trials were not previously treated with trastuzumab. The effectiveness of trastuzumab for women relapsing after adjuvant trastuzumab is still an open issue, although it is likely that it is offered to the majority of them.

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: patients with HER2-positive metastatic breast cancer
Settings: metastatic 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 Follow-up: median 2 years	**Moderate**[1]		**HR 0.82** (0.71 to 0.94)	1309 (5 studies)	⊕⊕⊕◯ **moderate**[2]	
	700 per 1000	**627 per 1000** (575 to 678)				
	High[1]					
	800 per 1000	**733 per 1000** (681 to 780)				
Progression-free survival Follow-up: median 2 years	**Moderate**		**HR 0.61** (0.54 to 0.70)	1489 (7 studies)	⊕⊕⊕◯ **moderate**[3]	
	700 per 1000	**520 per 1000** (478 to 569)				
	High					
	800 per 1000	**625 per 1000** (581 to 676)				
Congestive heart failure	**Low**		**RR 3.49** (1.88 to 6.47)[4]	1459 (7 studies)	⊕⊕⊕◯ **moderate**[3]	

	10 per 1000	**35 per 1000** (19 to 65)
	Moderate	
	20 per 1000	**70 per 1000** (38 to 129)
	High	
	50 per 1000	**175 per 1000** (94 to 323)

*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% confidence interval) 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]Moderate risk derived from Slamon 2001 first-line treatment. High risk: estimated from moderate risk increased by 10% absolute risk.
[2]Gasparini 2006, Huober 2012 and von Minckwitz 2009 did not report the overall survival data stratified by arm.
[3]All the studies were open-label.
[4]Confidence interval 90%.

BACKGROUND

Description of the condition

Breast cancer is the most commonly diagnosed cancer in women (Ferlay 2010), and the second leading cause of cancer-related death. Patients with breast cancer are classified as having cells that over-express the human epidermal growth factor receptor 2 (known as HER2-positive) or not (HER2-negative). The gene encoding the HER2 is amplified and the protein is over-expressed in 20% to 25% of women with metastatic breast cancer (Slamon 1987). 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 human epidermal growth factor receptor-2 (HER2) (Coussens 1985). The study by Baselga et al provided the first clinical evidence of the antitumour activity of this recombinant human monoclonal antibody against HER2 in patients with HER2 over-expressing breast carcinomas (Baselga 1996). Research by Baselga et al and other follow-up studies have documented an important difference between trastuzumab and most standard chemotherapy agents due to its tolerability, with a favourable risk-benefit profile in patients with metastatic breast cancer (Cobleigh 1999; Vogel 2001). The most common adverse events are fever, chills and other acute and self limiting symptoms that may accompany the initial infusion of trastuzumab. Cardiac dysfunction has been reported with trastuzumab, particularly when used in combination with anthracycline-based chemotherapy, but many patients will recover with standard treatment for congestive heart failure (Seidman 2002). Furthermore, it has been observed that patients with HER2 over-expressing metastatic breast cancer receiving trastuzumab are at an increased risk for isolated central nervous system progression (Burstein 2005; Pestalozzi 2006), possibly because they are living longer with improved systemic disease control.

Why it is important to do this review

Due to reported improvements in time to disease progression and survival, the US Food and Drug Administration rapidly approved trastuzumab in 1998 for the treatment of women with metastatic breast cancer (FDA 1998). Other drug regulatory agencies approved trastuzumab following a longer period of scrutiny of the evidence - the UK National Institute for Health and Clinical Excellence recommended trastuzumab for women with HER2-positive advanced breast cancer in 2002 (NICE 2002). The evidence supporting trastuzumab regimens mostly relied upon surrogate endpoints (e.g. progression-free survival). The strength of this evidence has been questioned (Apolone 2005; Joppi 2005), and other uncertainties have been raised about the net benefit of trastuzumab, particularly related to transient cardiac toxicity and secondly to a long-term increased risk of metastasis to the central nervous system.

The purpose of this review is to systematically evaluate the evidence for the efficacy and safety of the use of trastuzumab alone or in combination with chemotherapy in women with metastatic breast cancer using evidence from randomised controlled trials. We recognise that since some of the adverse events of interest are rare but serious, and occur during long-term use of trastuzumab, we need to look also at non-randomised studies to address our question fully. We plan to carry out a systematic review of non-randomised studies as a second phase of this project.

OBJECTIVES

To assess the evidence on the efficacy and safety of therapy with trastuzumab (overall) and in relation to the type of co-administered regimen and the line of treatment, i.e. first-line or beyond progression, in women with HER2-positive metastatic breast cancer.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs).

Types of participants

Women with HER2-positive metastatic breast cancer, of any age, menopausal status or hormone receptor status.

Types of interventions

1. Intervention group: trastuzumab alone or in combination with chemotherapy, hormonal therapy or targeted agents.
2. Comparator: the same regimen used in the intervention group without trastuzumab.

Trials could include both women with metastatic disease and women with locally advanced/recurrent disease, as long as the data on the patients with metastatic disease could be extracted from the data reported.

Trials could or could not specify recommended treatment upon disease progression or initial treatment failure. We included trials where patients crossed over to the other treatment arm at the time

of progression or received other treatment off-study in this review, and analysed these according to the treatment they were originally randomised to receive.

Types of outcome measures

Primary outcomes

- Overall survival on intention-to-treat analysis.
- Progression-free survival.

Secondary outcomes

- Overall response rate.
- Cardiac toxicity per protocol analysis (all patients who received the experimental treatment, regardless of compliance).
- Other toxicities (defined and graded according to the World health Organization (WHO)/National Cancer Institute (NCI) toxicity Criteria.
- Recurrence in central nervous system.
- Treatment-related deaths.
- Quality of life.

We applied the following definitions of the outcomes:

- Overall survival: time from randomisation to death (from any cause).
- Progression-free survival: time from randomisation to date of progression or death (from any cause). We considered time to progression (TTP - time from randomisation to date of progression) when progression-free survival was not reported.
- Overall response rate: the proportion of patients with a complete or partial response. Partial response is defined as a decrease in the size of a tumour, or in the extent of cancer in the body, in response to treatment.
- Cardiac toxicity: congestive heart failure and decline of left ventricular ejection fraction (LVEF). We considered the following definitions of congestive heart failure: cardiac dysfunction New York Heart Association class III-IV; severe, symptomatic or confirmed congestive heart failure. The decline of LVEF was defined as reported by the authors, as different thresholds were used.
- Other toxicities: neutropenic fever (grade 3/4),; anaemia (grade 3/4) and neutropenia (grade 3/4).
- Recurrence in central nervous system (CNS): the proportion of patients with disease progression due to metastases to CNS. Time to recurrence (also referred to as disease-free interval): time from date randomised to date of first CNS recurrence. Isolated metastasis to CNS confirmed radiologically by computed tomography (CT) or magnetic resonance imaging (MRI) scanning in patients with new brain or leptomeningeal metastasis.
- Treatment-related death is defined as death due to drug toxicity not due to disease progression, reported as 'treatment-related', 'toxic death' or 'lethal toxicity'.
- Quality of life: expression of well-being, measured through a validated scale (i.e. SF-36, European Organisation for Research and Treatment of Cancer (EORTC), Functional Assessment of Cancer Therapy (FACT)).

Search methods for identification of studies

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

Electronic searches

For RCTs, see: Cochrane Breast Cancer Group search strategy (http://www.mrw.interscience.wiley.com/cochrane/clabout/articles/BREASTCA/frame.html).
We searched the following databases:

1. Cochrane Breast Cancer Group (CBCG) Specialised Register on 14 January 2013. Details of the search strategy applied to create the register and the procedure used to code references are described in the Group's module in *The Cochrane Library*. The register includes both published and unpublished trials (including ongoing). We applied the CBCG codes 'advanced' and 'immunotherapy' to the Specialised Register and combined with the following keywords (imported with the references from MEDLINE): 'trastuzumab' [Substance Name], and a search of all non-indexed fields for the following text words: Trastuzumab, Herceptin or monoclonal antibod* AND HER2.
2. Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library*) on 17 January 2013 (Issue 1). See Appendix 1 for the search strategy;
3. MEDLINE (via OVID) (searched on 17 January 2013). Refer to Appendix 2.
4. EMBASE (via EMBASE.com) (searched on 17 January 2013). Refer to Appendix 3.
5. WHO International Clinical Trials Registry Platform (ICTRP) search portal (http://apps.who.int/trialsearch/AdvSearch.aspx), for all prospectively registered and ongoing trials (searched on 17 January 2013). Refer to Appendix 4).
6. ClinicalTrials.gov (http://www.clinicaltrials.gov/) (searched on 23 January 2013). Refer to Appendix 5.
7. BIOSIS (host: ISI Web of Knowledge), January 1996 to current (searched on 23 January 2013). Refer to Appendix 6.

We used 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', 'Drug tolerance', 'Causality', 'Risk', 'Adverse event', 'Adverse drug reaction', 'Breast cancer', 'Breast tu-

mour', 'Breast tumor' and 'Breast neoplasm'. We included reports irrespective of the language in which they were reported.

Searching other resources

We searched the Health Technology Assessment (HTA) Database and the Database of Abstracts of Reviews of Effects (DARE) to identify existing systematic reviews. We scanned the lists of studies included in these systematic reviews to assemble a list of known RCTs.

Data collection and analysis

The methods of this systematic review partially overlap with another Cochrane review exploring the efficacy and safety of trastuzumab in early breast cancer (Moja 2012).

Selection of studies

Three review authors (SB, SM and LT) 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 (SB, SM and LT) independently extracted information from the included trials using the pro-forma process piloted on a random sample of papers investigating other chemotherapy agents. Another review author (LM) checked data for correctness. 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). 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 the 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 the other treatment off-study and were managed according to the arm where they were originally randomised.

Assessment of risk of bias in included studies

We based the 'Risk of bias' assessment 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). Three review authors (SB, SM and LT) independently assessed trials according to the predefined quality criteria. Another review author (LM) checked data for correctness. We evaluated the impact of methodological quality only on the primary outcomes by considering the allocation concealment item.

We assessed the overall quality of evidence using the GRADE approach (Guyatt 2008). The GRADE approach appraises the quality of a body of evidence based on the extent to which one can be confident that an estimate of effect or association reflects the item being assessed. Randomised trials start as high-quality evidence, but may be downgraded due to: risk of bias (methodological quality), indirectness of evidence, unexplained heterogeneity, imprecision (sparse data) and publication bias. We determined the overall quality of the evidence for each outcome after considering each of these factors and judged this as follows.

- High: further research is very unlikely to change our confidence in the estimate of effect.
- Moderate: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
- Low: 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: any estimate of effect is very uncertain.

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

Survival-type outcomes

The measure of association chosen for overall survival and progression-free survival 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.

Dichotomous outcomes

The measure of association chosen for combining overall response rate and toxicities was the risk ratio (RR). For overall response rate, a RR greater than 1.0 favoured regimens containing trastuzumab, and less than 1.0 favoured regimens that do not contain trastuzumab. For toxicities, 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 the experimental treatment.

Dealing with missing data

Where possible, we sought any missing data or unclear information using the Internet, by contacting the authors and by checking for the best available resource or publication.

Assessment of heterogeneity

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

Assessment of reporting biases

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

Data synthesis

We directly extracted the HRs and their variances for overall survival and progression-free survival 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.
For all adverse events and brain metastases, treated as binary data, we used the RR as the measure of association and fixed a higher type I error (α = 0.10, two-sided) (Shadish 2002).
We pooled the HRs and RRs on the log scale through the generic inverse variance approach, using the random-effects model (DerSimonian 1986).

Subgroup analysis and investigation of heterogeneity

We pre-specified two subgroup analyses:

1. analysis by type of regimen (anthracycline-based, taxane-based, other chemotherapy-based, other targeted agents-based);
2. line of chemotherapy for metastatic breast cancer (first-line versus other).

Sensitivity analysis

We conducted a sensitivity analysis in order to assess the impact of methodological quality on the primary outcomes, i.e. overall survival and progression-free survival, by considering the allocation concealment item.

RESULTS

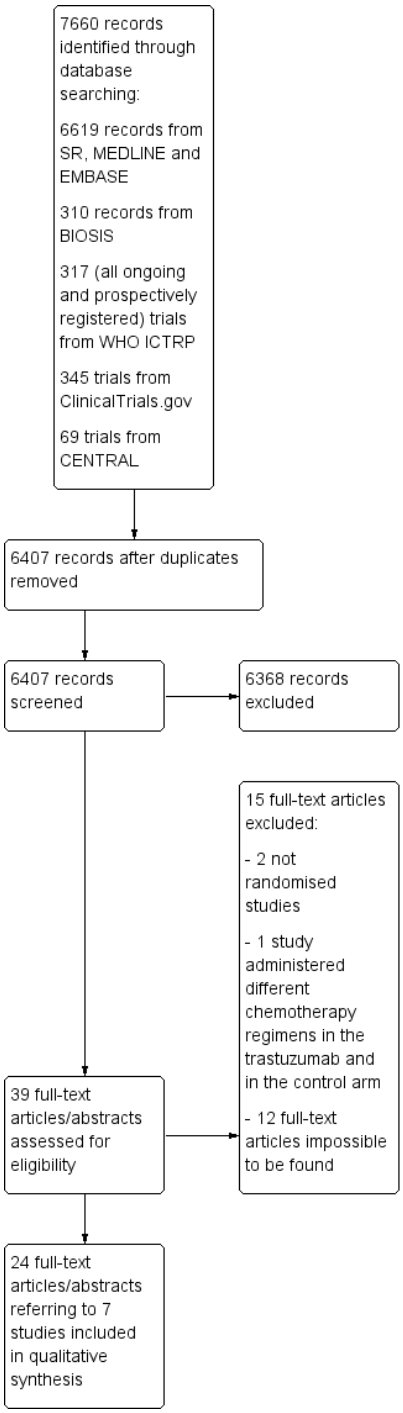
Description of studies

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

Results of the search

Randomised trials evaluating the efficacy of the therapy with trastuzumab in metastatic breast cancer therapy first started accruing patients in the early 1990s, as the first study on this topic was published in 1996 (Baselga 1996). Since then, research has rapidly moved forward on 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 trial registers provided 7660 citations. After removing duplicates, there were 6407 citations remaining. Of these, we discarded 6368 after reviewing the titles and abstracts because they clearly did not meet the inclusion criteria. We examined the full text of the remaining 39 citations: three references did not meet the inclusion criteria (see: Characteristics of excluded studies) and we excluded 12 references as we could not find the full text. Twenty-four publications (corresponding to seven trials) met the inclusion criteria and we included them in this systematic review.

Included studies

See: Characteristics of included studies.

We identified and defined seven eligible studies evaluating the efficacy or safety of trastuzumab in patients with HER2-over-expressing metastatic breast cancer as RCTs (Slamon 2001; Marty 2005; Gasparini 2006; Kaufman 2009; von Minckwitz 2009; Blackwell 2010; Huober 2012). All studies were fully published in peer-reviewed journals. For two trials additional unpublished data were provided by the investigators or obtained from regulatory agency reports or trial registries (Slamon 2001; Gasparini 2006).

The study by Slamon 2001 had four arms; two of them were experimental (anthracyclines or taxane plus trastuzumab) and two were control arms (anthracyclines or taxane alone). Data were reported for all arms, allowing us to lump together the two arms in which trastuzumab was administrated and the other two control arms.

Characteristics of patients

The seven studies randomised a total of 1497 HER2-positive women; 752 women were allocated to the trastuzumab-containing arm and 745 to the non-trastuzumab-containing arm. All studies included women aged between 24 and 88 years and the reported median ages ranged from 51 to 59 years.

Five studies enrolled untreated metastatic patients and excluded those with brain metastases (Slamon 2001; Marty 2005; Gasparini 2006; Kaufman 2009; Huober 2012). Previous adjuvant treatment with anthracyclines was permitted in Marty 2005 and Gasparini 2006. Gasparini 2006 also included patients previously treated with taxanes. Kaufman 2009 considered eligible patients treated with tamoxifen or anastrozole. Kaufman 2009, Slamon 2001 and Huober 2012 did not clearly report the inclusion criteria with respect to prior treatment with anthracyclines or taxanes. Huober 2012 enrolled postmenopausal women with newly diagnosed hormone receptor (HR)-positive metastatic breast cancer or locally advanced breast cancer; none of the patients in this trial received aromatase inhibitors or trastuzumab in the adjuvant setting.

Two studies enrolled metastatic breast cancer patients who progressed during prior trastuzumab-based therapy (von Minckwitz 2009; Blackwell 2010). In the von Minckwitz trial, the median duration of previous trastuzumab treatment was 45 weeks (range: 7 to 235 weeks) in the control arm and 44 weeks (range: 10 to 284 weeks) in the trastuzumab arm. In the trial by Blackwell, both groups had received a median of three prior trastuzumab regimens for metastatic disease. In the study by von Minckwitz, 3% of patients in the control arm and 1% of the patients in the trastuzumab arm had central nervous system metastases. Blackwell 2010 did not clearly report the inclusion criteria with respect to patients with central nervous system metastases.

As an inclusion criterion all the trials required normal heart function, with the exception of Slamon 2001, although patients were monitored for cardiac dysfunction.

Six RCTs included only HER2-positive patients (Slamon 2001; Marty 2005; Gasparini 2006; Kaufman 2009; von Minckwitz 2009; Blackwell 2010). In the study by Huober, an amendment for German sites permitted the implementation of a third arm where patients with HER2-negative and HR-positive tumours were assigned to receive letrozole alone as first-line treatment (Huober 2012). We only considered the data from HER2-positive patients for our analyses.

Interventions used in the trials

Five trials evaluated trastuzumab as first-line treatment and administered it until progression (Slamon 2001; Marty 2005; Gasparini 2006; Kaufman 2009; Huober 2012). Three trials combined trastuzumab with a taxane (Slamon 2001; Marty 2005; Gasparini 2006). A second arm of Slamon 2001 combined trastuzumab with an anthracycline plus cyclophosphamide. Kaufman 2009 used the regimen of trastuzumab with anastrozole. Huober 2012 used the regimen of trastuzumab with letrozole.

Two studies evaluated trastuzumab in patients with metastatic breast cancer who progressed after treatment with trastuzumab (von Minckwitz 2009; Blackwell 2010); von Minckwitz combined trastuzumab with capecitabine while Blackwell combined trastuzumab with lapatinib. In the von Minckwitz study, patients could have received up to one chemotherapy drug for metastatic disease: 4% of patients in the trastuzumab arm received first-line treatment during the study; all the remaining included patients received second-line treatment. Blackwell combined trastuzumab in a heavily pretreated patient population (median number of prior trastuzumab-based regimens: three). In this trial, patients were randomised to receive either oral lapatinib 1500 mg daily or oral lapatinib 1000 mg daily in combination with trastuzumab. Although the treatment regimen in the control arm was not the same as in the trastuzumab arm (i.e. a 30% relative increase in

the lapatinib dose), we decided to include the study in the meta-analysis and to exclude it in a sensitivity analysis.

In five studies the protocol prescribed trastuzumab at 2 mg/kg weekly doses (with a loading dose of 4 mg/kg) (Slamon 2001; Marty 2005; Gasparini 2006; Kaufman 2009; Blackwell 2010). In the study by von Minckwitz 2009, trastuzumab was administered at a dose of 6 mg/kg every three weeks (after a loading dose of 8 mg/kg). In the study by Huober 2012, the protocol prescribed trastuzumab at 2 mg/kg weekly doses (with a loading dose of 4 mg/kg), but approximately two years after the start of the study trastuzumab was allowed to be given as a three-weekly application with a dose of 6 mg/kg (after a loading dose of 8 mg/kg).

The median number of doses of trastuzumab differed among studies: Slamon 2001 prescribed a median of 36 doses, Marty 2005 39 doses and Gasparini 2006 and Kaufman 2009 25 doses each; for von Minckwitz 2009 the median number of doses was nine. Blackwell 2010 and Huober 2012 did not clearly report information on the number of administered doses.

All studies reported detailed safety data with details of toxicities encountered in each arm.

Quality of life data were properly reported in two papers only, referring to the studies by Slamon 2001 and von Minckwitz 2009 (see Osoba 2002 and Wu 2011 sub-references).

All the trials were funded by pharmaceutical companies.

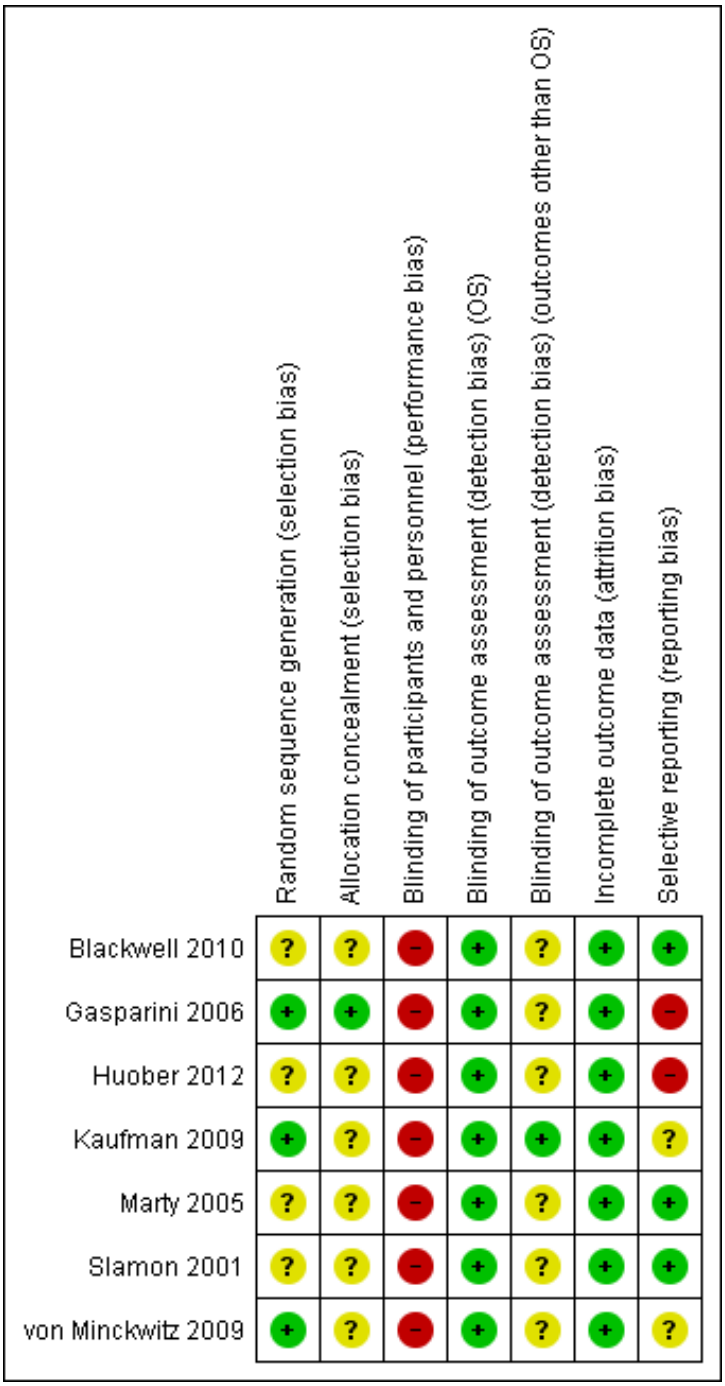
Excluded studies

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

Risk of bias in included studies

See: Figure 2 ('Risk of bias' summary table).

Figure 2. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



Since the trials were 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 alternative presentations (i.e. meeting proceedings or regulatory agency reports). 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 overall survival was measured, as this outcome was not subject to observer or patient bias in interpretation.

Allocation

All studies were described as randomised. We assessed the generation of a random sequence as adequate for three trials (Gasparini 2006; Kaufman 2009; von Minckwitz 2009); four studies did not report sufficient details (Slamon 2001; Marty 2005; Blackwell 2010; Huober 2012). We assessed allocation concealment as adequate in one trial (Gasparini 2006).

Treatment groups were well balanced in four studies (Slamon 2001; Gasparini 2006; Kaufman 2009; Blackwell 2010). Clinically relevant imbalances were reported by:

- Marty 2005: compared to the trastuzumab group, the control group had more patients with positive oestrogen and progesterone receptors (56% versus 41%) and fewer patients receiving prior adjuvant anthracyclines (55% versus 64%);
- Huober 2012: more patients in the control arm (71%) than in the trastuzumab arm (42%) had received adjuvant systemic treatment, and tamoxifen as adjuvant endocrine treatment was given in 65% and 31% of patients, respectively;
- von Minckwitz 2009: T3-4 stage at first diagnosis was more frequent in the capecitabine and trastuzumab arm than in the capecitabine alone arm (respectively: 34% and 14%).

These imbalances may have introduced some biases in the estimated intervention effect.

Blinding

All the studies were open-label, so performance bias cannot be ruled out. Outcome assessment may be influenced by unblinded investigators or patients. While overall survival is unlikely to be influenced by a lack of blinding, open-label trials might be at high risk of bias, particularly trials using subjective outcomes such as quality of life or pain reduction. However, in trials testing trastuzumab, outcomes were assessed by combining subjective and objective dimensions: progression-free survival or congestive heart failure were confirmed through imaging and biochemical tests. The independence that these tests ensure from the investigator's subjective assessment is difficult to predict. We reasoned that the risk of detection bias in open-label trials for progression-free survival, overall response rate and congestive heart failure was marginal: we decided to rate studies as having unclear risk of bias for these outcomes. We suggest that trialists use central independent adjudication committees to evaluate these outcomes independently from the study site and completely blinded to the treatment allocation of the patient. This would eliminate any subjective element from the outcome assessment, guaranteeing a low risk of bias for outcome determination. Only Kaufman 2009 declared that they relied upon a blinded Response Evaluation committee.

Incomplete outcome data

The rate of loss to follow-up was minimal (less than 3%) and accounted for in all of the trials. In Blackwell 2010, although 26 patients (9%) had withdrawn consent or were lost to follow-up before death, only eight patients (2.7%) were lost to follow-up before progression (progression-free survival is the primary outcome of the study).

Selective reporting

The protocols for the studies were not available for Gasparini 2006 and Kaufman 2009.

At the moment, Gasparini 2006 and Huober 2012 have not published or released their results for overall survival, therefore the presence of selective outcome reporting bias cannot be ruled out. Gasparini 2006 reported that the total number of deaths occurring in both arms was 42, but they did not provide information on how many patients died in the treatment and the control arms. Huober 2012 reported that there was no significant difference in overall survival between arms, without showing data. If the overall survival data are released, we will include these data in the review update. Kaufman 2009 reported results for the primary and secondary outcomes: it is likely that reporting bias has not occurred. In the paper published in 2011, von Minckwitz 2009 reported updated results only for overall survival.

Other potential sources of bias

Two trials were closed prematurely because of slow recruitment (von Minckwitz 2009; Huober 2012). Another trial was stopped early because data from other trials suggested that only patients with strong HER2 over-expression (3+) gain benefit from trastuzumab (Gasparini 2006). Three trials allowed patients in the control arm experiencing progression to cross over to the trastuzumab arm: 52%, 56% and 57% of patients in the control arm crossed over to the experimental arm, respectively (Marty

2005; Kaufman 2009; Blackwell 2010). Sixty-six per cent of the patients in the Slamon 2001 trial, upon documented disease progression, were entered into the extension study H0659g, a non-randomised, open-label study in which they could receive either trastuzumab alone or in combination with a chemotherapy of choice.

The possibility of switching from the control arm to the experimental arm at progression makes it more difficult to interpret the results for overall survival (Moja 2012).

Effects of interventions

See: **Summary of findings for the main comparison**
See: Summary of findings for the main comparison.

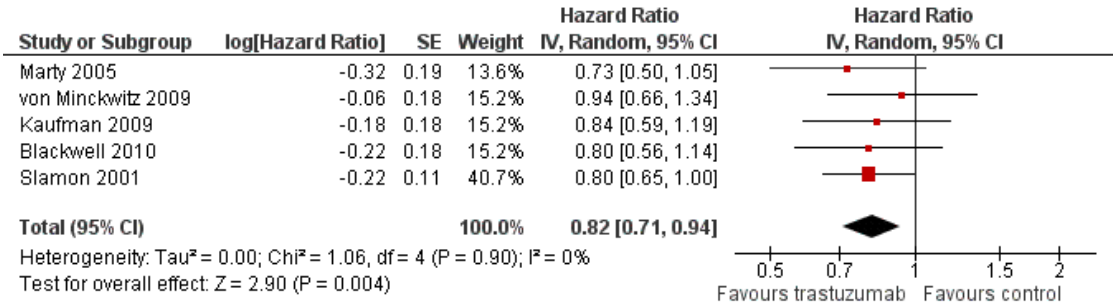
Efficacy of trastuzumab

Overall survival

Overall survival was reported in five out of the seven included trials (Slamon 2001; Marty 2005; Kaufman 2009; von Minckwitz 2009; Blackwell 2010). Trastuzumab extended time to death by between five and eight months. We indirectly estimated the hazard ratio (HR) for the Kaufman 2009 trial by using the number of events occurring in each arm and the P value of the log-rank test. We indirectly estimated the HR for the Marty 2005 trial as the ratio of the medians for the time to death in the trastuzumab and control groups; we estimated its variance by dividing the total number of deaths by four, as suggested in Parmar 1998. For Blackwell 2010, we considered the analysis that censored patient data at the time of cross-over after progression, as reported in the paper published in 2012. For the study by von Minckwitz 2009, we considered the data reported in the paper published in 2011, which focused on overall survival: 119 deaths were observed.

Although each single study reported a non-statistically significant difference between groups, our meta-analysis showed a statistically significant improvement in overall survival among patients treated with trastuzumab-containing regimens compared to the control group (HR 0.82, 95% confidence interval (CI) 0.71 to 0.94, P = 0.004; Analysis 1.1). There was no heterogeneity among studies (I^2 = 0%). The results are reported in Figure 3 and in Summary of findings for the main comparison.

Figure 3. Forest plot of comparison: 1 Efficacy of trastuzumab, outcome: 1.1 Overall survival - all studies.



We conducted a sensitivity analysis by excluding Blackwell 2010, because the lapatinib dose was higher in the control arm compared to the trastuzumab arm. The result did not change substantially (HR 0.82, 95% CI 0.70 to 0.95, P = 0.009; Analysis 1.2).

Overall survival stratified by type of regimen

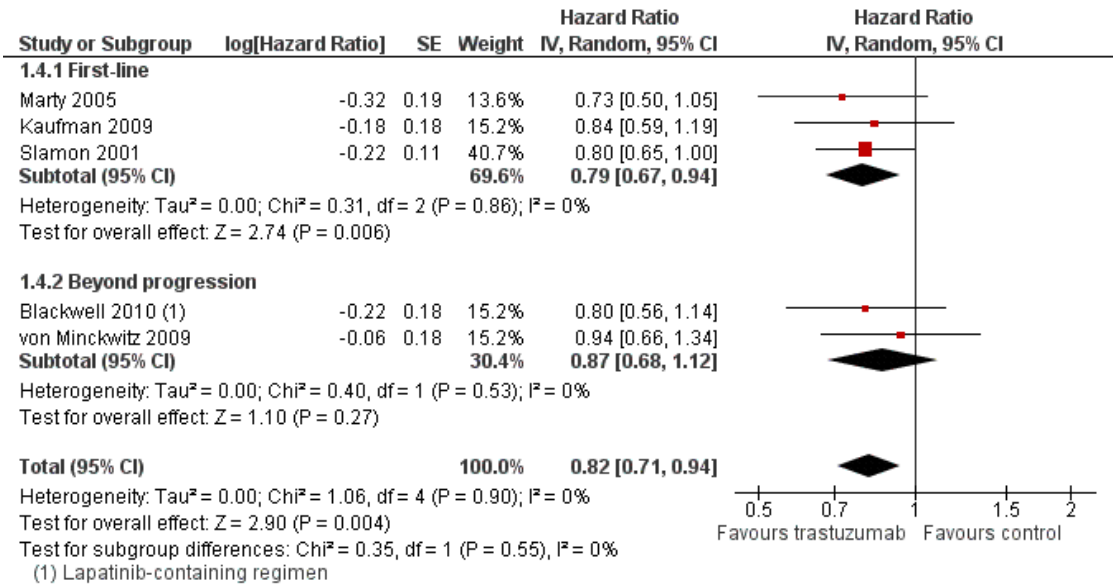
The taxane-containing regimen subgroup consisted of two studies (Marty 2005 and the paclitaxel arms of Slamon 2001), while the other subgroups included one study each: in the anthracycline-containing regimen subgroup, there was the anthracycline arms of Slamon 2001; in the aromatase inhibitor-containing regimen subgroup, there was Kaufman 2009; and in the lapatinib-containing regimen subgroup, there was the study by Blackwell 2010. The taxane-containing regimen reported a statistically significant improvement in overall survival (HR 0.80, 95% CI 0.65 to 0.99, P = 0.04; Analysis 1.3).

Overall survival stratified by treatment line

The studies that administered trastuzumab as first-line treatment were Slamon 2001, Marty 2005 and Kaufman 2009. Blackwell 2010 and von Minckwitz 2009 considered trastuzumab beyond progression. The analysis showed that trastuzumab as first-line treatment improved overall survival (HR 0.79, 95% CI 0.67 to 0.94, P = 0.006; Analysis 1.4). The difference in overall survival

did not reach statistical significance in the studies administering trastuzumab beyond progression (P = 0.27). The test for differences between treatment line subgroups was not statistically significant (P = 0.55). The results are reported in Figure 4.

Figure 4. Forest plot of comparison: 1 Efficacy of trastuzumab, outcome: 1.4 Overall survival - stratified by treatment line.



Progression-free survival

Progression-free survival was provided by or estimated from all seven included trials (Slamon 2001; Marty 2005; Gasparini 2006; Kaufman 2009; von Minckwitz 2009; Blackwell 2010; Huober 2012). For Slamon 2001, Marty 2005 and Gasparini 2006 we considered the time to progression. Although there were some differences in the definitions of time to progression (i.e. not considering death as an event contributing to the composite outcome), we judged these to have a minor impact on the overall analysis of progression-free survival (in Marty 2005 and Slamon 2001), due to the lack of heterogeneity between studies. Indeed we decided to pool the data irrespective of the progression-free survival definition adopted. Trastuzumab extended time to disease progression with gains varying between two and 11 months. We indirectly estimated the HR for the Marty 2005 trial as the ratio of the medians for the time to progression in the trastuzumab and control groups; we estimated its variance by using the relationship between the Chi^2 test and the log HR. It was not possible to report the total number of progression-free survival events since Marty 2005 and Slamon 2001 did not report this basic information. For the study by Blackwell 2010, we considered the intention-to-treat (ITT) analysis reported in the paper published in 2012. The analysis showed a statistically significant improvement in progression-free survival among patients treated with trastuzumab-containing regimens compared to the control group (HR 0.61, 95% CI 0.54 to 0.70, P < 0.00001; Analysis 1.5). We found low heterogeneity among studies (I^2 = 12%). The results are reported in the Summary of findings for the main comparison.

The sensitivity analysis excluding Blackwell 2010 provided a very similar benefit favouring trastuzumab (HR 0.58, 95% CI 0.50 to 0.66, P < 0.00001; Analysis 1.6).

Progression-free survival stratified by type of regimen

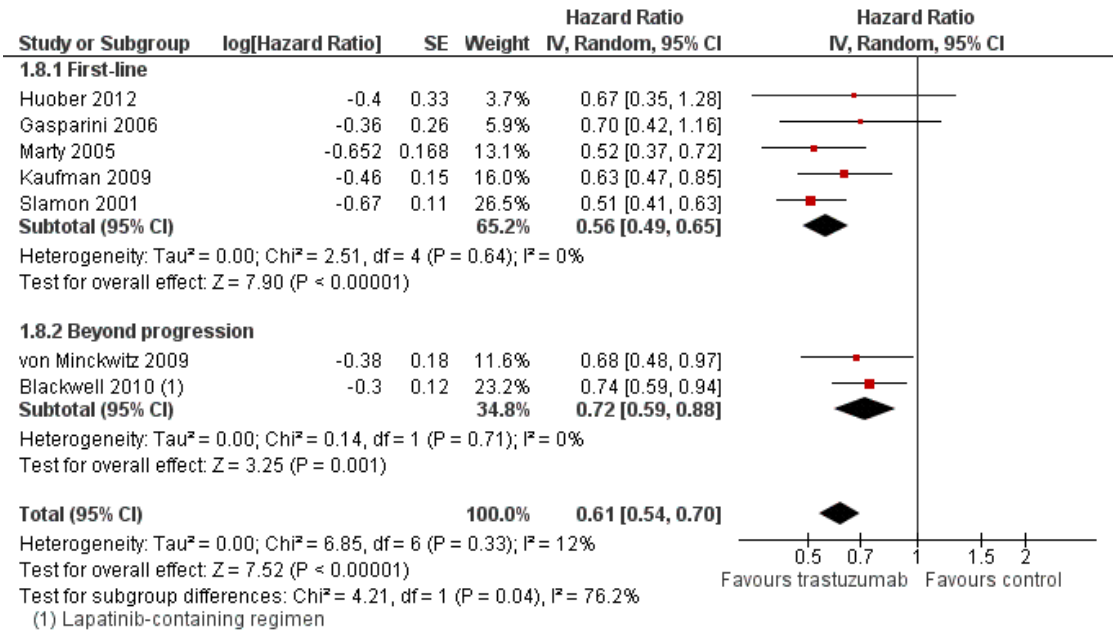
The taxane-containing regimen subgroup was composed of three studies (the paclitaxel arms of Slamon 2001, Marty 2005 and Gasparini 2006), with a significant difference in progression-free

survival (HR 0.53, 95% CI 0.42 to 0.68). In the anthracycline-containing regimen subgroup there were the anthracycline arms of Slamon 2001, with a significant improvement in progression-free survival (HR 0.78, 95% CI 0.68 to 0.91). In the subgroup who received aromatase inhibitor-containing regimens, the pooled hazard ratio for the Huober 2012 and Kaufman 2009 trials was statistically significant (HR 0.64, 95% CI 0.49 to 0.83). In the subgroup who received other types of regimens, the pooled hazard ratio for the Blackwell 2010 and von Minckwitz 2009 trials was statistically significant (HR 0.72, 95% CI 0.59 to 0.88). Heterogeneity was not detected among studies in each subgroup (I^2 = 0%). The variability among subgroups was high (I^2 = 60.8%). Refer to Analysis 1.7 for these results.

Progression-free survival stratified by treatment line

The studies that administered trastuzumab as first-line treatment were Slamon 2001, Marty 2005, Gasparini 2006, Kaufman 2009 and Huober 2012. von Minckwitz 2009 and Blackwell 2010 considered trastuzumab beyond progression. The analysis showed that trastuzumab significantly improved progression-free survival, both as first-line treatment (HR 0.56, 95% CI 0.49 to 0.65, P < 0.00001) and beyond progression (HR 0.72, 95% CI 0.59 to 0.88, P = 0.001). No heterogeneity was found among studies in each subgroup (I^2 = 0%). As expected, the test for differences between treatment line subgroups showed that trastuzumab seems to be more effective as first-line treatment compared to beyond progression (P = 0.04). The results are reported in Figure 5 (Analysis 1.8).

Figure 5. Forest plot of comparison: 1 Efficacy of trastuzumab, outcome: 1.8 Progression-free survival - stratified by treatment line.



Overall response rate

The seven included trials reported information on overall response rates (Slamon 2001; Marty 2005; von Minckwitz 2009; Huober 2012 according to ITT analysis; Gasparini 2006; Kaufman 2009; Blackwell 2010 according to per protocol analysis). There were 293 cases (41.3%) out of 710 in the trastuzumab group and 178 (25.1%) out of 709 in the control group who had an overall response. The overall response rate was higher in patients treated with trastuzumab (risk ratio (RR) 1.58, 95% CI 1.38 to 1.82, P < 0.00001) (Analysis 1.9).

Overall response rate stratified by type of regimen

The analysis showed an overall response rate favouring the trastuzumab group for all subgroups. In the taxane-containing groups, the RR was 1.71 (95% CI 1.23 to 2.38, P = 0.002). In the anthracycline-containing groups, the RR was 1.33 (95% CI 1.04 to 1.70, P = 0.02). In the aromatase inhibitor-containing groups, the RR was 2.55 (95% CI 1.23 to 5.27, P = 0.01). In the subgroup that administered other types of regimens, the RR was 1.70 (95% CI 1.16 to 2.49, P = 0.006). See Analysis 1.10 for these results. There was no heterogeneity among studies in each subgroup, with the exception of the taxane-containing subgroup (substantial heterogeneity, I^2 = 62%).

Overall response rate stratified by treatment line

The analysis showed that trastuzumab significantly improved overall response rate both as first-line treatment (RR 1.57, 95% CI 1.34 to 1.84, P < 0.00001) and beyond progression (RR 1.70, 95% CI 1.16 to 2.49, P = 0.006; see Analysis 1.11). We found low heterogeneity among studies in the first-line subgroup (I^2 = 5%); we found no heterogeneity among studies in the beyond progression subgroup (I^2 = 0%).

Safety of trastuzumab

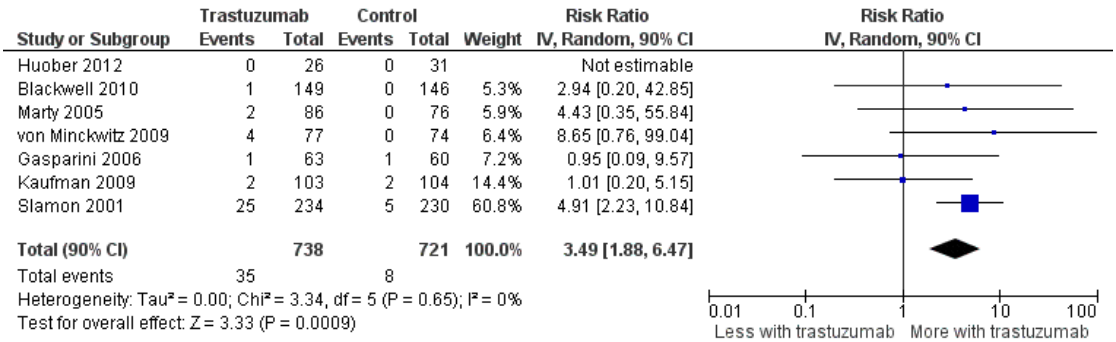
Data on cardiac dysfunction were reported in different ways among the included studies. We decided to combine data on major cardiac toxicities (e.g. congestive heart failure and cardiac dysfunction NYHA class III and IV) under the outcome congestive heart failure and to combine data on left ventricular ejection fraction (LVEF) decline, considering definitions for relevant decline as reported in the original study irrespective of the threshold used, under the outcome LVEF decline.

Congestive heart failure

All seven included trials reported data on congestive heart failure or severe cardiac events, totaling 1459 patients with HER2-positive metastatic breast cancer. Blackwell 2010 reported a fatal cardiac event in the trastuzumab arm. From Gasparini 2006 two events were reported, one acute myocardial infarction occurred in the control arm and one ischaemic heart attack occurred in the trastuzumab arm. No symptomatic congestive heart failure was observed in Huober 2012. Kaufman 2009 reported one grade 3 cardiac failure and one grade 4 myocardial ischaemia in the trastuzumab arm, while one grade 3 sinus tachycardia and one grade 4 myocardial ischaemia occurred in the control arm. Marty 2005 reported two symptomatic congestive heart failures in the trastuzumab arm. Slamon 2001 observed cardiac dysfunction NYHA class III/IV in 25 in the trastuzumab arms and five in the control arms. In von Minckwitz 2009, four patients in the trastuzumab arm experienced severe cardiac events.

There were 35 cases (4.7%) of severe cardiac event out of 738 in the trastuzumab group and 8/721 (1.1%) in the control group. The overall result indicated an increased risk of severe cardiac event with trastuzumab (RR 3.49, 90% CI 1.88 to 6.47, P = 0.0009; Analysis 2.1). We detected no heterogeneity (I^2 = 0%). The results are reported in Figure 6 and in Summary of findings for the main comparison.

Figure 6. Forest plot of comparison: 2 Cardiac toxicity of trastuzumab, outcome: 2.1 Congestive heart failure - all studies.



Congestive heart failure stratified by type of regimen

Based on two arms in Slamon 2001, trastuzumab in combination with an anthracycline significantly increased the risk of a severe cardiac event compared with an anthracycline alone (RR 5.43, 90% CI 2.28 to 12.94, P = 0.001). There was a trend for such an increase for the taxane-containing regimens (RR 1.98, 90% CI

0.54 to 7.26, P = 0.39) and in the subgroup of studies administering other types of regimens (RR 5.31, 90% CI 0.87 to 32.20, P = 0.13), with the possible exception of those including aromatase inhibitors (RR 1.01, 90% CI 0.20 to 5.15, P = 0.99). Where applicable, we found no heterogeneity among studies in each subgroup (I^2 = 0%). The test for subgroup differences showed that the observed cardiotoxicity does not depend on type of regimen used (P = 0.40). Excluding from the analysis the anthracycline-containing arms of Slamon 2001, the RR failed to reach statistical significance (RR 2.06, 90% CI 0.85 to 4.99, P = 0.18). The results are likely to be influenced by the low number of events observed in most subgroups and differences between regimens have not been ruled out. Refer to Analysis 2.2.

Congestive heart failure stratified by treatment line

Trastuzumab as first-line treatment seemed to significantly increase the risk of a severe cardiac event (RR 3.30, 90% CI 1.71 to 6.37, P = 0.003). We observed a larger increase in the subgroup of studies which administered trastuzumab beyond progression (RR 5.31, 90% CI 0.87 to 32.20, P = 0.13), although it did not reach the threshold for statistical significance. We found no heterogeneity among studies in each subgroup (I^2 = 0%). The test for subgroup differences showed that the observed cardiotoxicity does not depend on the treatment line (P = 0.68). Refer to Analysis 2.3.

Decline in left ventricular ejection fraction

Data on decline in LVEF could be extracted from six trials (Marty 2005; Gasparini 2006; Kaufman 2009; von Minckwitz 2009; Blackwell 2010; Huober 2012). Blackwell 2010 observed 10 events of grade $\geq$ 3 left ventricular systolic dysfunction or decrease in LVEF $\geq$ 20% relative to the baseline value and below the normal lower limit (as defined by the institution) in the trastuzumab arm and three such events in the control arm. Kaufman 2009 observed one confirmed decrease of $\geq$ 15 LVEF percentage points from baseline to < 50% in the trastuzumab arm. In the study by von Minckwitz 2009, a decrease in LVEF to less than 40% (or by greater than 10% from baseline) was observed in one patient in the trastuzumab group. No cases of significant decrease in LVEF occurred in Gasparini 2006. Huober 2012 observed a mean decrease of 3% for patients in the control arm and of 7% in the trastuzumab arm. The different reporting meant that we could not include the data from Huober 2012 in the pooled analysis.
Based on five trials (Marty 2005; Gasparini 2006; Kaufman 2009; von Minckwitz 2009; Blackwell 2010), there were 28 cases (5.9%) of LVEF decline out of 478 women in the trastuzumab group and nine (2.0%) out of 460 in the control group. The pooled analysis indicated an increased risk of decline in LVEF with trastuzumab (RR 2.65, 90% CI 1.48 to 4.74, P = 0.006; Analysis 2.4). No heterogeneity was detected (I^2 = 0%).

Decline in left ventricular ejection fraction stratified by type of regimen

The analyses for the taxane-containing subgroups and the other regimens (that is capecitabine and lapatinib) showed a statistically significant increase in the risk of LVEF decline (respectively RR 2.36, 90% CI 1.12 to 4.96, P = 0.06; RR 3.21, 90% CI 1.19 to 8.64, P = 0.05). The results are inconclusive for the aromatase inhibitor-containing subgroup (RR 3.03, 90% CI 0.21 to 44.02, P = 0.50). The results are likely to be influenced by the low number of events observed in most subgroups. Where applicable, we observed no heterogeneity (I^2 = 0%). Refer to Analysis 2.5.

Decline in left ventricular ejection fraction stratified by treatment line

Trastuzumab seemed to increase the risk of LVEF decline both as first-line treatment and administered beyond progression (respectively RR 2.40, 90% CI 1.17 to 4.91, P = 0.04; RR 3.21, 90% CI 1.19 to 8.64, P = 0.05; Analysis 2.6). We found no heterogeneity among studies in both subgroups (I^2 = 0%).

Other toxicities

Neutropenic fever

Three studies reported information on neutropenic fever (Marty 2005; Gasparini 2006; von Minckwitz 2009). There were 24 cases (10.3%) out of 232 in the trastuzumab group and 17 (7.5%) out of 228 in the control group. The increased risk of neutropenic fever in patients treated with trastuzumab was not statistically significant (RR 1.38, 90% CI 0.86 to 2.21, P = 0.26; Analysis 3.1). We detected no heterogeneity (I^2 = 0%).

Neutropenic fever stratified by type of regimen/treatment line

A low number of studies reported data on neutropenic fever, therefore the subgroup analysis by type of regimen and the subgroup analysis by treatment line were the same. The subgroup composed of Gasparini 2006 and Marty 2005, which administered trastuzumab along with a taxane-containing regimen and as first-line treatment, reported an increased risk of neutropenic fever which failed to reach statistical significance (RR 1.32, 90% CI 0.82 to 2.13, P = 0.34). In von Minckwitz 2009, which administered trastuzumab along with capecitabine and beyond progression, a non-significant increased risk of neutropenic fever was observed (RR 4.81, 90% CI 0.38 to 60.61, P = 0.31). Where applicable, we found no heterogeneity (I^2 = 0%). Refer to Analysis 3.2 and Analysis 3.3.

Anaemia

Four studies reported information on anaemia (Slamon 2001; Marty 2005; Gasparini 2006; von Minckwitz 2009). No events occurred in Gasparini 2006. There were six cases (1.3%) out of 466 in the trastuzumab group and seven (1.5%) out of 458 in the control group. There was no evidence of an increased risk of anaemia in patients treated with trastuzumab (RR 0.93, 90% CI 0.37 to 2.35, P = 0.90) (Analysis 3.4). We observed no heterogeneity (I^2 = 0%).

Anaemia stratified by type of regimen

There was no evidence of an increased risk of developing anaemia in any of the subgroups defined by different regimens. For the taxane-containing regimen, the RR was 1.03 (90% CI 0.20 to 5.30, P = 0.97). For the anthracycline-containing regimen, the RR was 1.26 (90% CI 0.36 to 4.35, P = 0.76). For the subgroup of studies administering other types of regimens, the RR was 0.19 (90% CI 0.02 to 2.42, P = 0.28). The results are likely to be influenced by the low number of events observed in each subgroup. Where applicable, there was no heterogeneity (I^2 = 0%). Refer to Analysis 3.5.

Anaemia stratified by treatment line

There was no evidence of an increased risk of anaemia in either subgroup. For the first-line subgroup, the RR was 1.19 (90% CI 0.44 to 3.19, P = 0.77) and for the beyond progression subgroup, the RR was 0.19 (90% CI 0.02 to 2.42, P = 0.28). Where applicable, we found no heterogeneity (I^2 = 0%). Refer to Analysis 3.6.

Neutropenia

Three studies reported information on neutropenia (Marty 2005; Gasparini 2006; von Minckwitz 2009). There were 41 cases (17.7%) out of 232 in the trastuzumab group and 28 (12.3%) out of 228 in the control group. The increased risk of neutropenia in patients treated with trastuzumab was of borderline statistical significance (RR 1.46, 90% CI 1.02 to 2.08, P = 0.08; Analysis 3.7). We detected no heterogeneity (I^2 = 0%).

Neutropenia stratified by type of regimen/treatment line

A low number of studies reported data on neutropenia, therefore the subgroup analysis by type of regimen and the subgroup analysis by treatment line were the same. The subgroup composed by Gasparini 2006 and Marty 2005, which administered trastuzumab along with a taxane-containing regimen and as first-line treatment, reported an increased risk of neutropenia of borderline statistical significance (RR 1.48, 90% CI 1.02 to 2.14, P = 0.09). In von Minckwitz 2009, which administered trastuzumab along with capecitabine and beyond progression, the observed increased risk of neutropenia was not significant (RR 1.28, 90% CI 0.38 to 4.37, P = 0.74). Where applicable, we found no heterogeneity (I^2 = 0%). Refer to Analysis 3.8 (type of regimen) and Analysis 3.9 (treatment line).

Recurrence in central nervous system

One study reported information on brain metastases (Slamon 2001). There were 42 cases (17.9%) out of 235 in the trastuzumab-containing group and 21/234 (9.0%) in the control group (RR 1.99, 95% CI 1.32 to 3.01). Blackwell 2010, which allowed the accrual of patients with known brain metastases, reported that nine (6.2%) out of the 146 patients treated with trastuzumab experienced central nervous system progression, whereas 15 patients (10.3) out of the 145 treated with lapatinib alone experienced progression while on study (RR 0.60, 90% CI 0.31 to 1.16). We decided against pooling the data.

Treatment-related deaths

Slamon 2001 reported that two deaths, both in the trastuzumab arm, were possibly related to the therapy. In the Marty 2005 trial, two drug-related deaths occurred in the control arm. Blackwell 2010 reported that one patient died in the trastuzumab arm with cardiac failure (concurrent with pulmonary thromboembolism) considered to be treatment-related. No drug-related deaths were observed in the Gasparini 2006, Kaufman 2009 and von Minckwitz 2009 trials. Huober 2012 did not report data on treatment-related deaths.

We decided against pooling these data.

Quality of life

Quality of life, measured by the European Organization for Research and Treatment Care Quality of Life Questionnaire, was assessed in the study by Osoba 2002, which used data previously published in Slamon 2001. The authors showed that higher proportions of patients treated with a combination of trastuzumab and chemotherapy achieved improvement in global quality of life than did patients treated by chemotherapy alone.

Blackwell 2010 reported results on quality of life, assessed using the Functional Assessment of Cancer Therapy-Breast (FACT-B) questionnaire (version 4). It was reported that changes from baseline in the combination arm were comparable to the changes from baseline in the monotherapy arm for all of the subscales, so none of the differences between the two treatment arms were statistically significant, but data were not shown. Quality of life was also assessed in the study by Wu 2011, which used data from the Blackwell study, using the Functional Assessment of Cancer Therapy-Breast (FACT-B) questionnaire (version 4). The analyses presented showed that comparable quality of life was maintained in both arms during the investigational treatment period.

We decided against pooling these data.

Sensitivity analysis: trastuzumab efficacy according to allocation concealment

We judged allocation concealment to be adequate only for Gasparini 2006, which did not report data on overall survival. Thus, it was not possible to conduct a sensitivity analysis by allocation concealment for overall survival with unclear/inadequate allocation concealment. The HR for progression-free survival in Gasparini 2006 was 0.70 (95% CI 0.42 to 1.16, P = 0.17); the HR in the unclear/inadequate subgroup, which represents 94.1% of the total weight in the meta-analysis, was 0.61 (95% CI 0.53 to 0.70, P < 0.00001; Analysis 4.1). There was no statistically significant difference between the two subgroups.

DISCUSSION

Summary of main results

This systematic review allowed us to measure the benefits of trastuzumab-based therapy in terms of response, progression-free survival and overall survival, and to quantify the risk of cardiac toxicities. The majority of studies were of fairly long duration, with the median duration of follow-up being two years, and were of moderate quality, the main weaknesses being the lack of blinding for progression-free survival and toxicity, and the potential outcome reporting bias for overall survival in two studies. The results should therefore be considered with this background in mind. All studies reported that trastuzumab extends time to disease progression, with gains varying between two and 11 months, and in five studies it extended time to death by between five and eight months. The meta-analysis showed a significant improvement in overall survival and progression-free survival for trastuzumab-containing regimens, which is possibly greater when considering patients treated as first-line compared to its use beyond progression, or patients receiving taxane-based regimens. For severe cardiac toxicities, we combined data across studies according to the premise that the adverse event profile of trastuzumab would be similar irrespective of the specific toxicity definition. We found that compared with control treatments, trastuzumab was associated with statistically significantly higher rates of serious cardiac toxicity. Subgroup comparisons revealed that the regimens did not differ from each other with respect to the relative risk of serious cardiac toxicities. The overall result of the meta-analysis on cardiotoxicity is influenced by the study of Slamon 2001, which is the first one that evaluated trastuzumab in addition to an anthracycline-containing regimen. Cardiotoxicity was unexpected at that time. The other studies do not show the same levels of cardiotoxicity, mainly due to the adoption of stricter enrolment criteria with respect to the baseline cardiovascular risk of patients. Indeed, in the subsequent trastuzumab studies, a normal baseline cardiac function was mandatory for study entry.

Human epidermal growth factor receptor 2 (HER2) positivity is associated with poor prognosis. Trastuzumab represents the paradigm of successfully developed targeted agents. Indeed, the target is measurable, the presence of the target is associated with a poorer outcome and target inhibition led to increased activity of standard therapy (Gschwind 2004). Moreover, trastuzumab has shown synergistic interaction with several cytotoxic agents (Pegram 2004), and its overall tolerability facilitated the combination of trastuzumab with the majority of agents registered for metastatic breast cancer, with the exception of anthracyclines. Trastuzumab used in combination therapies produced gains in absolute survival over older single agents and the magnitude of these gains was around 7%, as showed in our sensitivity analysis excluding the study by Blackwell. Strong survival benefits are also obtained in early breast cancer (Moja 2012). Other molecular targeted therapies, such as bevacizumab or lapatinib, did not produced similar gains (Mauri 2008; Ioannidis 2010; Gelmon 2012; Wagner 2012). For instance, in 2008 the FDA granted the accelerated approval of bevacizumab plus paclitaxel for advanced breast cancer (Miller 2007), on the basis of a supposed progression-free survival benefit. However, in 2010 the FDA removed the indication due to the lack of overall survival benefit in light of the possible side effects. A recent interim analysis of a trial designed to compare lapatinib or trastuzumab in combination with taxane-based chemotherapy for patients with metastatic breast cancer revealed that patients receiving trastuzumab had a statistically significant increase in progression-free survival (Gelmon 2012). As a matter of fact, trastuzumab remains a key, milestone drug in routine clinical practice and should be preferred over other treatment choices. The possibility of developing cardiac toxicity (left ventricular ejection fraction (LVEF) decline or congestive heart failure) is a well known side effect of trastuzumab-based regimens (Moja 2012), and should be balanced against the benefits. In advanced disease, however, where the majority of the patients will eventually die with progressive disease, patients and doctors are likely to accept a higher risk of toxicity even for a slight survival benefit (Simes 2001). The absence of a statistically significant difference in the risk of developing severe cardiac toxicity reported in our subgroup analyses for the combination of trastuzumab with taxanes, aromatase inhibitors, capecitabine and lapatinib should not be interpreted as evidence of lack of cardiac toxicities, particularly if compared with the significant increase when trastuzumab is combined with anthracyclines. The increased risk of developing significant cardiotoxicity means that the concomitant administration of anthracyclines and trastuzumab is not recommended in clinical practice. Indeed, more recently, relatively small neoadjuvant studies conducted in selected patients with an earlier disease stage were reassuring in terms of the cardiac safety of combining anthracycline-based regimens and trastuzumab (Guarneri 2012; Buzdar 2013; Schneeweiss 2013). However, the overall limited number of patients does not allow recommendation of the use of this combination outside a clinical trial. Moreover, in the Z1041 phase III

randomised study, the concurrent administration of trastuzumab with anthracyclines resulted in no additional benefit as compared to the standard administration of anthracyclines followed by taxanes-trastuzumab (Buzdar 2013). Currently, the results from this review inform us about an overall increase of the risk ratio of congestive heart failure of 3.49 in patients receiving trastuzumab-based therapy in metastatic breast cancer, irrespective of type of regimen used in combination with trastuzumab. Excluding the anthracycline-containing regimens, the RR lowers to 2.06 and fails to reach statistical significance. This toxicity might be mainly reversible and overall has little clinical relevance in the setting of advanced disease. On the other hand, in a large cohort of patients with breast cancer treated with trastuzumab outside clinical trials, cardiotoxicity varied considerably across subgroups of patients (e.g. age and history of cardiac disease were strong predictors of cardiotoxicity) and the long-term safety profile was less favourable (Bonifazi 2013).

Trastuzumab does not increase the risk of haematologic toxicities, such as neutropenic fever and anaemia; it seems to raise the risk of neutropenia.

Central nervous system (CNS) metastases are another important issue in HER2-positive disease. Breast cancer is the second most common cancer that can metastasise to the CNS (Tabouret 2012). Early studies reported an increased incidence of brain metastases in patients receiving trastuzumab. Historically, CNS progression has occurred late in the clinical course of the disease and survival has been mainly affected by the lack of systemic disease control. More recently, the availability of several lines of therapy, along with a better knowledge of the disease biology, has resulted in a substantial change in the natural history of the disease. Today, it is well known that breast cancer is a heterogeneous disease, with a distinct clinical behaviour and pattern of relapse (Kennecke 2010). The improvement in systemic control is increasing the prevalence of metastatic breast cancer patients who develop CNS recurrence while having good extra-CNS disease control. Only one study reported data on CNS progression, precluding the possibility of drawing definitive conclusions on this issue. This study showed an almost doubled incidence of CNS progression in trastuzumab-treated patients. The observed increase of brain metastases in trastuzumab-treated patients is reasonably a consequence of improved extra-CNS disease control along with low or no effect on CNS anatomical site.

Few data were found on treatment-related deaths or on quality of life, making it difficult to understand the impact of trastuzumab on these.

Overall completeness and applicability of evidence

The results of our meta-analysis cannot be generalised to all women with metastatic breast cancer in clinical practice. Most of the women in the studies included in our review were younger than women usually affected by the disease (median age ranging from 51 to 59 years). Furthermore, the present analysis combines the results from the registrative RCT, which included women with different baseline risks for cardiotoxicity, and the results from more recent RCTs, which instead included only women with a normal baseline risk. The overall results for congestive heart failure are influenced by the anthracycline-containing regimens of the registrative study, which would not be regarded as standard of care if in combination with trastuzumab, but the lack of generalisability of the other included studies may have an impact on the estimates and the risks may be slightly increased in real-practice settings (Bonifazi 2013). Therefore, careful cardiac monitoring is required before and while on trastuzumab-based therapy.

Quality of the evidence

Two trials were closed prematurely because of slow recruitment and another trial was stopped early for apparent benefit. Three trials allowed patients in the control arm experiencing progression to cross over to the trastuzumab arm and in one trial, upon documented disease progression, two-thirds of the patients were entered into an extension non-randomised study, in which they could receive either trastuzumab alone or in combination with chemotherapy of choice. This can have an impact on the estimates, since one arm has a continuous exposure to the biologic, whereas in the other arm the exposure is first to the control treatment and then the biologic. The consequences of the cross-over lead to problems in data analysis and the interpretation of results (D'Amico 2011), especially for overall survival in a metastatic setting, so the risk-benefit profile of trastuzumab might have been modified by the switch.

Potential biases in the review process

This systematic review has several strengths. We asked a specific clinical question and the search strategy was comprehensive. We included any publication of all relevant trials irrespective of language. We investigated the potential interaction between the treatment effect and potential effect modifiers. Finally, we rigorously applied the GRADE criteria for each of the relevant outcomes (Guyatt 2008).

This review also has limitations. Some readers may question the pooling of different cardiac toxicities. Since the overall numbers are relatively small, even limited changes in the definition of severe congestive heart failure or in LVEF thresholds might be associated with a higher or lower RR for adverse events.

A limitation of the current subgroup analyses is that they are based on published group data, rather than individual patient information. Individual patient data might allow a more detailed appraisal of outcomes for each regimen and for patients with different baseline risks. Still, the power to detect effect modification might still be limited even with individual patient information, particularly

for cardiac toxicities. Thus, strong inferences about the specific efficacy or safety superiority of one particular regimen over the others should be avoided.

For this review, we limited inclusion to RCTs and their open-label extensions. Long-term observational studies, including population-based registries, can provide realistic estimates of the risks of trastuzumab in real settings. We are completing the analyses of a second phase of this project, which will include observational studies, to address the issue of the frequency of cardiac events outside clinical trials, their cumulative incidence over a longer period and the potential for reversibility.

Agreements and disagreements with other studies or reviews

We found four other systematic reviews evaluating trastuzumab for the treatment of HER2-positive metastatic breast cancer (Mannocci 2010; Fleeman 2011; Harris 2011; Liao 2011). Although the outcomes and the methods do not totally overlap, there are no major disagreements with the results and conclusions of our systematic review. Mannocci 2010 considered trastuzumab beyond progression: the review included 12 observational studies and the only RCT published at that time (von Minckwitz 2009); safety was not evaluated. Quality assessment was not done. From analyses of the observational studies, the authors found no significant differences among subgroups defined by type of regimen (capecitabine or vinorelbine). Fleeman 2011 considered lapatinib or trastuzumab in combination with an aromatase inhibitor as first-line treatment. This health technology report included three RCTs, one assessing the efficacy and safety of lapatinib, and two assessing the efficacy and safety of trastuzumab (Kaufman 2009, and a 2009 conference abstract of the trial by Huober 2012). The authors decided against pooling the data. Harris 2011 evaluated only the efficacy of HER2-targeted agents. The meta-analysis included eight trials, three of them assessing lapatinib and five assessing trastuzumab (Slamon 2001; Marty 2005; Gasparini 2006; Kaufman 2009; von Minckwitz 2009). There were no subgroup analyses by type of drug. The results for efficacy were similar to the results of our systematic review in terms of overall survival (hazard ratio (HR) 0.78, 95% confidence interval (CI) 0.67 to 0.91), progression-free survival (HR 0.63, 95% CI 0.53 to 0.74) and overall response rate (RR 1.67, 95% CI 1.46 to 1.90). Liao 2011 reported the results in a letter to the editor. The meta-analysis included four trials, mixing metastatic and early breast cancer patients. Results on overall survival, overall response rate and cardiac toxicity did not differ from our results. None of the other reviews reported results in absolute terms.

AUTHORS' CONCLUSIONS

Implications for practice

The included trials had limitations: recruitment in three out of seven studies was slow or stopped early, in three trials more than 50% of patients in the control groups were permitted to switch to the trastuzumab arms at progression, all studies were open-label and there is potential selective outcome reporting bias with regard to overall survival in two studies. There is therefore moderate-quality evidence that the use of trastuzumab for metastatic breast cancer: a) improves both overall survival and progression-free survival in HER2-positive women; and b) increases by between three and four times the risk of severe cardiac toxicities.

From seven trials involving nearly 1500 women with HER2-positive metastatic breast cancer, the overall finding was that overall mortality was reduced by one-fifth after an average of two years of follow-up, but the risk of heart toxicity was more than three times greater for women in the group receiving trastuzumab compared to those receiving standard therapy alone. In absolute terms, if 1000 women were given standard therapy without trastuzumab then about 300 would survive for at least two years, but if 1000 women were treated with standard chemotherapy and trastuzumab until or beyond progression, about 373 would be alive two years after their diagnosis. However, about 35 in every 1000 women taking trastuzumab would experience severe heart toxicity, which is 25 more than for 1000 women taking the standard therapy alone. These heart problems are often reversible if the treatment is stopped straight away and, in the context of advanced disease, patients might choose to accept this risk given the potential benefit.

Treatment beyond progression might involve a greater risk of severe heart toxicities than first-line treatment, although these results are based on only two studies and few events.

The review did not identify a trastuzumab-containing regimen that may have been more or less effective or toxic, with the exception of the combination with anthracyclines that raises the risk of severe cardiac toxicity.

The evidence to support the use of trastuzumab beyond progression in metastatic disease is limited.

Implications for research

Trastuzumab is widely used for women with early breast cancer.

However, despite adjuvant trastuzumab, between 61 and 340 patients so treated are predicted to relapse and be considered for further anti-HER2 directed therapy in the metastatic setting in the first three years (Moja 2012). The effectiveness of trastuzumab for these patients is still an open issue since patients enrolled in trials in the metastatic setting were naive to trastuzumab. It is likely that the majority of the patients relapsing after adjuvant trastuzumab will be offered trastuzumab again even if the evidence of benefit is indirect. In our GRADE analysis we did not downgrade the overall

quality of the evidence for the primary endpoints for indirectness. Guideline panellists need to explore carefully the implications of this issue.

In women with metastatic cancer progressing while on trastuzumab it is important to test either the continuation or stopping of trastuzumab or to switch to other options: lapatinib, pertuzumab and TDM-1. The optimal therapeutic strategy for this growing group of patients is still a matter of debate.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

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

Blackwell 2010

Methods	Accrual time: November 2005 to November 2006 Multi-centre, international Baseline comparability: balanced
Participants	296 female enrolled Age: trastuzumab arm: median 52, range from 26 to 81; control arm: median 51, range from 29 to 78 Diagnosis: metastatic breast cancer progressed during prior trastuzumab-based therapy Inclusion criteria: women $\geq$ 18 years of age with histologically or cytologically confirmed BC. Patients must have metastatic disease that progressed on their most recent treatment regimen, which must have contained trastuzumab. Tumours with ErbB2 gene amplification as measured by fluorescence in situ hybridisation or ErbB2 over-expression as measured by immunohistochemistry (3+). Patients must have received prior anthracycline- and taxane-based regimens in either the adjuvant or metastatic setting. Eligible patients had at least 1 measurable lesion according to Response Evaluation Criteria in Solid Tumors (RECIST) or bone-only disease. ECOG performance status of $\leq$ 2; adequate haematologic, renal and hepatic function; and a cardiac ejection fraction within the institutional normal range. Written informed consent Exclusion criteria: see inclusion criteria HER2+: 100%
Interventions	2-arm RCT Trastuzumab arm (randomised N = 148): oral lapatinib (1000 mg daily) in combination with intravenous trastuzumab (2 mg/kg weekly, after the initial 4 mg/kg loading dose) Control arm (randomised N = 148): oral lapatinib (1500 mg daily)
Outcomes	Primary: progression-free survival Secondary: ORR (confirmed complete response plus partial response), clinical benefit response rate (confirmed complete response plus partial response at any time, plus stable disease for $\geq$ 24 weeks), OS, quality of life, safety
Notes	Study ID: EGF104900 Median length of time on study: 12.8 months (range 0.4 to 31.3) for patients receiving lapatinib + trastuzumab; 8.7 months (range 0.8 to 29.2) for patients receiving lapatinib alone 1 patient randomly assigned to the combination group did not receive study treatment, and 2 patients randomly assigned to lapatinib monotherapy received lapatinib in combination with trastuzumab, accounting for small differences between the ITT and safety populations Patients analysed for ORR: trastuzumab arm N = 146; control arm N = 145 Patients analysed for safety: trastuzumab arm N = 149; control arm N = 146 Patients with objective disease progression after receiving at least 4 weeks of study treatment with lapatinib monotherapy were permitted to cross over to combination therapy: 77 patients (52%) crossed over after progression

Blackwell 2010 (*Continued*)

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 of participants and personnel (performance bias) Overall Survival	High risk	Open-label
Blinding of outcome assessment (detection bias) (OS)	Low risk	Open-label, but our primary outcome (i.e. OS) is not likely to be influenced by lack of blinding
Blinding of outcome assessment (detection bias) (outcomes other than OS)	Unclear risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	Although 26 patients (9%) had withdrawn consent or were lost to follow-up before death, only 8 patients (2.7%) were lost to follow-up before progression (PFS is the primary outcome of the study)
Selective reporting (reporting bias)	Low risk	The protocol for the study is available (http://www.clinicaltrials.gov/ct2/show/NCT00320385). All of the study pre-specified primary outcomes that are of interest in the review have been reported

Gasparini 2006

Methods	Accrual time: December 2000 to September 2004 Multi-centre, national (Italy) Baseline comparability: balanced
Participants	123 female enrolled Age: trastuzumab arm: median 56, range from 32 to 72; control arm: median 54.3, range from 30 to 71 Diagnosis: untreated metastatic breast cancer Inclusion criteria: women over-expressing HER2/*neu* by the Hercep Test assay (score 2+ or 3+), measurable disease, age $\geq$ 18 years, performance status $\leq$ 2 according to the ECOG scale, life expectancy > 3 months and adequate organ function, defined as follows: LVEF > 50% or within normal limits, AST and ALT levels $\leq$ 2.5 times the normal value, total bilirubin < 1.5 the normal value, serum creatinine levels $\leq$ 1.5 mg/dl,

	neutrophils $\geq$ 2000/mm^3, platelets $\geq$ 100.000 mm^3, haemoglobin > 10 g/dl. Patients may have received an anthracycline and/or taxane-containing regimen given as adjuvant chemotherapy and relapsed > 12 months following the end of chemotherapy Exclusion criteria: patients were excluded if they had received previous chemotherapy for metastatic disease, brain, leptomeningeal or bone metastases as the only site of disease, a positive history for other types of cancer, with the exception of *in situ* cervix cancer radically resected and non melanoma skin cancer, prior history of myocardial infarction, unstable angina pectoris, cardiac insufficiency, uncontrolled arrhythmia or hypertension, peripheral neuropathy of grade $\geq$ 2 or pregnancy HER2+: 100%
Interventions	2-arm RCT Trastuzumab arm (randomised N = 63): paclitaxel (80 mg/m^2 weekly) plus trastuzumab (first loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg until disease progression) Control arm (randomised N = 60): paclitaxel (80 mg/m^2 weekly until disease progression)
Outcomes	Primary: ORR Secondary: safety profile, TTP and duration of response
Notes	Median follow-up for efficacy: 16.6 for both arms Trial stopped early for benefit. Interim analyses not planned in the RCT protocol Stopping characteristics: - Interim analyses: after the first 124 patients enrolled (sample size planned not reported) - Outcome: ORR - Details of stop: trialists interrupted the accrual but monitored follow-up of enrolled patients - Monitoring methods/stopping boundaries: not planned - the decision was based on: 1. data from other trials suggesting that only the patients with strong HER2 over-expression (3+) gain benefit from trastuzumab; 2. trial results confirmed a statistically significant superior outcome for the patients HER2-3+; 3. the planned statistical difference of 15% for ORR was reached - Role of monitoring committee: not described

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	1:1 randomisation stratified according to visceral involvement, HER2/neu over-expression and centre
Allocation concealment (selection bias)	Low risk	The patients were allocated by the independent monitoring agency to treatment group by randomisation code envelopes
Blinding of participants and personnel (performance bias) Overall Survival	High risk	Open-label

Gasparini 2006 (*Continued*)

Blinding of outcome assessment (detection bias) (OS)	Low risk	Open-label, but our primary outcome (i.e. OS) is not likely to be influenced by lack of blinding
Blinding of outcome assessment (detection bias) (outcomes other than OS)	Unclear risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	124 patients enrolled: 123 patients assessable for efficacy and safety and 118 for ORR
Selective reporting (reporting bias)	High risk	Protocol not found Results for OS not shown

Huober 2012

Methods	Accrual time: 2003 to 2007 Multi-centre, international Baseline comparability: more patients in the control arm (71%) than in the trastuzumab arm (42%) had received adjuvant systemic treatment. Tamoxifen was exclusively used for adjuvant endocrine treatment (given in 65% and 31% of patients, respectively)
Participants	57 female enrolled Age: trastuzumab arm: median 61.5, range from 39 to 87; control arm: median 61, range from 47 to 88 Diagnosis: HER2 and hormone receptor-positive metastatic breast cancer or locally advanced breast cancer Inclusion criteria: postmenopausal women with newly diagnosed hormone receptor-positive MBC or LABC defined as ER and/or PgR $\geq$ 10 fmol/mg cytosol protein, or $\geq$ 10% of the tumour cells positive as assessed by immunohistochemical evaluation of the primary tumour. For the trastuzumab and control arms, the primary tumour had to reveal HER2 over-expression defined as 3+ staining by IHC or HER2 amplification (ratio > 2) by FISH or an equivalent method. No prior treatment for metastatic or locally advanced breast cancer; LVEF $\geq$ 50% at baseline; adequate hepatic, renal and bone marrow function; an ECOG performance status of 0 or 1. Furthermore, patients were required to have at least 1 measurable tumour lesion (patients with bone only disease were eligible) Exclusion criteria: clinical or radiological signs of CNS metastasis; inflammatory breast cancer; other concurrent or previous malignant disease; uncontrolled cardiac diseases; prior anti-HER2 therapy apart from trastuzumab in the adjuvant setting HER2+: 61.3% (57/93). We considered HER2+ women alone
Interventions	2-arm RCT Trastuzumab arm (randomised N = 26): letrozole (2.5 mg once daily) plus trastuzumab (first loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg until disease progression) iv until progression of disease. As of May 2005 trastuzumab was allowed to be given as 3-weekly application with the typical dose of 6 mg/kg (after a loading dose of 8 mg/kg)

	Control arm (randomised N = 31): letrozole (2.5 mg once daily until disease progression) In addition, in an amendment for German sites, a third arm (N = 35) was implemented where patients with HER2-negative and hormone receptor-positive tumours were assigned to receive letrozole alone as first-line treatment
Outcomes	Primary: time to disease progression (interval between the date of randomisation/start of treatment and the earliest date of progression, or of death due to the underlying breast cancer, or of death from a cause thought to be connected to the underlying disease) Secondary: TTF, ORR/CBR, duration of response/clinical benefit and OS
Notes	Study ID: eLEcTRA Median follow-up: not specified Closed prematurely due to slow recruitment: the total number of patients to be recruited in the trastuzumab and the control arms was planned to be 300 (150 per arm) Trastuzumab was admitted as second-line treatment in 31% and the 52% of the treatment and control arms respectively ClinicalTrials identifier: NCT00171847

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 of participants and personnel (performance bias) Overall Survival	High risk	Open-label
Blinding of outcome assessment (detection bias) (OS)	Low risk	Open-label, but our primary outcome (i.e. OS) is not likely to be influenced by lack of blinding
Blinding of outcome assessment (detection bias) (outcomes other than OS)	Unclear risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	No missing outcome data
Selective reporting (reporting bias)	High risk	The protocol for the study is available (http://www.clinicaltrials.gov/ct2/show/NCT00171847). Results on OS not shown

Kaufman 2009

Methods	Accrual time: 2001 to 2004 Multi-centre, international Baseline comparability: balanced Co-intervention: in the trastuzumab plus anastrozole arm, patients had almost twice the duration of exposure to anastrozole compared with patients receiving anastrozole alone (median, 189 days compared to 98 days, respectively)
Participants	208 female enrolled Age: trastuzumab arm: median 56, range from 31 to 85; control arm: median 54, range from 27 to 77 Diagnosis: metastatic breast cancer Inclusion criteria: postmenopausal women over-expressing HER2/*neu* by the Hercep Test assay (score 2+ or 3+), and/or fluorescence in situ hybridisation positive with two-fold amplification, hormone receptor-positive (ER-positive and/or PgR-positive). Previous treatment with tamoxifen as adjuvant or hormonal therapy or anastrozole was permitted. Other requirements included a LVEF greater than 50%; adequate baseline hepatic, renal and bone marrow function; an ECOG performance status of 0 to 1; and measurable or evaluable disease Exclusion criteria: prior chemotherapy for MBC or adjuvant chemotherapy within 6 months, clinical or radiologic evidence of CNS metastases; history of another malignancy, CHF or uncontrolled cardiac disease (angina, arrhythmias, hypertension); uncontrolled serious intercurrent illness; and severe dyspnoea at rest. Patients with previous radiotherapy to indicator lesions were excluded from the response evaluation HER2+: not reported
Interventions	2-arm RCT Trastuzumab arm (randomised N = 104): anastrozole (1 mg/d orally) plus trastuzumab (first loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg) until disease progression Control arm (randomised N = 104): anastrozole (1 mg/d orally) until disease progression
Outcomes	Primary: progression-free survival Secondary: clinical benefit rate, overall response rate, time to disease progression, time to treatment failure, duration of response
Notes	Median follow-up for efficacy: not reported Upon documented disease progression, 58 of 103 patients (56.3%) crossed over from anastrozole alone to trastuzumab while 15 patients (14.6%) received trastuzumab after study withdrawal Response data were reported for only 147 patients (70.6%; trastuzumab plus anastrozole, n = 74; anastrozole alone, n = 73). Hormone receptor positivity was confirmed in the central laboratory for 150 patients (trastuzumab plus anastrozole, n = 77; anastrozole alone, n = 73). ER/PgR negativity was found centrally in 44 patients (trastuzumab plus anastrozole, n = 21; anastrozole alone, n = 23) and central confirmation was not possible in 13 patients (trastuzumab plus anastrozole, n = 5; anastrozole alone, n = 8)
Risk of bias	

Kaufman 2009 (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Random assignment was conducted using a minimisation procedure, with stratification according to presence of liver metastases; measurable versus evaluable disease; time to relapse after adjuvant tamoxifen, if administered; and bisphosphonate therapy at enrolment
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants and personnel (performance bias) Overall Survival	High risk	Open-label
Blinding of outcome assessment (detection bias) (OS)	Low risk	Open-label, but our primary outcome (i.e. OS) is not likely to be influenced by lack of blinding
Blinding of outcome assessment (detection bias) (outcomes other than OS)	Low risk	Open-label, but it is stated that "A reconciled data set, which integrated the tumor response/date of progression as determined by the investigator and a blinded Response Evaluation Committee, was used for the primary analyses. An independent oncologist reconciled cases where investigator and committee assessments differed."
Incomplete outcome data (attrition bias) All outcomes	Low risk	208 patients enrolled: 1 patient (trastuzumab plus anastrozole arm) withdrew before receiving the first dose. 207 patients assessable for efficacy and toxicity (although response data were reported for only 147 patients (70. 6%))
Selective reporting (reporting bias)	Unclear risk	Protocol not found Results for primary and secondary outcomes are reported in published papers: it is likely that reporting bias has not occurred

Marty 2005

Methods	Accrual time: April 2000 to October 2002 Multi-centre, international Baseline comparability: baseline patients characteristics were generally balanced between the 2 arms, although there were more patients with oestrogen receptor- or progesterone receptor-positive disease in the docetaxel alone arm compared with the combination arm (56% versus 41%) and more patients had received prior (neo)adjuvant anthracyclines in the combination arm (64% versus 55%)
Participants	188 female enrolled Age: trastuzumab arm: median 53, range from 32 to 80; control arm: median 55; range from 24 to 79 Diagnosis: metastatic breast cancer Inclusion criteria: women age 18 to 70 years with HER2 over-expression (IHC 3+) and/or gene amplification (FISH positive) who had not previously received chemotherapy for metastatic disease. Patients could have received prior (neo)adjuvant anthracyclines (maximum cumulative dose, 360 mg/m^2 doxorubicin or 750 mg/m^2 epirubicin). Baseline LVEF had to be more than 50%. Hormonal therapy had to be discontinued before the first dose of study drug. Previous radiotherapy was allowed only if treatment had ended at least 14 days before enrolment into the trial and the patient had fully recovered from all acute adverse effects. ECOG performance status of $\leq$ 2, life expectancy $\geq$ 12 weeks, and at least one bidimensionally measurable lesion (according to WHO criteria). Bone marrow, renal and hepatic function: haemoglobin $\geq$ 10 g/dL and no blood transfusion within the previous 2 weeks; neutrophil count $\geq$ 2.0 x 109 cells/L; platelet count $\geq$ 100 x 109 cells/L; no evidence of myelodysplastic syndrome or abnormal bone marrow reserve; creatinine $\leq$ 1.5 x upper limit of normal (ULN) or creatinine clearance $\geq$ 60 mL/min; total bilirubin less than 1 x ULN; AST and/or ALT $\leq$ 2.5 x ULN; and alkaline phosphatase $\leq$ 5 x ULN (patients with AST and/or ALT > 1.5 x ULN concomitantly with alkaline phosphatase > 2.5 x ULN were ineligible for the study) Exclusion criteria: patients who had received prior chemotherapy for their metastatic disease or any prior taxanes or anti-HER therapy were excluded. Patients who had brain or leptomeningeal metastases, significant cardiac insufficiency (New York Heart Association III or IV), myocardial infarction within the previous 6 months, unstable angina pectoris, uncontrolled arrhythmia, or advanced pulmonary disease or severe dyspnoea at rest due to complications of advanced malignancy, or who required supplementary oxygen therapy, were ineligible for the trial HER2+: group 1: 97%; group 2: 94%
Interventions	2-arm RCT Trastuzumab arm (randomised N = 94): docetaxel (100 mg/m^2 every 21 days for 6 cycles) plus trastuzumab (first loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg until disease progression) Control arm (randomised N = 94): docetaxel (100 mg/m^2 every 21 days for 6 cycles)
Outcomes	Primary: ORR Secondary: OS, duration of response, time to disease progression, time to treatment failure
Notes	Study ID: M77001 Study Group Median follow-up for efficacy: 24 months; minimum, maximum not reported Upon documented disease progression, 53 of 94 patients (57%) crossed over from doc-

	etaxel alone to trastuzumab Patients analysed: trastuzumab arm N = 92; control arm N = 94 Patients analysed for cardiac safety: trastuzumab arm N = 86; control arm N = 76 OS: HR estimated using the ratio of the medians. HR variance estimated using number of deaths in the 2 groups. Time to progression: HR estimated using the ratio of the medians. HR variance estimated using the relationship between Chi^2 and the log of the HR

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Random assignment to treatment, block by country
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants and personnel (performance bias) Overall Survival	High risk	Open-label
Blinding of outcome assessment (detection bias) (OS)	Low risk	Open-label, but our primary outcome (i.e. OS) is not likely to be influenced by lack of blinding
Blinding of outcome assessment (detection bias) (outcomes other than OS)	Unclear risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	2 patients (both in the trastuzumab plus docetaxel arm) withdrew before receiving the first dose. 7 patients in the docetaxel alone arm withdrew at cycle 1 or 2
Selective reporting (reporting bias)	Low risk	The protocol for the study is available (http://www.roche-trials.com/studyResultGet.action?studyResultNumber=M77001)

Slamon 2001

Methods	Accrual time: June 1995 to March 1997 Multi-centre, international Baseline comparability: balanced
Participants	469 female enrolled Age: trastuzumab + anthracycline arm: mean 54, range from 27 to 76; anthracycline control arm: mean 54, range from 25 to 75; trastuzumab + taxane arm: mean 51, range from 25 to 77; taxane control arm: mean 51, range from 26 to 73

	Diagnosis: progressive metastatic breast cancer Inclusion criteria: women over-expressing HER2 who had not previously received chemotherapy for metastatic disease. Only patients who had weak-to-moderate staining of the entire tumour cell membrane for HER2 (referred to as a score of 2+) or more than moderate staining (referred to as a score of 3+) in more than 10% of tumour cells on immunohistochemical analysis were eligible Exclusion criteria: patients were excluded if they had bilateral breast cancer, untreated brain metastases, osteoblastic bone metastases, pleural effusion or ascites as the only evidence of disease, a second type of primary cancer, or a Karnofsky score of less than 60, if they were pregnant or had received any type of investigational agent within 30 days before the study began HER2+: 100%
Interventions	4-arm RCT Trastuzumab + anthracycline arm (randomised N = 143): doxorubicin (or epirubicin) plus cyclophosphamide (60 mg/m^2 (75 mg/m^2) and 600 mg/m^2 every 21 days for 6 cycles) plus trastuzumab (first loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg until disease progression) Anthracycline control arm (randomised N = 138): doxorubicin (or epirubicin) plus cyclophosphamide (60 mg/m^2 (75 mg/m^2) and 600 mg/m^2 every 21 days for 6 cycles) Trastuzumab + taxane arm (randomised N = 92): paclitaxel (175 mg/m^2 every 21 days for 6 cycles) plus trastuzumab (first loading dose of 4 mg/kg, followed by weekly doses of 2 mg/kg until disease progression) Taxane control arm (randomised N = 96): paclitaxel (175 mg/m^2 every 21 days for 6 cycles) Trastuzumab + anthracycline and anthracycline control arms: 100% patients naive to previous anthracycline chemotherapy Trastuzumab + taxane and taxane control arm: 97% patients previously treated with adjuvant anthracycline chemotherapy
Outcomes	Primary: time to disease progression (disease progression was defined as an increase of more than 25% in the dimensions of any measurable lesion) Secondary: OS, ORR, the duration of a response, the time to treatment failure (a composite of disease progression, death, discontinuation of treatment and the use of other types of antitumour therapy)
Notes	Study ID: HO648g Median follow-up for efficacy: 30 months (30 min to 51 max) Upon documented disease progression patients (66%) were entered into the extension study H0659g, a non-randomised, open-label study in which they could receive either trastuzumab alone or in combination with chemotherapy of choice We found possible inconsistencies among different data reports (FDA Statistical Review - Trastuzumab Product Approval Information - Licensing Action 9/25/98, EMEA EPAR H-C-278 Scientific discussion for the approval of Herceptin 2004). For this systematic review we considered the NEJM publication as our primary data source OS: HR estimated using the ratio of the medians. HR variance estimated using the relationship between Chi^2 and the log of the HR. Time to progression: HR estimated using the ratio of the medians. HR variance estimated using the relationship between Chi^2 and the log of the HR

Slamon 2001 (*Continued*)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Not reported Stratification on the basis of history of adjuvant anthracycline treatment
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants and personnel (performance bias) Overall Survival	High risk	Open-label
Blinding of outcome assessment (detection bias) (OS)	Low risk	Open-label (original double-blind design was abandoned due to ethical considerations), but our primary outcome (i.e. OS) is not likely to be influenced by lack of blinding
Blinding of outcome assessment (detection bias) (outcomes other than OS)	Unclear risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	Enrolled 469 patients. 5 patients were never treated; 2 decline treatment; 1 died before treatment; 1 had disease progression at enrolment; 1 was enrolled inadvertently
Selective reporting (reporting bias)	Low risk	The protocol for the study is available (http://www.cancer.gov/clinicaltrials/search/view?cdrid=64329&version=HealthProfessional&protocolsearchid=6378103)

von Minckwitz 2009

Methods	Accrual time: September 2003 to June 2007 Multi-centre, international Baseline comparability: 3 to 4 T stage at first diagnosis more frequent in the capecitabine + trastuzumab arm than in the capecitabine alone arm (respectively: 34% and 14%)
Participants	156 female enrolled Age: capecitabine arm: median 59, range from 33 to 82; capecitabine + trastuzumab arm: median 52.5, range from 28 to 78 Diagnosis: pathologically confirmed, HER2-positive, locally advanced or metastatic breast cancer Inclusion criteria: women with pathologically confirmed, HER2-positive, locally advanced or metastatic breast cancer. HER2 status was considered positive if over-expres-

	sion was detected in either the primary or metastatic tumour tissue by local immunohistochemistry (grade 3+ staining intensity) or by fluorescence in situ hybridisation. Duration of previous trastuzumab treatment had to be 12 weeks or greater, and the time since the end of the last trastuzumab cycle had to be less than 6 weeks. Patients could have received up to 1 chemotherapy drug for metastatic disease. Karnofsky performance status of 60% or greater; a life expectancy of greater than 3 months; and adequate haematologic, renal, hepatic and cardiac function sonographically confirmed by a LVEF of 50% or greater. Written informed consent Exclusion criteria: see inclusion criteria HER2+: 100%
Interventions	2-arm RCT Trastuzumab arm (randomised N = 78): capecitabine (2500 mg/m^2 (1250 mg/m^2 semi-daily) on days 1 through 14 followed by 1 week of rest) plus trastuzumab (6 mg/kg every 3 weeks until disease progression or unacceptable toxicity) Control arm (randomised N = 78): capecitabine (2500 mg/m^2 (1250 mg/m^2 semi-daily) on days 1 through 14 followed by 1 week of rest)
Outcomes	Primary: time to progression (time period between random assignment and documented disease progression or disease-related death) Secondary: response of tumour lesions; clinical benefit (complete response, partial response or disease stabilisation for greater than 24 weeks); duration of response (time period between first notification of a response and the date of documented progression, disease-related death or withdrawal); OS
Notes	Study ID: GBG-26 Median follow-up: 20.7 months As accrual was slowing down, the trial was closed in agreement with the IDMC on 1 July 2007

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random assignment list was prepared before stratification (by pretreatment and participating centre); a block permutation method with a block size of 4 was used
Allocation concealment (selection bias)	Unclear risk	Not reported
Blinding of participants and personnel (performance bias) Overall Survival	High risk	Open-label
Blinding of outcome assessment (detection bias) (OS)	Low risk	Open-label, but our primary outcome (i.e. OS) is not likely to be influenced by lack of blinding

von Minckwitz 2009 (*Continued*)

Blinding of outcome assessment (detection bias) (outcomes other than OS)	Unclear risk	Open-label
Incomplete outcome data (attrition bias) All outcomes	Low risk	Enrolled 156 patients. 5 patients were never treated; 151 patients received at least 1 cycle of allocated treatment
Selective reporting (reporting bias)	Unclear risk	The protocol is available (http://www.clinicaltrials.gov/ct2/show/NCT00320385) In the paper published in 2011, only updated results for OS are reported

ALT: alanine aminotransferase
AST: aspartate aminotransferase
BC: breast cancer
CBR: clinical benefit rate
CHF: congestive heart failure
CNS: central nervous system
ECOG: Eastern Cooperative Oncology Group
ER: oestrogen receptor
FDA: (US) Food and Drug Administration
FISH: fluorescence in situ hybridisation
HER2: human epidermal growth factor receptor 2
HR: hazard ratio
IDMC: Independent Data Monitoring Committee
IHC: immunohistochemistry
ITT: intention-to-treat
iv: intravenous
LABC: locally advanced breast cancer
LVEF: left ventricular ejection fraction
MBC: metastatic breast cancer
NEJM: *New England Journal of Medicine*
ORR: overall response rate
OS: overall survival
PFS: progression-free survival
PgR: progesterone receptor
RCT: randomised controlled trial
TTF: time to failure
TTP: time to progression
ULN: upper limit of normal
WHO: World Health Organization

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

Study	Reason for exclusion
Gelmon 2012	Head-to-head comparison of trastuzumab and lapatinib
Papaldo 2006	Not randomised, all HER2-positive patients were given trastuzumab
Raff 2004	Not randomised, all HER2-positive patients were given trastuzumab

HER2: human epidermal growth factor receptor 2

DATA AND ANALYSES

Comparison 1. Efficacy of trastuzumab

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Overall survival - all studies	5		Hazard Ratio (Random, 95% CI)	0.82 [0.71, 0.94]
2 Overall survival - excluding Blackwell	4		Hazard Ratio (Random, 95% CI)	0.82 [0.70, 0.95]
3 Overall survival - stratified by type of regimen	4		Hazard Ratio (Random, 95% CI)	0.81 [0.70, 0.93]
3.1 Taxane-containing regimen	2		Hazard Ratio (Random, 95% CI)	0.80 [0.65, 0.99]
3.2 Anthracycline-containing regimen	1		Hazard Ratio (Random, 95% CI)	0.80 [0.59, 1.10]
3.3 Aromatase inhibitor-containing regimen	1		Hazard Ratio (Random, 95% CI)	0.84 [0.59, 1.19]
3.4 Other	1		Hazard Ratio (Random, 95% CI)	0.80 [0.56, 1.14]
4 Overall survival - stratified by treatment line	5		Hazard Ratio (Random, 95% CI)	0.82 [0.71, 0.94]
4.1 First-line	3		Hazard Ratio (Random, 95% CI)	0.79 [0.67, 0.94]
4.2 Beyond progression	2		Hazard Ratio (Random, 95% CI)	0.87 [0.68, 1.12]
5 Progression-free survival - all studies	7		Hazard Ratio (Random, 95% CI)	0.61 [0.54, 0.70]
6 Progression-free survival - excluding Blackwell	6		Hazard Ratio (Random, 95% CI)	0.58 [0.50, 0.66]
7 Progression-free survival - stratified by type of regimen	7		Hazard Ratio (Random, 95% CI)	0.67 [0.59, 0.77]
7.1 Taxane-containing regimen	3		Hazard Ratio (Random, 95% CI)	0.53 [0.42, 0.68]
7.2 Anthracycline-containing regimen	1		Hazard Ratio (Random, 95% CI)	0.78 [0.68, 0.91]
7.3 Aromatase inhibitor-containing regimen	2		Hazard Ratio (Random, 95% CI)	0.64 [0.49, 0.83]
7.4 Other	2		Hazard Ratio (Random, 95% CI)	0.72 [0.59, 0.88]
8 Progression-free survival - stratified by treatment line	7		Hazard Ratio (Random, 95% CI)	0.61 [0.54, 0.70]
8.1 First-line	5		Hazard Ratio (Random, 95% CI)	0.56 [0.49, 0.65]
8.2 Beyond progression	2		Hazard Ratio (Random, 95% CI)	0.72 [0.59, 0.88]
9 Overall response rate - all studies	7	1419	Risk Ratio (IV, Random, 95% CI)	1.58 [1.38, 1.82]
10 Overall response rate - stratified by type of regimen	7	1419	Risk Ratio (IV, Random, 95% CI)	1.61 [1.35, 1.91]
10.1 Taxane-containing regimen	3	492	Risk Ratio (IV, Random, 95% CI)	1.71 [1.23, 2.38]
10.2 Anthracycline-containing regimen	1	281	Risk Ratio (IV, Random, 95% CI)	1.33 [1.04, 1.70]
10.3 Aromatase inhibitor-containing regimen	2	204	Risk Ratio (IV, Random, 95% CI)	2.55 [1.23, 5.27]

10.4 Other	2	442	Risk Ratio (IV, Random, 95% CI)	1.70 [1.16, 2.49]
11 Overall response rate - stratified by treatment line	7	1419	Risk Ratio (IV, Random, 95% CI)	1.58 [1.38, 1.82]
11.1 First-line	5	977	Risk Ratio (IV, Random, 95% CI)	1.57 [1.34, 1.84]
11.2 Beyond progression	2	442	Risk Ratio (IV, Random, 95% CI)	1.70 [1.16, 2.49]

Comparison 2. Cardiac toxicity of trastuzumab

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Congestive heart failure - all studies	7	1459	Risk Ratio (IV, Random, 90% CI)	3.49 [1.88, 6.47]
2 Congestive heart failure - stratified by type of regimen	7	1459	Risk Ratio (IV, Random, 90% CI)	3.37 [1.81, 6.27]
2.1 Taxane-containing regimen	3	471	Risk Ratio (IV, Random, 90% CI)	1.98 [0.54, 7.26]
2.2 Anthracycline-containing regimen	1	278	Risk Ratio (IV, Random, 90% CI)	5.43 [2.28, 12.94]
2.3 Aromatase inhibitor-containing regimen	2	264	Risk Ratio (IV, Random, 90% CI)	1.01 [0.20, 5.15]
2.4 Other	2	446	Risk Ratio (IV, Random, 90% CI)	5.31 [0.87, 32.20]
3 Congestive heart failure - stratified by treatment line	7	1459	Risk Ratio (IV, Random, 90% CI)	3.49 [1.88, 6.47]
3.1 First-line	5	1013	Risk Ratio (IV, Random, 90% CI)	3.30 [1.71, 6.37]
3.2 Beyond progression	2	446	Risk Ratio (IV, Random, 90% CI)	5.31 [0.87, 32.20]
4 Left ventricular ejection fraction (LVEF) decline - all studies	5	938	Risk Ratio (IV, Random, 90% CI)	2.65 [1.48, 4.74]
5 LVEF decline - stratified by type of regimen	5	938	Risk Ratio (IV, Random, 90% CI)	2.65 [1.48, 4.74]
5.1 Taxane-containing regimen	2	285	Risk Ratio (IV, Random, 90% CI)	2.36 [1.12, 4.96]
5.2 Aromatase inhibitor-containing regimen	1	207	Risk Ratio (IV, Random, 90% CI)	3.03 [0.21, 44.02]
5.3 Other	2	446	Risk Ratio (IV, Random, 90% CI)	3.21 [1.19, 8.64]
6 LVEF decline - stratified by treatment line	5	938	Risk Ratio (IV, Random, 90% CI)	2.65 [1.48, 4.74]
6.1 First-line	3	492	Risk Ratio (IV, Random, 90% CI)	2.40 [1.17, 4.91]
6.2 Beyond progression	2	446	Risk Ratio (IV, Random, 90% CI)	3.21 [1.19, 8.64]

Comparison 3. Other toxicities

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Neutropenic fever - all studies	3	460	Risk Ratio (IV, Random, 90% CI)	1.38 [0.86, 2.21]
2 Neutropenic fever - stratified by type of regimen	3	460	Risk Ratio (IV, Random, 90% CI)	1.38 [0.86, 2.21]
2.1 Taxane-containing regimen	2	309	Risk Ratio (IV, Random, 90% CI)	1.32 [0.82, 2.13]
2.2 Other	1	151	Risk Ratio (IV, Random, 90% CI)	4.81 [0.38, 60.61]
3 Neutropenic fever - stratified by treatment line	3	460	Risk Ratio (IV, Random, 90% CI)	1.38 [0.86, 2.21]
3.1 First-line	2	309	Risk Ratio (IV, Random, 90% CI)	1.32 [0.82, 2.13]
3.2 Beyond progression	1	151	Risk Ratio (IV, Random, 90% CI)	4.81 [0.38, 60.61]
4 Anaemia - all studies	4	924	Risk Ratio (IV, Random, 90% CI)	0.93 [0.37, 2.35]
5 Anaemia - stratified by type of regimen	4	924	Risk Ratio (IV, Random, 90% CI)	0.92 [0.37, 2.32]
5.1 Taxane-containing regimen	3	495	Risk Ratio (IV, Random, 90% CI)	1.03 [0.20, 5.30]
5.2 Anthracycline-containing regimen	1	278	Risk Ratio (IV, Random, 90% CI)	1.26 [0.36, 4.35]
5.3 Other	1	151	Risk Ratio (IV, Random, 90% CI)	0.19 [0.02, 2.42]
6 Anaemia - stratified by treatment line	4	924	Risk Ratio (IV, Random, 90% CI)	0.93 [0.37, 2.35]
6.1 First-line	3	773	Risk Ratio (IV, Random, 90% CI)	1.19 [0.44, 3.19]
6.2 Beyond progression	1	151	Risk Ratio (IV, Random, 90% CI)	0.19 [0.02, 2.42]
7 Neutropenia - all studies	3	460	Risk Ratio (IV, Random, 90% CI)	1.46 [1.02, 2.08]
8 Neutropenia - stratified by type of regimen	3	460	Risk Ratio (IV, Random, 90% CI)	1.46 [1.02, 2.08]
8.1 Taxane-containing regimen	2	309	Risk Ratio (IV, Random, 90% CI)	1.48 [1.02, 2.14]
8.2 Other	1	151	Risk Ratio (IV, Random, 90% CI)	1.28 [0.38, 4.37]
9 Neutropenia - stratified by treatment line	3	460	Risk Ratio (IV, Random, 90% CI)	1.46 [1.02, 2.08]
9.1 First-line	2	309	Risk Ratio (IV, Random, 90% CI)	1.48 [1.02, 2.14]
9.2 Beyond progression	1	151	Risk Ratio (IV, Random, 90% CI)	1.28 [0.38, 4.37]

Comparison 4. Sensitivity analysis: progression-free survival - by allocation concealment

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Progression-free survival - by allocation concealment	7		Hazard Ratio (Random, 95% CI)	0.61 [0.54, 0.70]
1.1 Adequate	1		Hazard Ratio (Random, 95% CI)	0.70 [0.42, 1.16]
1.2 Unclear/inadequate	6		Hazard Ratio (Random, 95% CI)	0.61 [0.53, 0.70]

Analysis 1.1. Comparison 1 Efficacy of trastuzumab, Outcome 1 Overall survival - all studies.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 1 Overall survival - all studies

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
Marty 2005	-0.32 (0.19)		13.6 %	0.73 [0.50, 1.05]
von Minckwitz 2009	-0.06 (0.18)		15.2 %	0.94 [0.66, 1.34]
Kaufman 2009	-0.18 (0.18)		15.2 %	0.84 [0.59, 1.19]
Blackwell 2010	-0.22 (0.18)		15.2 %	0.80 [0.56, 1.14]
Slamon 2001	-0.22 (0.11)		40.7 %	0.80 [0.65, 1.00]
Total (95% CI)			**100.0 %**	**0.82 [0.71, 0.94]**

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 1.06$, df = 4 (P = 0.90); $I^2 = 0.0\%$

Test for overall effect: Z = 2.90 (P = 0.0038)

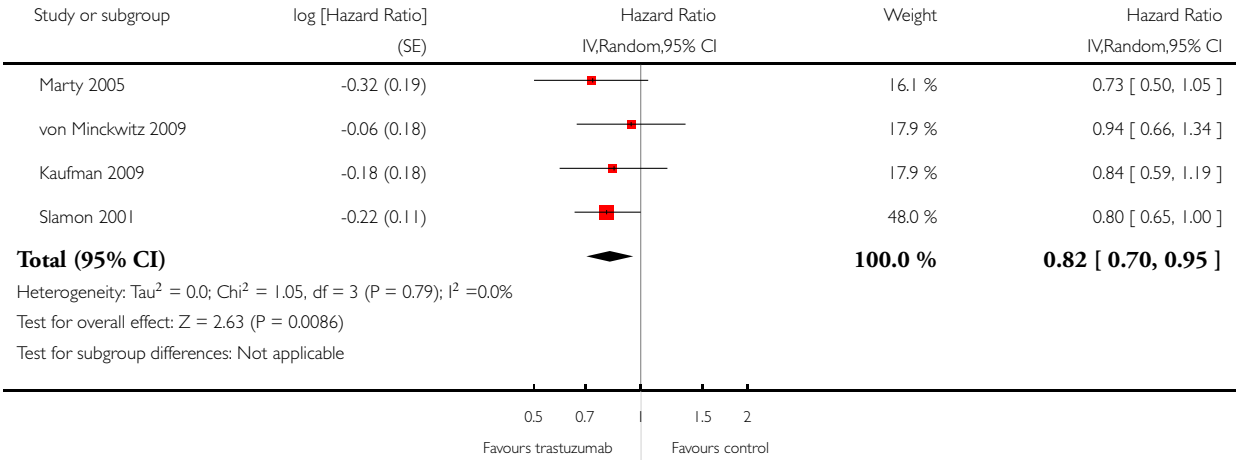
Test for subgroup differences: Not applicable

Analysis 1.2. Comparison 1 Efficacy of trastuzumab, Outcome 2 Overall survival - excluding Blackwell.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 2 Overall survival - excluding Blackwell

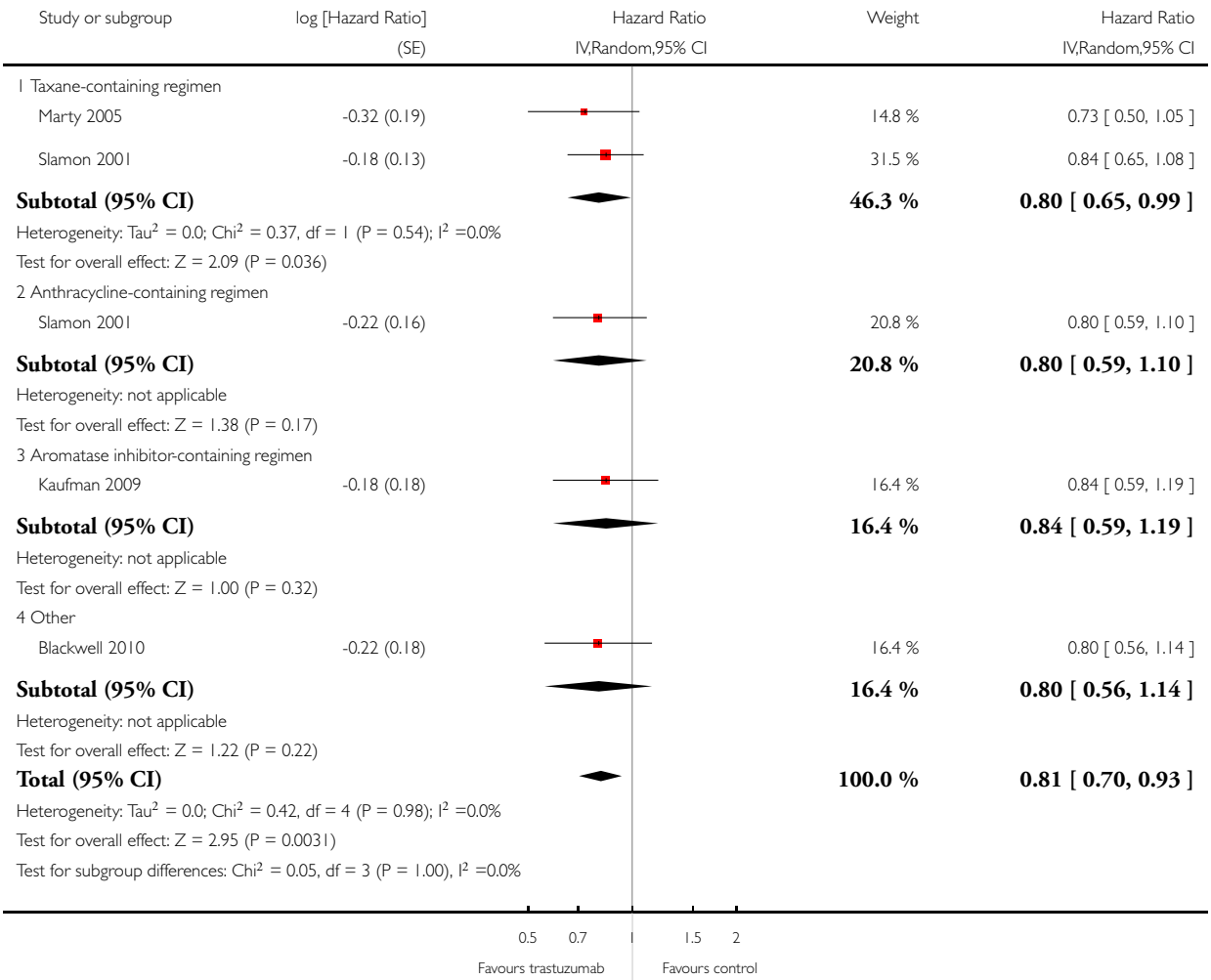


Analysis 1.3. Comparison 1 Efficacy of trastuzumab, Outcome 3 Overall survival - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 3 Overall survival - stratified by type of regimen

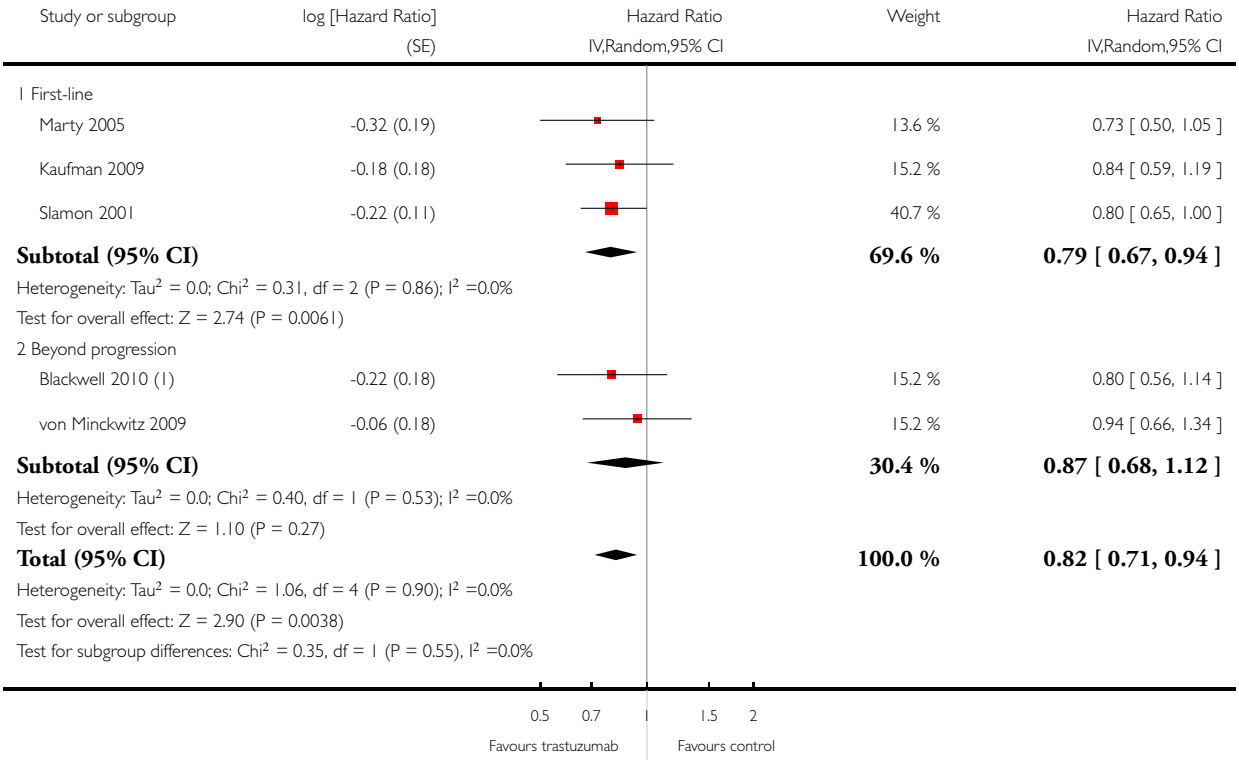


Analysis 1.4. Comparison 1 Efficacy of trastuzumab, Outcome 4 Overall survival - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 4 Overall survival - stratified by treatment line



(1) Lapatinib-containing regimen

Analysis 1.5. Comparison 1 Efficacy of trastuzumab, Outcome 5 Progression-free survival - all studies.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

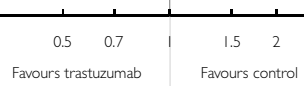
Outcome: 5 Progression-free survival - all studies

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
Huober 2012	-0.4 (0.33)		3.7 %	0.67 [0.35, 1.28]
Gasparini 2006	-0.36 (0.26)		5.9 %	0.70 [0.42, 1.16]
von Minckwitz 2009	-0.38 (0.18)		11.6 %	0.68 [0.48, 0.97]
Marty 2005	-0.652 (0.168)		13.1 %	0.52 [0.37, 0.72]
Kaufman 2009	-0.46 (0.15)		16.0 %	0.63 [0.47, 0.85]
Blackwell 2010	-0.3 (0.12)		23.2 %	0.74 [0.59, 0.94]
Slamon 2001	-0.67 (0.11)		26.5 %	0.51 [0.41, 0.63]
Total (95% CI)			**100.0 %**	**0.61 [0.54, 0.70]**

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 6.85$, df = 6 (P = 0.33); $I^2 = 12\%$

Test for overall effect: Z = 7.52 (P < 0.00001)

Test for subgroup differences: Not applicable



Analysis 1.6. Comparison 1 Efficacy of trastuzumab, Outcome 6 Progression-free survival - excluding Blackwell.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

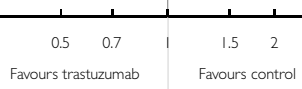
Outcome: 6 Progression-free survival - excluding Blackwell

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
Huober 2012	-0.4 (0.33)		4.2 %	0.67 [0.35, 1.28]
Gasparini 2006	-0.36 (0.26)		6.8 %	0.70 [0.42, 1.16]
von Minckwitz 2009	-0.38 (0.18)		14.2 %	0.68 [0.48, 0.97]
Marty 2005	-0.652 (0.168)		16.3 %	0.52 [0.37, 0.72]
Kaufman 2009	-0.46 (0.15)		20.4 %	0.63 [0.47, 0.85]
Slamon 2001	-0.67 (0.11)		38.0 %	0.51 [0.41, 0.63]
Total (95% CI)			**100.0 %**	**0.58 [0.50, 0.66]**

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 3.55$, df = 5 (P = 0.62); $I^2 = 0.0\%$

Test for overall effect: Z = 8.12 (P < 0.00001)

Test for subgroup differences: Not applicable



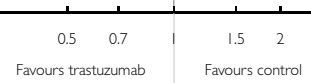
Analysis 1.7. Comparison 1 Efficacy of trastuzumab, Outcome 7 Progression-free survival - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 7 Progression-free survival - stratified by type of regimen

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
1 Taxane-containing regimen				
Gasparini 2006	-0.36 (0.26)		5.6 %	0.70 [0.42, 1.16]
Slamon 2001	-0.832 (0.252)		6.0 %	0.44 [0.27, 0.71]
Marty 2005	-0.652 (0.168)		11.7 %	0.52 [0.37, 0.72]
Subtotal (95% CI)			**23.3 %**	**0.53 [0.42, 0.68]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 1.74, df = 2 (P = 0.42); I^2 =0.0%				
Test for overall effect: Z = 5.11 (P < 0.00001)				
2 Anthracycline-containing regimen				
Slamon 2001	-0.246 (0.075)		30.1 %	0.78 [0.68, 0.91]
Subtotal (95% CI)			**30.1 %**	**0.78 [0.68, 0.91]**
Heterogeneity: not applicable				
Test for overall effect: Z = 3.28 (P = 0.0010)				
3 Aromatase inhibitor-containing regimen				
Huober 2012	-0.4 (0.33)		3.7 %	0.67 [0.35, 1.28]
Kaufman 2009	-0.46 (0.15)		13.8 %	0.63 [0.47, 0.85]
Subtotal (95% CI)			**17.5 %**	**0.64 [0.49, 0.83]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.03, df = 1 (P = 0.87); I^2 =0.0%				
Test for overall effect: Z = 3.29 (P = 0.00099)				
4 Other				
von Minckwitz 2009	-0.38 (0.18)		10.5 %	0.68 [0.48, 0.97]
Blackwell 2010	-0.3 (0.12)		18.7 %	0.74 [0.59, 0.94]
Subtotal (95% CI)			**29.1 %**	**0.72 [0.59, 0.88]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.14, df = 1 (P = 0.71); I^2 =0.0%				
Test for overall effect: Z = 3.25 (P = 0.0011)				
Total (95% CI)			**100.0 %**	**0.67 [0.59, 0.77]**
Heterogeneity: Tau^2 = 0.01; Chi^2 = 9.56, df = 7 (P = 0.22); I^2 =27%				
Test for overall effect: Z = 6.00 (P < 0.00001)				
Test for subgroup differences: Chi^2 = 7.66, df = 3 (P = 0.05), I^2 =61%				



Analysis 1.8. Comparison 1 Efficacy of trastuzumab, Outcome 8 Progression-free survival - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 8 Progression-free survival - stratified by treatment line

Study or subgroup	log [Hazard Ratio] (SE)	Hazard Ratio IV,Random,95% CI	Weight	Hazard Ratio IV,Random,95% CI
1 First-line				
Huober 2012	-0.4 (0.33)		3.7 %	0.67 [0.35, 1.28]
Gasparini 2006	-0.36 (0.26)		5.9 %	0.70 [0.42, 1.16]
Marty 2005	-0.652 (0.168)		13.1 %	0.52 [0.37, 0.72]
Kaufman 2009	-0.46 (0.15)		16.0 %	0.63 [0.47, 0.85]
Slamon 2001	-0.67 (0.11)		26.5 %	0.51 [0.41, 0.63]
Subtotal (95% CI)			**65.2 %**	**0.56 [0.49, 0.65]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 2.51, df = 4 (P = 0.64); I^2 =0.0%				
Test for overall effect: Z = 7.90 (P < 0.00001)				
2 Beyond progression				
von Minckwitz 2009	-0.38 (0.18)		11.6 %	0.68 [0.48, 0.97]
Blackwell 2010 (1)	-0.3 (0.12)		23.2 %	0.74 [0.59, 0.94]
Subtotal (95% CI)			**34.8 %**	**0.72 [0.59, 0.88]**
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.14, df = 1 (P = 0.71); I^2 =0.0%				
Test for overall effect: Z = 3.25 (P = 0.0011)				
Total (95% CI)			**100.0 %**	**0.61 [0.54, 0.70]**
Heterogeneity: Tau^2 = 0.00; Chi^2 = 6.85, df = 6 (P = 0.33); I^2 =12%				
Test for overall effect: Z = 7.52 (P < 0.00001)				
Test for subgroup differences: Chi^2 = 4.21, df = 1 (P = 0.04), I^2 =76%				

(1) Lapatinib-containing regimen

Analysis 1.9. Comparison 1 Efficacy of trastuzumab, Outcome 9 Overall response rate - all studies.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 9 Overall response rate - all studies

Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,95% CI	Weight	Risk Ratio IV,Random,95% CI
Huober 2012	7/26	4/31		1.6 %	2.09 [0.69, 6.35]
Kaufman 2009	15/74	5/73		2.1 %	2.96 [1.13, 7.72]
Blackwell 2010	15/146	10/145		3.3 %	1.49 [0.69, 3.21]
von Minckwitz 2009	37/77	20/74		10.0 %	1.78 [1.14, 2.76]
Marty 2005	56/92	32/94		18.3 %	1.79 [1.29, 2.48]
Gasparini 2006	45/60	33/58		27.1 %	1.32 [1.01, 1.72]
Slamon 2001	118/235	74/234		37.6 %	1.59 [1.26, 1.99]
Total (95% CI)	**710**	**709**		**100.0 %**	**1.58 [1.38, 1.82]**

Total events: 293 (Trastuzumab), 178 (Control)

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 4.50$, df = 6 (P = 0.61); $I^2 = 0.0\%$

Test for overall effect: Z = 6.47 (P < 0.00001)

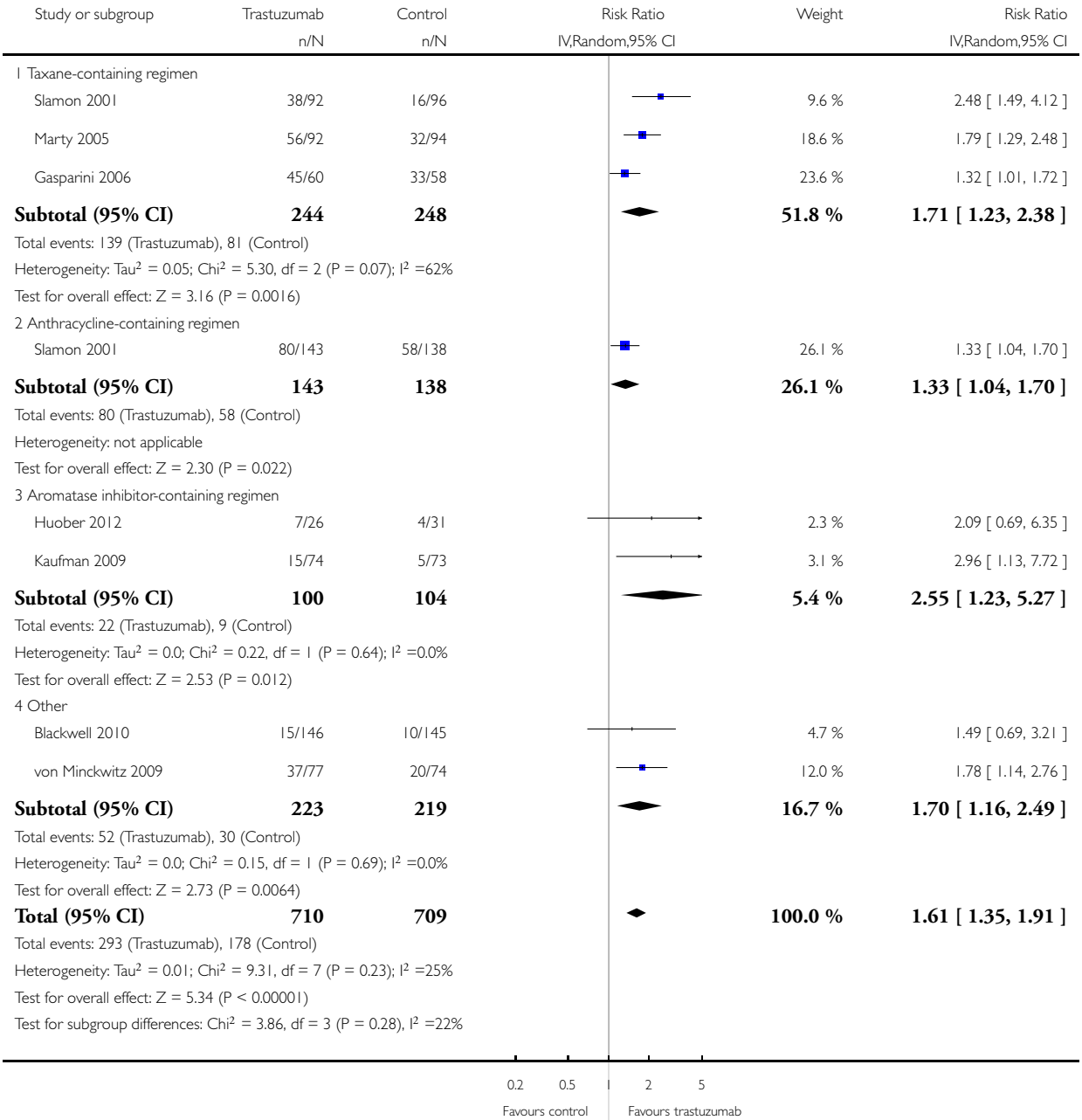
Test for subgroup differences: Not applicable

Analysis 1.10. Comparison 1 Efficacy of trastuzumab, Outcome 10 Overall response rate - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 10 Overall response rate - stratified by type of regimen



Analysis 1.11. Comparison 1 Efficacy of trastuzumab, Outcome 11 Overall response rate - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 1 Efficacy of trastuzumab

Outcome: 11 Overall response rate - stratified by treatment line

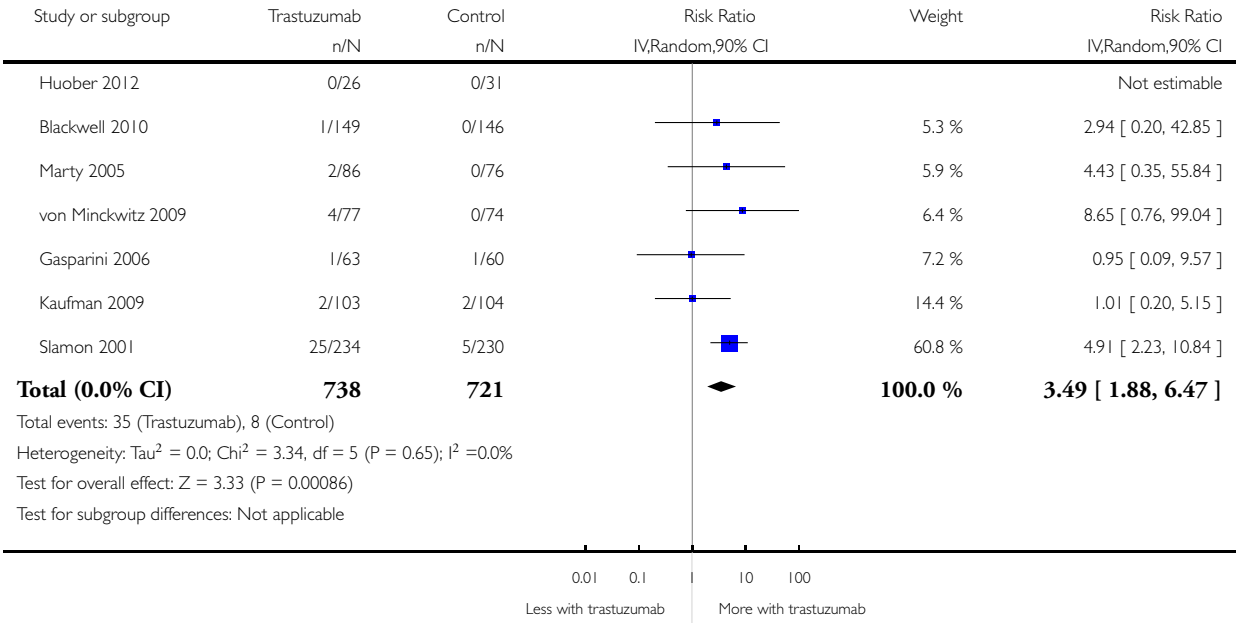
Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,95% CI	Weight	Risk Ratio IV,Random,95% CI
1 First-line					
Huober 2012	7/26	4/31		1.6 %	2.09 [0.69, 6.35]
Kaufman 2009	15/74	5/73		2.1 %	2.96 [1.13, 7.72]
Marty 2005	56/92	32/94		18.3 %	1.79 [1.29, 2.48]
Gasparini 2006	45/60	33/58		27.1 %	1.32 [1.01, 1.72]
Slamon 2001	118/235	74/234		37.6 %	1.59 [1.26, 1.99]
Subtotal (95% CI)	**487**	**490**		**86.7 %**	**1.57 [1.34, 1.84]**
Total events: 241 (Trastuzumab), 148 (Control)					
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 4.19$, df = 4 (P = 0.38); $I^2 = 5\%$					
Test for overall effect: Z = 5.65 (P < 0.00001)					
2 Beyond progression					
Blackwell 2010	15/146	10/145		3.3 %	1.49 [0.69, 3.21]
von Minckwitz 2009	37/77	20/74		10.0 %	1.78 [1.14, 2.76]
Subtotal (95% CI)	**223**	**219**		**13.3 %**	**1.70 [1.16, 2.49]**
Total events: 52 (Trastuzumab), 30 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.15$, df = 1 (P = 0.69); $I^2 = 0.0\%$					
Test for overall effect: Z = 2.73 (P = 0.0064)					
Total (95% CI)	**710**	**709**		**100.0 %**	**1.58 [1.38, 1.82]**
Total events: 293 (Trastuzumab), 178 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 4.50$, df = 6 (P = 0.61); $I^2 = 0.0\%$					
Test for overall effect: Z = 6.47 (P < 0.00001)					
Test for subgroup differences: $Chi^2 = 0.14$, df = 1 (P = 0.70), $I^2 = 0.0\%$					

Analysis 2.1. Comparison 2 Cardiac toxicity of trastuzumab, Outcome 1 Congestive heart failure - all studies.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 2 Cardiac toxicity of trastuzumab

Outcome: 1 Congestive heart failure - all studies

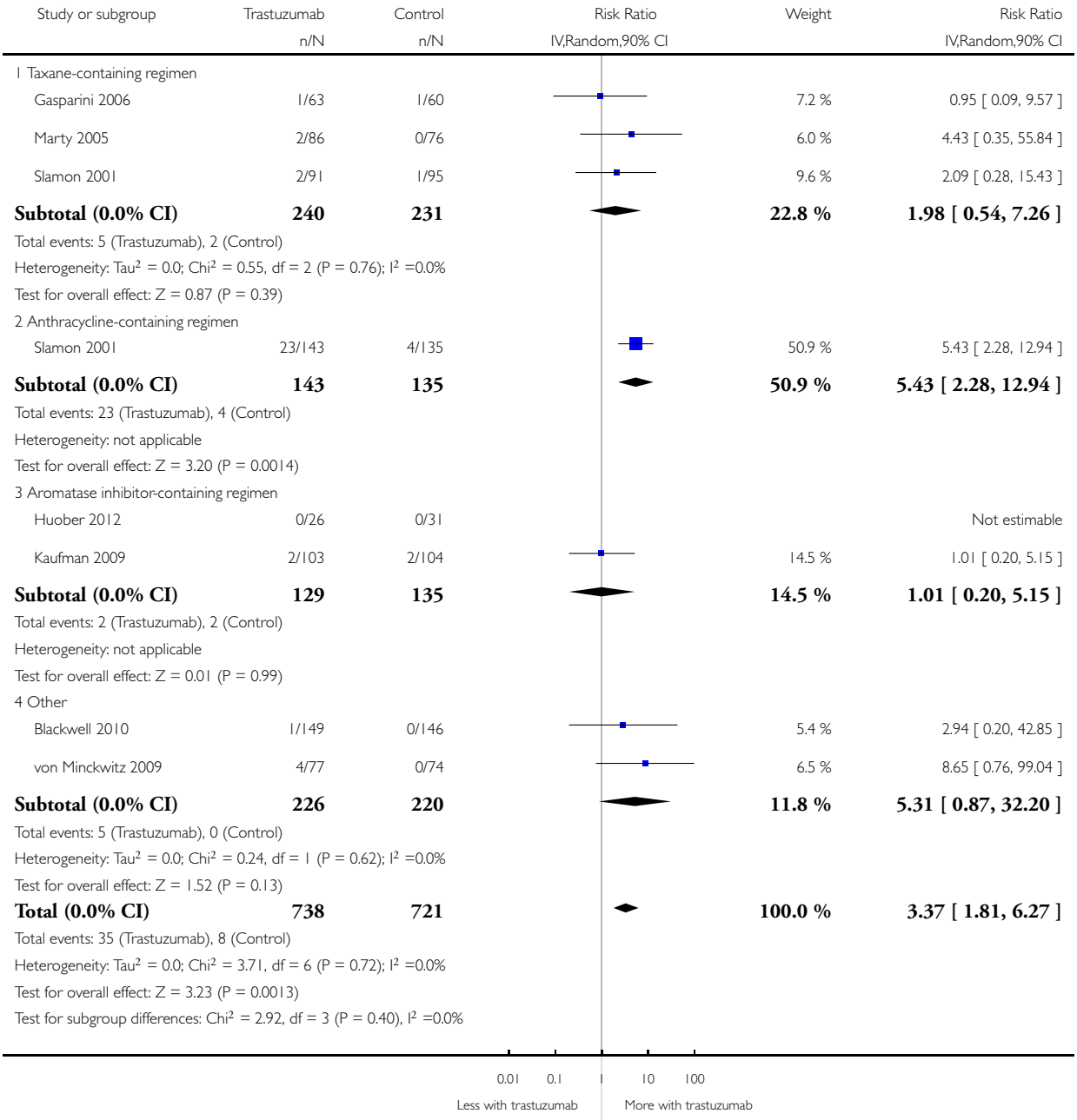


Analysis 2.2. Comparison 2 Cardiac toxicity of trastuzumab, Outcome 2 Congestive heart failure - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 2 Cardiac toxicity of trastuzumab

Outcome: 2 Congestive heart failure - stratified by type of regimen



Analysis 2.3. Comparison 2 Cardiac toxicity of trastuzumab, Outcome 3 Congestive heart failure - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 2 Cardiac toxicity of trastuzumab

Outcome: 3 Congestive heart failure - stratified by treatment line

Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
1 First-line					
Huober 2012	0/26	0/31			Not estimable
Marty 2005	2/86	0/76		5.9 %	4.43 [0.35, 55.84]
Gasparini 2006	1/63	1/60		7.2 %	0.95 [0.09, 9.57]
Kaufman 2009	2/103	2/104		14.4 %	1.01 [0.20, 5.15]
Slamon 2001	25/234	5/230		60.8 %	4.91 [2.23, 10.84]
Subtotal (0.0% CI)	**512**	**501**		**88.3 %**	**3.30 [1.71, 6.37]**
Total events: 30 (Trastuzumab), 8 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 2.94$, df = 3 (P = 0.40); $I^2 = 0.0\%$					
Test for overall effect: Z = 2.99 (P = 0.0028)					
2 Beyond progression					
Blackwell 2010	1/149	0/146		5.3 %	2.94 [0.20, 42.85]
von Minckwitz 2009	4/77	0/74		6.4 %	8.65 [0.76, 99.04]
Subtotal (0.0% CI)	**226**	**220**		**11.7 %**	**5.31 [0.87, 32.20]**
Total events: 5 (Trastuzumab), 0 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.24$, df = 1 (P = 0.62); $I^2 = 0.0\%$					
Test for overall effect: Z = 1.52 (P = 0.13)					
Total (0.0% CI)	**738**	**721**		**100.0 %**	**3.49 [1.88, 6.47]**
Total events: 35 (Trastuzumab), 8 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 3.34$, df = 5 (P = 0.65); $I^2 = 0.0\%$					
Test for overall effect: Z = 3.33 (P = 0.00086)					
Test for subgroup differences: $Chi^2 = 0.17$, df = 1 (P = 0.68), $I^2 = 0.0\%$					

Analysis 2.4. Comparison 2 Cardiac toxicity of trastuzumab, Outcome 4 Left ventricular ejection fraction (LVEF) decline - all studies.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 2 Cardiac toxicity of trastuzumab

Outcome: 4 Left ventricular ejection fraction (LVEF) decline - all studies

Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
Gasparini 2006	0/63	0/60			Not estimable
Kaufman 2009	1/103	0/104		4.7 %	3.03 [0.21, 44.02]
von Minckwitz 2009	1/77	0/74		4.7 %	2.88 [0.20, 41.77]
Blackwell 2010	10/149	3/146		29.7 %	3.27 [1.13, 9.48]
Marty 2005	16/86	6/76		60.9 %	2.36 [1.12, 4.96]
Total (0.0% CI)	**478**	**460**		**100.0 %**	**2.65 [1.48, 4.74]**

Total events: 28 (Trastuzumab), 9 (Control)

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.18$, df = 3 (P = 0.98); $I^2 = 0.0\%$

Test for overall effect: Z = 2.76 (P = 0.0057)

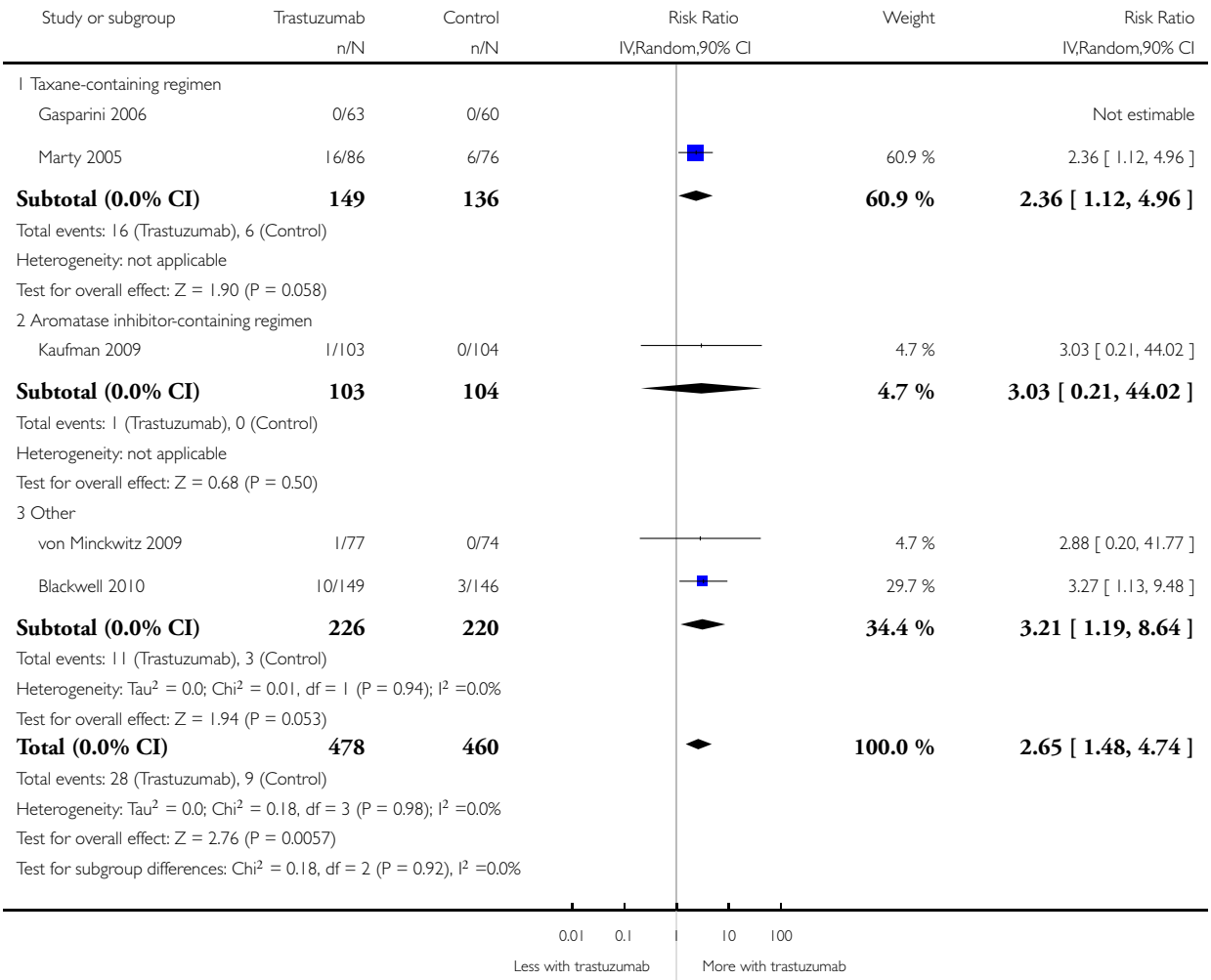
Test for subgroup differences: Not applicable

Analysis 2.5. Comparison 2 Cardiac toxicity of trastuzumab, Outcome 5 LVEF decline - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 2 Cardiac toxicity of trastuzumab

Outcome: 5 LVEF decline - stratified by type of regimen

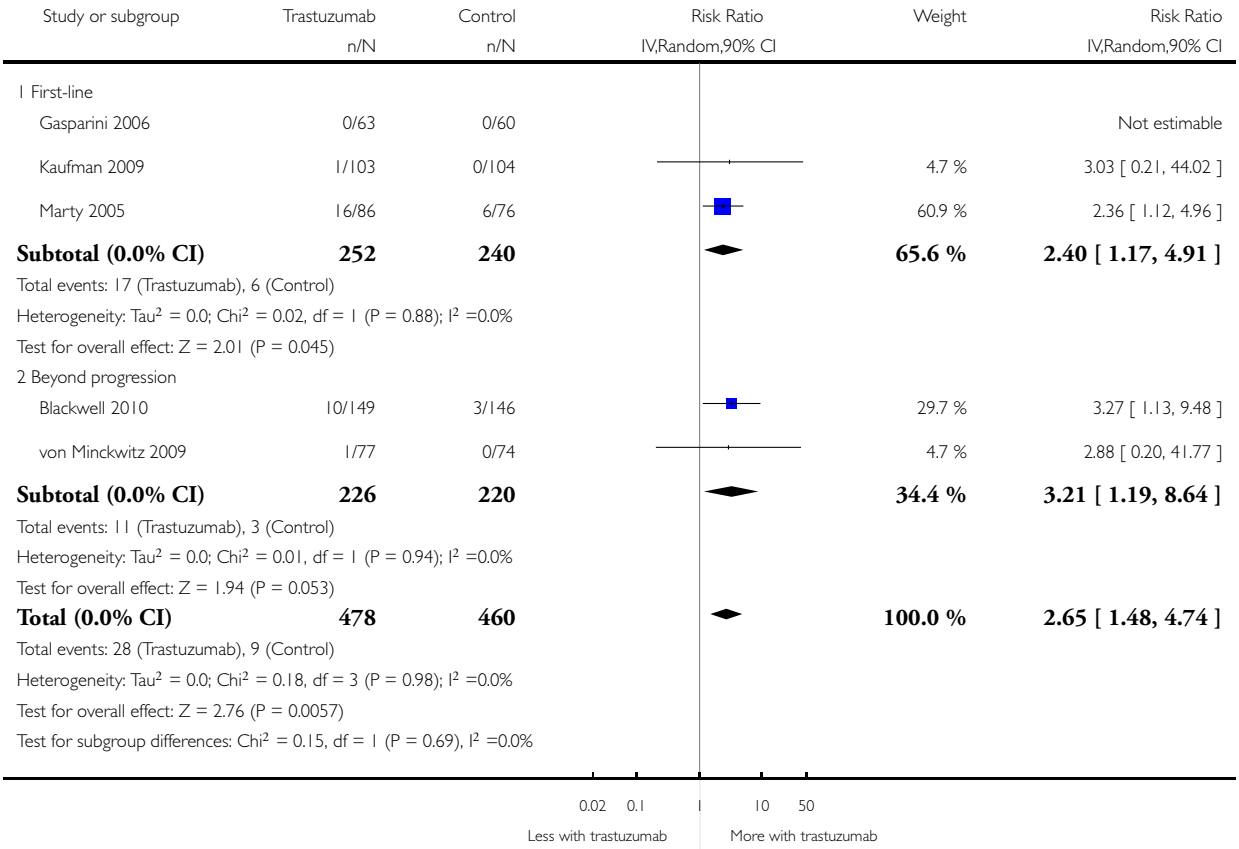


Analysis 2.6. Comparison 2 Cardiac toxicity of trastuzumab, Outcome 6 LVEF decline - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 2 Cardiac toxicity of trastuzumab

Outcome: 6 LVEF decline - stratified by treatment line



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

Review: Trastuzumab-containing regimens for metastatic 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
von Minckwitz 2009	2/77	0/74		3.4 %	4.81 [0.38, 60.61]
Gasparini 2006	1/63	1/60		4.2 %	0.95 [0.09, 9.57]
Marty 2005	21/92	16/94		92.4 %	1.34 [0.82, 2.19]
Total (0.0% CI)	**232**	**228**		**100.0 %**	**1.38 [0.86, 2.21]**

Total events: 24 (Trastuzumab), 17 (Control)

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.74$, df = 2 (P = 0.69); $I^2 = 0.0\%$

Test for overall effect: Z = 1.13 (P = 0.26)

Test for subgroup differences: Not applicable

Analysis 3.2. Comparison 3 Other toxicities, Outcome 2 Neutropenic fever - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 2 Neutropenic fever - stratified by type of regimen

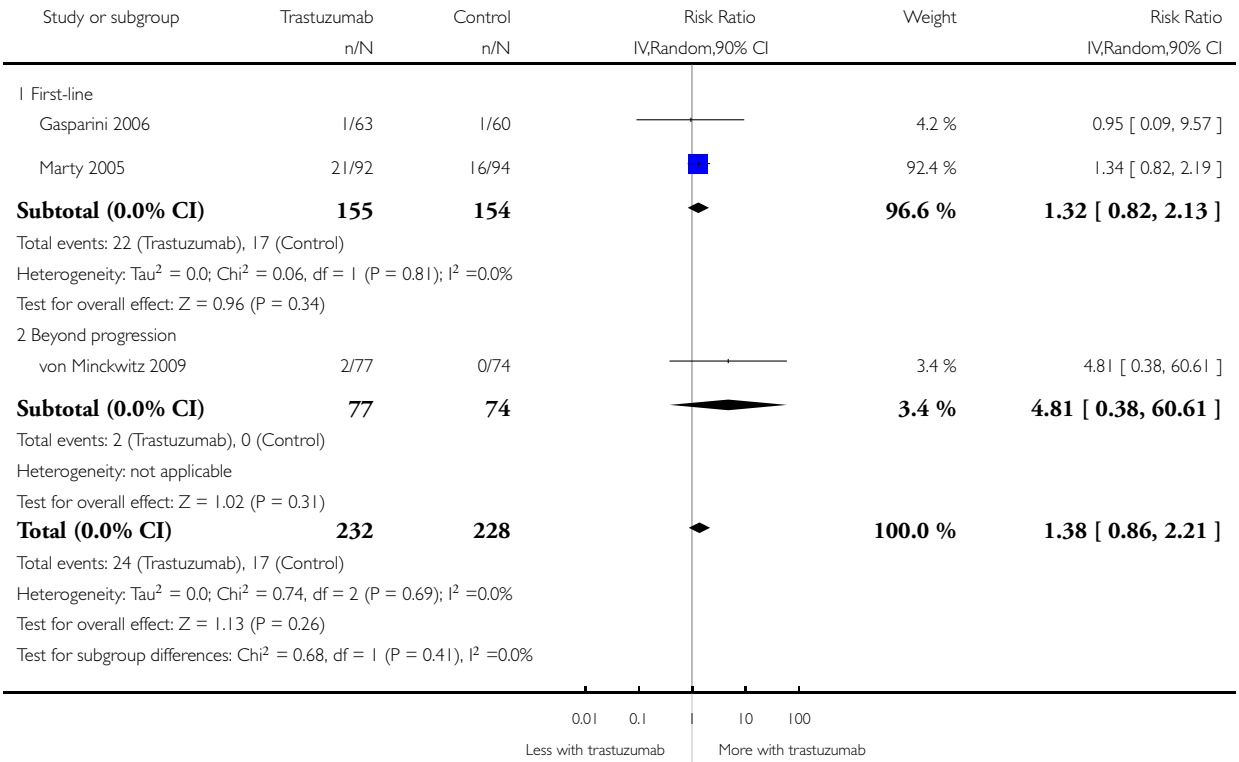
Study or subgroup	Trastuzumab n/N	Control n/N	Risk Ratio IV,Random,90% CI	Weight	Risk Ratio IV,Random,90% CI
1 Taxane-containing regimen					
Gasparini 2006	1/63	1/60		4.2 %	0.95 [0.09, 9.57]
Marty 2005	21/92	16/94		92.4 %	1.34 [0.82, 2.19]
Subtotal (0.0% CI)	**155**	**154**		**96.6 %**	**1.32 [0.82, 2.13]**
Total events: 22 (Trastuzumab), 17 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.06, df = 1 (P = 0.81); I^2 =0.0%					
Test for overall effect: Z = 0.96 (P = 0.34)					
2 Other					
von Minckwitz 2009	2/77	0/74		3.4 %	4.81 [0.38, 60.61]
Subtotal (0.0% CI)	**77**	**74**		**3.4 %**	**4.81 [0.38, 60.61]**
Total events: 2 (Trastuzumab), 0 (Control)					
Heterogeneity: not applicable					
Test for overall effect: Z = 1.02 (P = 0.31)					
Total (0.0% CI)	**232**	**228**		**100.0 %**	**1.38 [0.86, 2.21]**
Total events: 24 (Trastuzumab), 17 (Control)					
Heterogeneity: Tau^2 = 0.0; Chi^2 = 0.74, df = 2 (P = 0.69); I^2 =0.0%					
Test for overall effect: Z = 1.13 (P = 0.26)					
Test for subgroup differences: Chi^2 = 0.68, df = 1 (P = 0.41), I^2 =0.0%					

Analysis 3.3. Comparison 3 Other toxicities, Outcome 3 Neutropenic fever - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 3 Neutropenic fever - stratified by treatment line

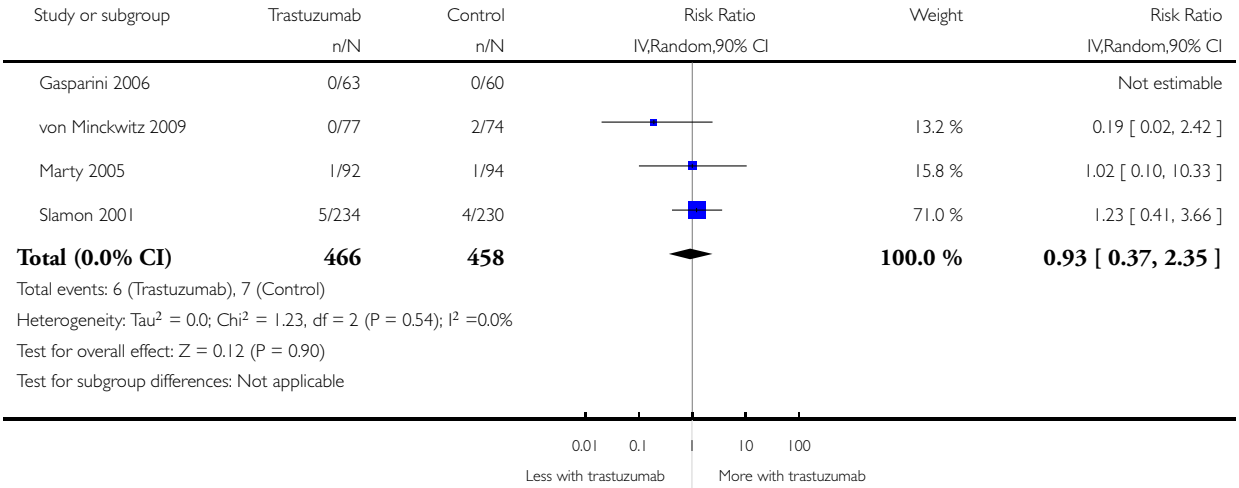


Analysis 3.4. Comparison 3 Other toxicities, Outcome 4 Anaemia - all studies.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 4 Anaemia - all studies

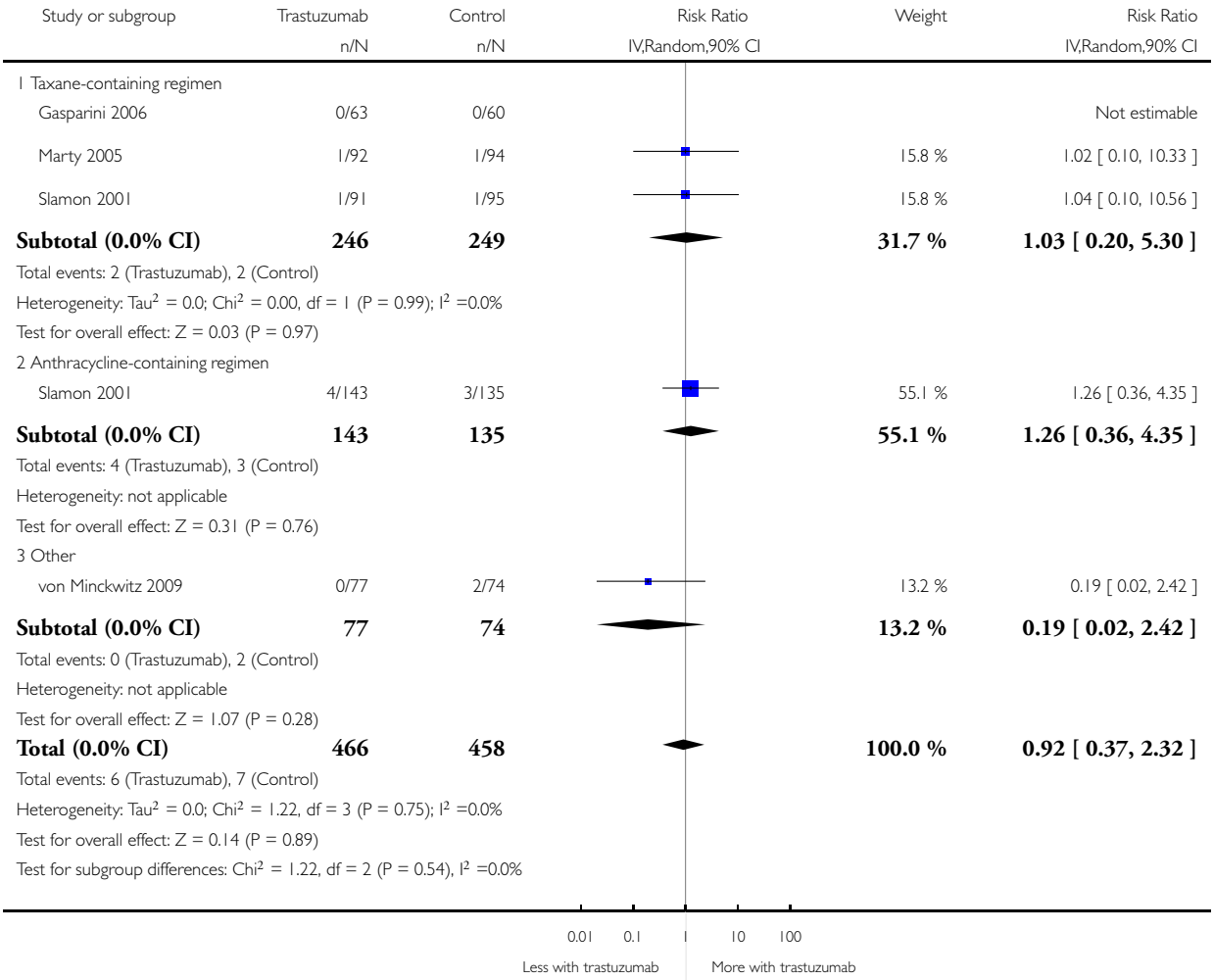


Analysis 3.5. Comparison 3 Other toxicities, Outcome 5 Anaemia - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 5 Anaemia - stratified by type of regimen

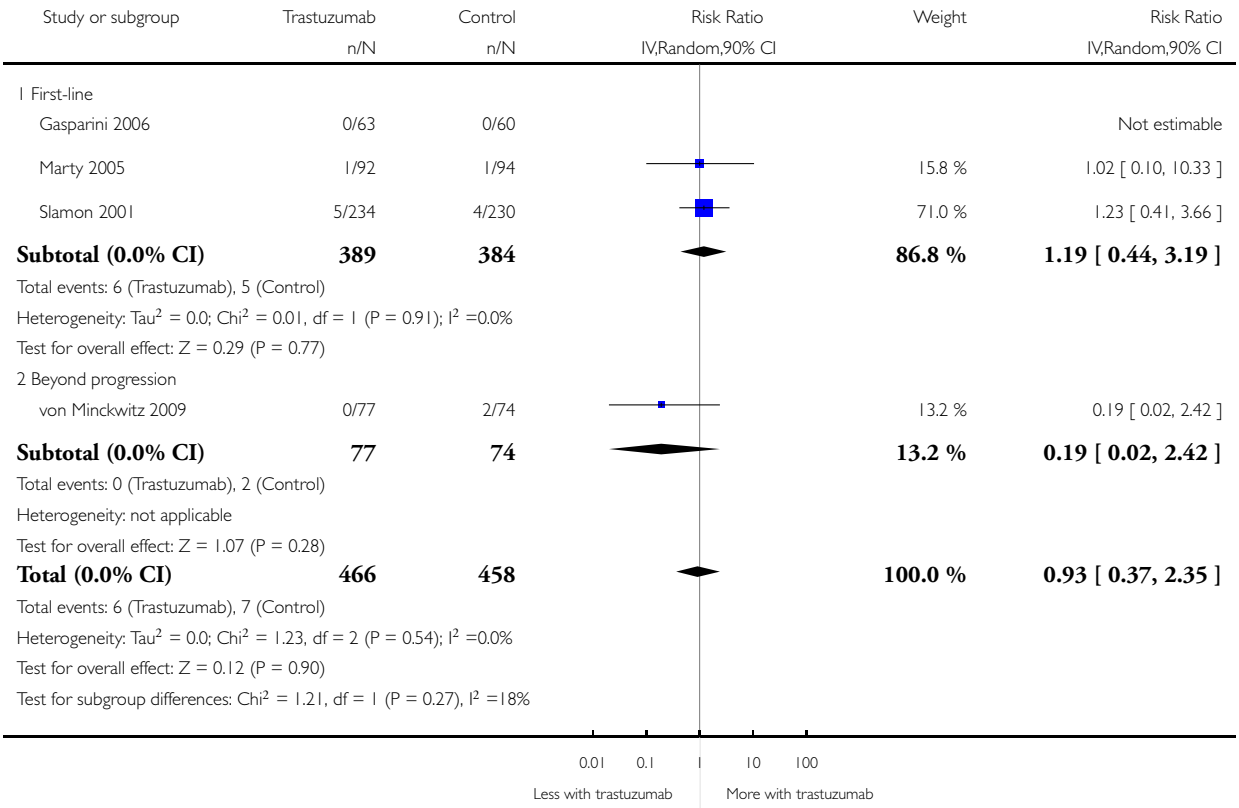


Analysis 3.6. Comparison 3 Other toxicities, Outcome 6 Anaemia - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 6 Anaemia - stratified by treatment line



Analysis 3.7. Comparison 3 Other toxicities, Outcome 7 Neutropenia - all studies.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 7 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
von Minckwitz 2009	4/77	3/74		8.5 %	1.28 [0.38, 4.37]
Gasparini 2006	8/63	4/60		13.8 %	1.90 [0.73, 4.99]
Marty 2005	29/92	21/94		77.8 %	1.41 [0.94, 2.12]
Total (0.0% CI)	**232**	**228**		**100.0 %**	**1.46 [1.02, 2.08]**

Total events: 41 (Trastuzumab), 28 (Control)

Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.26$, df = 2 (P = 0.88); $I^2 = 0.0\%$

Test for overall effect: Z = 1.74 (P = 0.082)

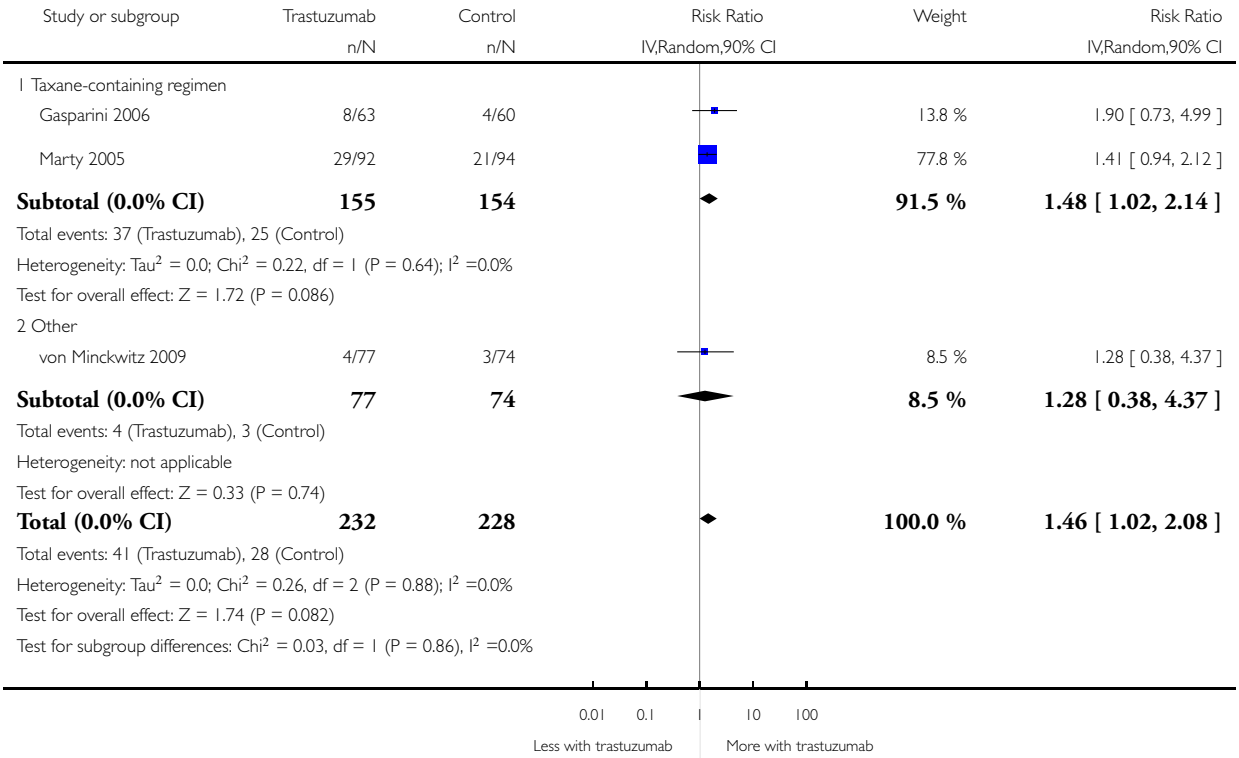
Test for subgroup differences: Not applicable

Analysis 3.8. Comparison 3 Other toxicities, Outcome 8 Neutropenia - stratified by type of regimen.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 8 Neutropenia - stratified by type of regimen

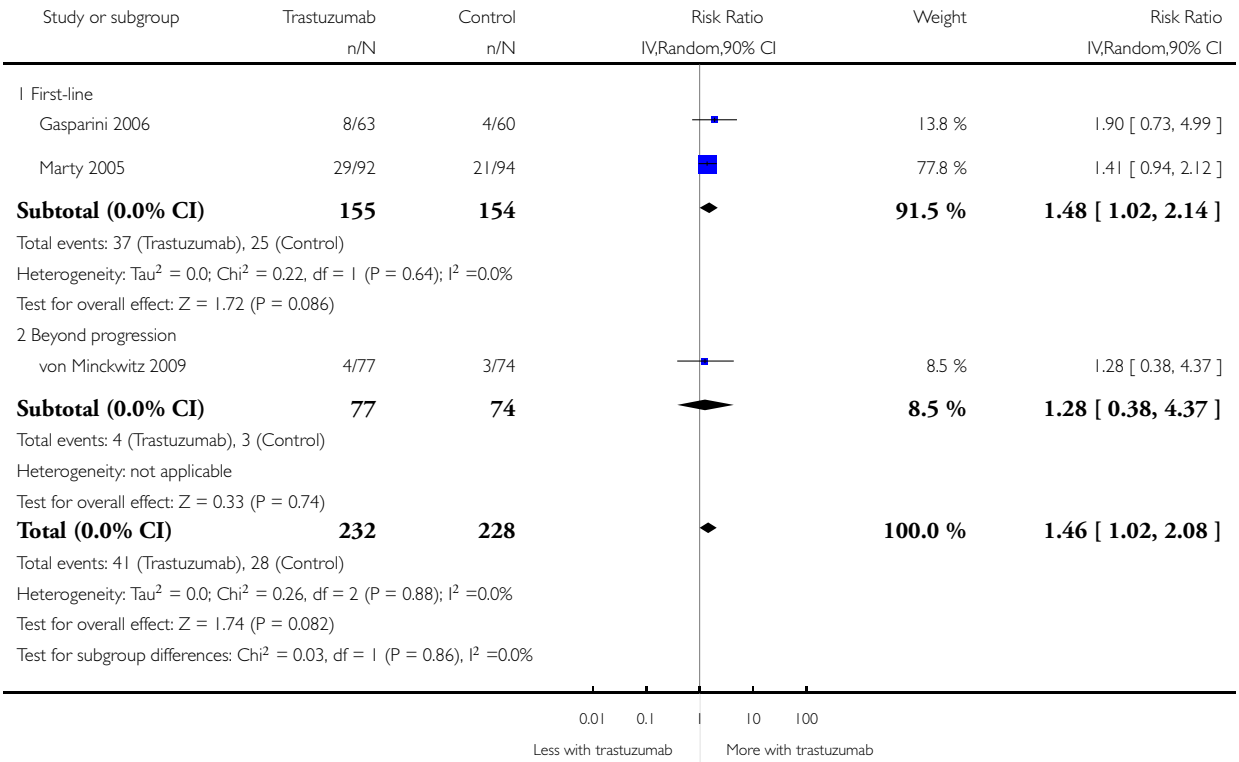


Analysis 3.9. Comparison 3 Other toxicities, Outcome 9 Neutropenia - stratified by treatment line.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 3 Other toxicities

Outcome: 9 Neutropenia - stratified by treatment line

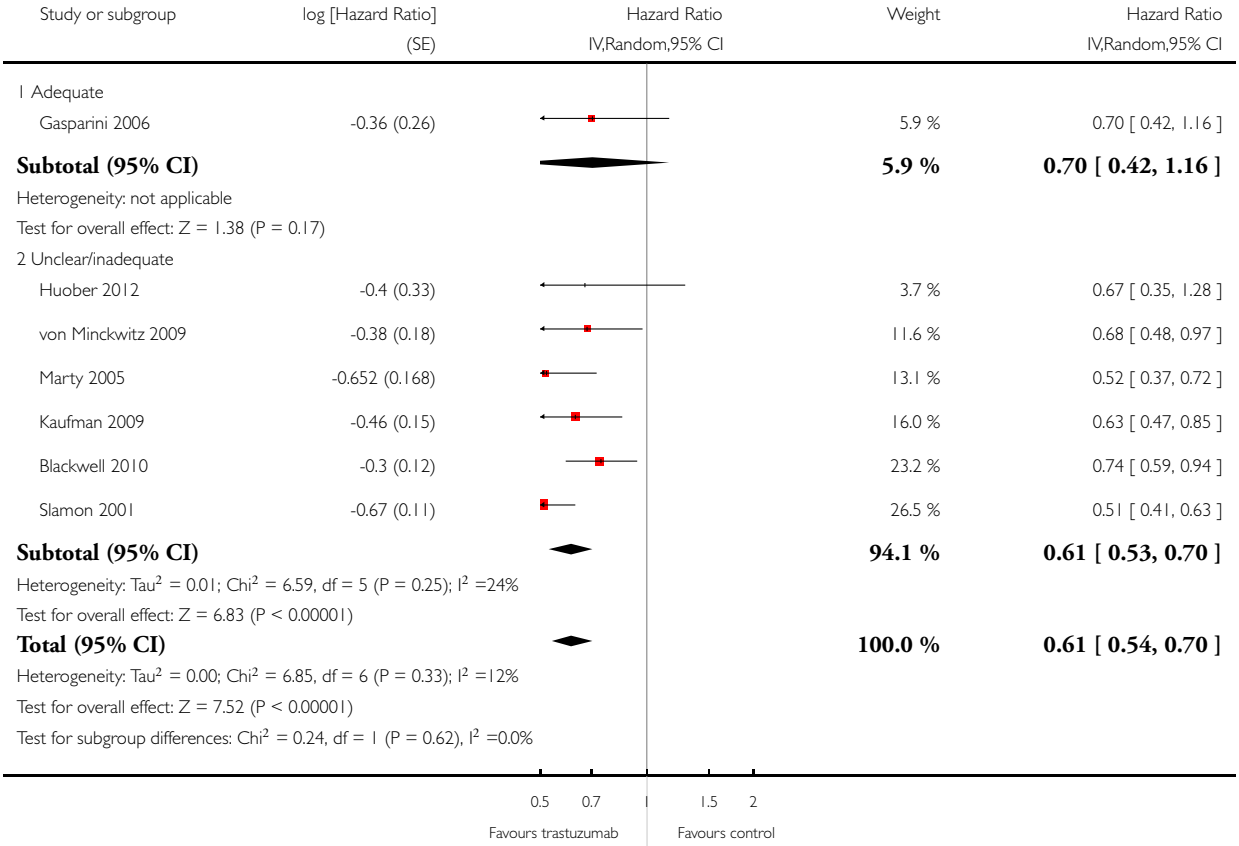


Analysis 4.1. Comparison 4 Sensitivity analysis: progression-free survival - by allocation concealment, Outcome 1 Progression-free survival - by allocation concealment.

Review: Trastuzumab-containing regimens for metastatic breast cancer

Comparison: 4 Sensitivity analysis: progression-free survival - by allocation concealment

Outcome: 1 Progression-free survival - by allocation concealment



APPENDICES

Appendix 1. Cochrane Central Register of Controlled Trials (CENTRAL)

#1 MeSH descriptor: [Breast Neoplasms] explode all trees
#2 breast and (cancer* or tumour* or tumor* or neoplas*)
#3 #1 or #2
#4 (adverse or toxic or side) and (effect* or event* or drug reaction*)
#5 MeSH descriptor: [Drug Toxicity] explode all trees
#6 #4 or #5
#7 trastuzumab or herceptin
#8 #3 and #6 and #7

Appendix 2. MEDLINE search strategy (1966 to current) (OVID)

1	exp Breast Neoplasms/
2	breast.ab,ti,tw.
3	mammary.ab,ti,tw.
4	2 or 3
5	cancer*.ab,ti,tw.
6	carcinoma*.ab,ti,tw.
7	neoplas*.ab,ti,tw.
8	tumo?r*.ab,ti,tw.
9	metastas*.ab,ti,tw.
10	5 or 6 or 7 or 8 or 9
11	4 and 10
12	metastatic breast cancer.ab,ti,tw,kw.
13	advance* breast cancer.ab,ti,tw,kw.
14	1 or 11 or 12 or 13
15	trastuzumab.mp.
16	herceptin.mp.
17	15 or 16

(*Continued*)

18	exp Antineoplastic Agents/ae, ct, de, to [Adverse Effects, Contraindications, Drug Effects, Toxicity]
19	exp Drug Hypersensitivity/
20	exp Drug Toxicity/
21	exp Drug Tolerance/
22	exp Causality/
23	exp Risk/
24	exp Product Surveillance, Postmarketing/
25	18 or 19 or 20 or 21 or 22 or 23 or 24
26	safe*.ab,ti.
27	adr.ab,ti.
28	adrs.ab,ti.
29	tolerabilit*.ab,ti.
30	toxicit*.ab,ti.
31	adverse reaction*.ab,ti.
32	hypersensitivit*.ab,ti.
33	adverse effect*.ab,ti.
34	undesirable effect*.ab,ti.
35	toxic effect*.ab,ti.
36	complication*.ab,ti.
37	causalit*.ab,ti.
38	risk*.ab,ti.
39	postmarketing.ab,ti.
40	post marketing.ab,ti.
41	side effect*.ab,ti.

(*Continued*)

42	side event*.ab,ti.
43	side outcome*.ab,ti.
44	adverse event*.ab,ti.
45	adverse outcome*.ab,ti.
46	26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45
47	25 or 46
48	14 and 17 and 47
49	limit 48 to (english language and humans and yr="1996 -Current")

Appendix 3. EMBASE (January 1996 to current) (host: Embase.com)

#23 #22 AND [humans]/lim AND [english]/lim AND [embase]/lim AND [1996-2013]/py
#22 #9 AND #20 AND #21
#21 #3 OR #6
#20 #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #19
#19 #17 AND #18
#18 effect*:ab,ti OR event*:ab,ti OR outcome*:ab,ti
#17 side:ab,ti OR adverse:ab,ti
#16 safe*:ab,ti OR adr:ab,ti OR adrs:ab,ti OR tolerabilit*: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
#15 'postmarketing surveillance'/exp
#14 'risk'/exp
#13 'drug tolerance'/exp
#12 'drug toxicity'/exp
#11 'drug hypersensitivity'/exp
#10 'antineoplastic agent'/exp/dd˙ae,dd˙to
#9 #7 OR #8
#8 trastuzumab:ab,ti OR herceptin:ab,ti
#7 'trastuzumab'/exp
#6 #4 AND #5
#5 cancer*:ab,ti OR tumour*:ab,ti OR tumor*:ab,ti OR neoplasm*:ab,ti OR metastas*:ab,ti OR carcinoma*:ab,ti
#4 breast:ab,ti OR mammary:ab,ti OR mammaries:ab,ti
#3 #1 OR #2
#2 'breast tumor'/exp
#1 'breast cancer'/exp

Appendix 4. WHO ICTRP search strategy

Basic search:
1. Trastuzumab containing regimens for metastatic breast cancer
2. Metastatic breast cancer AND trastuzumab
3. Advanced breast cancer AND trastuzumab
4. Metastatic breast cancer AND herceptin
5. Advanced breast cancer AND herceptin

Advanced search:
1. Title: Trastuzumab containing regimens for metastatic breast cancer
Recruitment Status: All
2. Condition: metastatic breast cancer
Intervention: trastuzumab OR herceptin OR trastuzumab containing regime*
Recruitment Status: All
3. Condition: advanced breast cancer
Intervention: trastuzumab OR herceptin OR trastuzumab containing regime*
Recruitment Status: All

Appendix 5. Clinicaltrials.gov

Basic search:
1. Trastuzumab containing regimens for metastatic breast cancer[ft1]
2. (metastatic breast cancer OR advanced breast cancer) AND (trastuzumab OR herceptin)[ft2]

Advanced search:
1. Title: Trastuzumab containing regimens for metastatic breast cancer
Recruitment: All studies
Study Results: All studies
Study Type: All studies
Gender: All studies
2. Condition: (metastatic breast cancer AND advanced breast cancer)
Intervention: trastuzumab OR herceptin OR trastuzumab containing regime*
Recruitment: All studies
Study Results: All studies
Study Type: All studies
Gender: All studies

Appendix 6. BIOSIS (January 1996 to current) (host: ISI Web of Knowledge)

# 7	#6 AND #5 *Databases=BIOSIS Previews Timespan=1996-2013*
# 6	#4 AND #3 *Databases=BIOSIS Previews Timespan=1996-2013*
# 5	#2 AND #1 *Databases=BIOSIS Previews Timespan=1996-2013*

(*Continued*)

# 4	(TS=(advanced breast cancer*) OR TS=(advanced breast neoplasm*) OR TS=(advanced breast tumor*) OR TS=(advanced breast tumour*)) AND Language=(English) AND Taxa Notes=(Humans) *Databases=BIOSIS Previews Timespan=1996-2013*
# 3	(TS=(metastatic breast cancer*) OR TS=(metastatic breast neoplasm*) OR TS=(metastatic breast tumor*) OR TS=(metastatic breast tumour*)) AND Language=(English) AND Taxa Notes=(Humans) *Databases=BIOSIS Previews Timespan=1996-2013*
# 2	(CC=(trastuzumab OR Toxicology - Pharmacology) OR CC=(herceptin OR Toxicology - Pharmacology)) AND Language=(English) AND Taxa Notes=(Humans) *Databases=BIOSIS Previews Timespan=1996-2013*
# 1	(MC=(trastuzumab OR Oncology) OR MC=(herceptin OR Oncology)) AND Language=(English) AND Taxa Notes=(Humans) *Databases=BIOSIS Previews Timespan=1996-2013*

CONTRIBUTIONS OF AUTHORS

Study concept: RD, LM.

Study protocol: RD, LM.

Search strategy: LM, VP.

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

Acquisition of data: SB, SM, LT.

'Risk of bias' assessment: SB, SM, LM, LT.

Analysis of data: SB, RD, LM.

Drafting of the manuscript: SB, RD, LM.

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

DECLARATIONS OF INTEREST

SB: nothing to declare.

SM: nothing to declare.

VG: she acted as speaker for GlaxoSmithkline, and as consultant for Novartis and AstraZeneca. VG institution received a grant from Roche to support a trial in which she is Principal Investigator.

LT: nothing to declare.

VP: nothing to declare.

LM: nothing to declare.

RD: nothing to declare.

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

External sources

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

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Protocol

Intervention group: trastuzumab alone or in combination with chemotherapy.
Comparator: the same chemotherapy alone or no trastuzumab.

Review

Intervention group: trastuzumab alone or in combination with chemotherapy, hormonal therapy or targeted agents.
Comparator: the same regimen used in the intervention group without trastuzumab.