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REVIEW

HER2 discordance between primary breast cancer and its paired metastasis: tumor biology or test artefact? Insights through meta-analysis

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Abstract The proto-oncogene, *HER2*, has prognostic and predictive relevance in invasive breast cancer (IBC). HER2 testing of primary IBC guides treatment selection and is assumed to reflect HER2 status of associated metastases, although HER2 discordance between IBC and metastasis has been reported. Systematic review and meta-analysis of HER2 status in IBC and its *paired* loco-regional or distant metastasis were done. Quality appraisal considered whether (within-subject) testing conditions were maintained for paired primary and metastasis. Random effects logistic regression models were used to estimate pooled within-subject HER2 discordant proportions and to examine study-level covariates, including tumor-related and testing-related variables, potentially associated with HER2 discordance differences *across* (between) studies. Modelled paired HER2 data for primary and metastatic cancer (2520 subjects, 26 studies) showed a pooled HER2 discordance of 5.5% (3.6–8.5%). Sensitivity analysis, excluding the only study not maintaining same conditions for paired testing, gave a pooled estimate of 5.2% (3.5–7.8%). Pooled discordant proportion was *not* associated with differences between studies in test type, test scoring or interpretation criteria, subjects' median age, study time-frame, or HER2 positivity in primary cancer (all $P > 0.05$). However, type of metastasis was significantly associated with estimated HER2 discordance ($P = 0.0017$): studies of primary tumor paired with *distant metastases* had higher discordance [11.5% (6.9–18.6%)] than studies of primary paired with lymph node metastases only [4.1% (2.4–7.2%)], *or* those paired with nodal or various metastases [3.3% (2.0–5.6%)]; $P < 0.01$. HER2 discordant proportion was higher where paired metastases were metachronous relative to synchronous to primary IBC ($P = 0.0024$). Sensitivity analysis provided weak evidence ($P = 0.074$) that discordance in the direction of change from HER2-negative primary cancer to HER2-positive paired metastasis was more likely than the reverse. Study-level meta-analysis suggests factors associated with the type of metastasis as underlying mechanisms for observed HER2 discordance between primary IBC and paired metastasis. Test-related factors did not account for differences across studies in the HER2 discordant proportion.

Keywords Breast cancer · Human epidermal growth factor receptor 2/HER2 · Metastasis · Meta-analysis · Test discordance

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Introduction

Gene amplification of the human epidermal growth factor receptor 2 (erbB2, or HER2/neu, or *HER2*) has been

intensively evaluated in contemporary oncology, because it has potential therapeutic implications for several human cancers, notably breast and gastric cancer [1–3]. *HER2* is amplified in approximately 20% of invasive breast cancers (IBC) [1]; an overview has reported HER2-positivity in breast cancer of 22.2% (range 9–74%) [4]. HER2, a proto-oncogene, has prognostic and predictive relevance in breast cancer in the adjuvant, neoadjuvant, and the metastatic setting [2, 5–11]. HER2 status refers to the extent of HER2 protein over-expression on immunohistochemistry (IHC), or *HER2* gene amplification determined by in situ hybridization (ISH) techniques. Guidelines recommend evaluation of HER2 status in every IBC [1, 12–14] primarily to guide treatment with molecularly targeted agents directed against the HER2 receptor. In practice, HER2 status of a primary breast cancer guides not only initial therapy but is often the basis for selection of patients with metastatic disease who may benefit from anti-HER2-directed therapies. This treatment approach makes the implicit assumption that HER2 status of the primary breast cancer reflects that of its corresponding metastasis.

A number of studies have examined HER2 status in primary breast cancer and associated metastases and have reported HER2 discordance in some cases, with variability in the reported discordance proportion, from none to 30% or higher [15–17]. Arguments for or against HER2 testing of metastases have therefore been based on divergent evidence and with little certainty on whether the reported HER2 discordance represents tumor-related behaviour or is largely a reflection of testing variability or measurement error [18, 19]. To date, there is little consensus on whether HER2 status discordance, *if any*, between primary breast cancer and its metastasis is a consistent finding or is of a magnitude warranting re-testing of metastases.

In this systematic review, we examine and summarise the evidence on HER2 discordance between primary IBC and its *paired* loco-regional or distant metastasis, with the aim of (1) providing summary estimates for HER2 discordance using all the available evidence and (2) exploring methodological, clinical, tumor, and testing-related variables that may be associated with differences *across* studies in the proportion of HER2 discordant cases. We hypothesized that this approach may potentially allow us to identify patterns in the proportion of HER2 discordance between primary and metastatic cancer which could point towards likely underlying mechanism(s).

Methods

We performed a systematic review and *study-level* meta-analysis of studies reporting HER2 status in primary breast cancer and its paired metastasis.

Study eligibility

Studies were eligible for inclusion if they reported HER2 status, based on IHC and/or ISH-based testing, in primary IBC and its *paired* loco-regional or distant metastases. We included studies reporting cross-classified data for HER2 status (primary vs. metastasis), and allowing calculation of discordance rates (proportion of discordant cases) *and* the direction of discordance between the primary cancer and associated metastasis. We also included studies where the cross-classified data could be derived from reported results.

We excluded studies reporting paired HER2 data in fewer than 25% of subjects *or* in <20 subjects. We excluded studies of circulating tumour cells, studies of sentinel node biopsy only (where metastases included micro-metastases or isolated tumour cells), or those based on post-mortem testing. Studies published as abstracts were ineligible for this review. Appendix 1 provides further information on eligible studies [15–17, 20–42] and on studies excluded [43–50] based on pre-defined criteria.

Literature search and data extraction

We systematically searched the literature (MEDLINE, EBM Reviews databases to August 2010) for primary studies that met eligibility criteria, using the search and study identification strategy summarised in Appendix 1. Descriptive and quantitative data were extracted by two of three authors (NH and either RB or MB), and disagreement was resolved by discussion and consensus; quantitative HER2 results were *independently* extracted into a spreadsheet. Extracted information included study and subjects' characteristics, type and site of metastases, time to metastasis, tumor grade, test-related parameters including scoring criteria, HER2 status for primary and metastatic tumor.

Quality appraisal

Evidence tables, summarising key study characteristics, methodology, and HER2 testing and interpretation parameters were developed with consideration of the American Society of Clinical Oncology/College of American Pathologists recommendations for human epidermal growth factor receptor 2 testing [1], and recommendations for reporting biomarker studies [51, 52]. For valid comparison of HER2 status in this scenario, we looked at whether testing methods and conditions (see "Testing parameters") were consistent between testing of the primary tumor and the metastasis, and whether paired testing was performed at the same time and laboratory. Studies not meeting this quality criterion (but otherwise meeting eligibility criteria) would, a priori, not be excluded but subjected to a sensitivity analysis. We also looked at whether

HER2 testing for each of the tumors was interpreted independently ("masked") of the other.

Testing parameters

We examined information on type of test (IHC or ISH based), testing methods (sampling, assays used, interpretation criteria, and interpreters), and test interpretation relative to consensus recommendations [1] while cognisant that current test-scoring standards were too recent to be included in studies published prior to 2008. We therefore accepted IHC 0–3+ scoring (based on HercepTest criteria) to be "standard" where staining was reported in >10% of tumor cells (further testing details, including sampling and assays used, are provided in Online-only Table A). For analytic purposes, we classified *study-specific* HER2 scoring/interpretation into pre-defined categories. For IHC, the categories (from most to least consistent with current standards) were: (i) either standard (HercepTest) scoring (0–3+) with 3+ counted as positive, equivocal results (2+) confirmed with ISH-based testing *or* standard scoring (0–3+) with most subjects tested/confirmed with ISH; (ii) standard scoring (0–3+) with ≥2+ counted as positive without ISH testing or ISH only in minority of equivocal results *or* ISH testing in all subjects but HER2 classified according to IHC alone, or (iii) semi-quantitative scoring system (other than standard) described in primary study without ISH testing, or other non-standard scoring.

HER2 scoring for gene-amplification in ISH-based testing was categorised in two groups, based on standards [1] (minimum threshold for FISH ratio was modified to 1.6 to allow classification of data): FISH ratio 1.6–2.2 or *HER2* gene copy 4.0–6.0; *or* FISH ratio >2.2 or HER2 *gene* copy >6.0. Study-specific information was extracted for the criteria used for HER2-positivity, type of antibody (monoclonal or polyclonal) for IHC assays, and DNA probe for ISH.

Statistical analysis

Descriptive data [median, inter-quartile range (IQR)] were examined for study-level variables: prevalence of HER2 positivity, subjects' age, time to metastasis, and tumor grade distribution. For each study, the proportion discordant in HER2 status and the corresponding exact 95% confidence limits were computed and displayed in forest plots. The overall *pooled* estimate of the proportion discordant was based on a random effects logistic regression model, which allows for both *within*-study sampling variability and *between*-study variability (heterogeneity) in estimates of the proportion discordant. Categorical covariates, such as test type and test-related parameters, or type of metastasis, were fitted (separately) in the model to obtain the random effects pooled estimates for the proportion discordant across studies and its confidence limits, for each category based on studies that provided covariate-specific data. Quantitative covariates, such as age, were fitted as continuous covariates in the model to examine their association with the proportion discordant. In sensitivity analysis, all modelled analyses were repeated excluding any study considered to be an "outlier" for *consistency in testing methodology* for paired tests.

HER2 discordance *directional* analysis was examined by classifying, at the study level, discordant pairs according to whether the HER2 positive result was from the primary or the metastasis; the difference (primary—metastasis) was then computed. The Wilcoxon signed rank test was used to assess whether there was any evidence of a systematic direction for the difference. McNemar's test was also used to analyse these data by classifying the directional difference for each study as positive, negative or equal.

Results

Twenty-six studies met our inclusion criteria [15–17, 20–42] (Appendix 1)—these provided *paired* HER2 data for primary and metastatic tumor in 2,520 subjects. The characteristics of these studies are summarised in Table 1, including definition of eligible subjects, site of metastases, and quality appraisal. Additional detailed testing methods (type of test and samples, assays, scoring and interpretation criteria) are in Online-only Table A. The median proportion of HER2 positivity in primary breast cancers in these studies was 26.1% (IQR 18.8–36.6%). Study-specific median age (21 studies) was 52.0 years (IQR 50.5–53.0 years). Median time to metachronous metastasis (13 studies) was 34.5 months (IQR 28.8–59.5 months). Median distribution for tumor grade (13 studies) was grade I in 8.2% (IQR 6.7–10.8%), grade II in 39.0% (IQR 29.0–45.2%), and grade III in 52.0% (IQR 48.1–63.8%).

All studies were retrospective except for two prospective studies [37, 41]. As shown in Table 1, most studies included subjects who had metastases biopsied and available tissue specimens for primary and metastasis; only six studies included or sampled subjects from a well-defined cohort or therapeutic clinical trial. Santinelli et al. [37] examined several groups of subjects and is included in *quantitative* analysis as two series—hence "27 studies" are reported in estimates.

Consistency of (within-subject) testing conditions for paired primary cancer and metastasis

Table 1 and Online-only Table A summarise the evidence on testing parameters: studies generally examined HER2 in primary tumor and its paired metastasis (*within* subjects) using the same test and testing methods, the same scoring

Table 1 Primary studies of HER2 in IBC and paired metastases: study characteristics and quality appraisal

Study (year) Timeframe [design][a]	Eligible subjects	Number of subjects (*N*); were subjects from a well-defined study base?	Median or mean age (range), years	Type of regional or distant metastasis (number[b] where reported)	Median time (range) to metastasis, months	Were the same tests and methods used for determining HER2 status of primary and metastasis?	Were paired tests performed at same time/ laboratory?	Was test interpretation masked?[c]
Idirisinghe [34] (2010); 1991–2007 [R]	Women with IBC and histology-proven distant metastases or local recurrence identified via a pathology database	$N = 117$; no	NR	Distant metastases (72): bone (35), lung/pleura (10), skin (10), brain (6), pericardium (3), other (5), omentum (3); LR (breast, chest wall) (45)	32, mean 46 (1–175)	Probably yes, same tests and methods but insufficient details on pathologists	Yes	NR
Aoyama [20] (2010); 2003–NR [R]	Women with IBC and node metastases: four groups of cases "randomly" selected to represent various HER2 scores on IHC: all re-tested with FISH	$N = 60$; no	53 (37–80)	Lymph nodes only (60)	Synchronous nodes	Probably yes, same tests and methods but insufficient details on pathologists	Yes	NR
Aitken [32] (2010); 1999–2002 [R]	Women with IBC and node metastases from a cohort of primary breast cancers treated in a breast service over a 4-year period	$N = 211$ (data for 190 paired tests) from 385 cases; yes	NR	Lymph nodes only (211)	Synchronous nodes	Yes, tested using same assays and methods, and interpreted by same observers (independent scoring: pathologist, 1 observer)	Yes	NR
Lower [17] (2009); 1998–2005 [R]	Women with biopsy-proven metastatic breast cancer, with both primary and metastatic HER2 data available, identified through clinical database of specialist physician	$N = 382$; no	50.5 (estimated)	Distant metastases only, site not further specified (382)	NR	No, tested as part of clinical care (no retesting), test applied by various pathologists over study timeframe	No	NR
Azam [31] (2009); 2004–2006 [R]	Women with IBC treated with mastectomy and had node metastases, identified via a pathology department	$N = 100$; no	46.3 (23–84)	Lymph nodes only (100)	Synchronous nodes	Probably yes, same tests and methods but insufficient details on pathologists	Yes	NR
Simmons [41] (2009); 2006–2007 [P]	Women attending a breast medical oncology practice over a 1-year period, and suspected of having (clinical or radiological) metastases recruited into a study of biopsy of metastases	$N = 40$ (data for 25 paired tests)[d]; yes	NR	Suspected distant metastases: bone (11), soft tissue (10), lung or pleural fluid (4), liver (3), cerebrospinal fluid (1)	28.8 (interquartile range 14–78)	Yes, same test and pathologist (primary cancer retested and metastasis tested using same methods), FISH methods not fully described	Yes	Yes

Table 1 continued

Study (year) Timeframe [design][a]	Eligible subjects	Number of subjects (*N*); were subjects from a well-defined study base?	Median or mean age (range), years	Type of regional or distant metastasis (number[b] where reported)	Median time (range) to metastasis, months	Were the same tests and methods used for determining HER2 status of primary and metastasis?	Were paired tests performed at same time/ laboratory?	Was test interpretation masked?[c]
Santiago [33] (2009); NR [R]	Consecutive cases of IBC with axillary lymph node metastasis identified via a pathology department	$N = 52$; no	57.4 (29–84)	Lymph nodes only (52)	Synchronous nodes	Yes, tested using same assays and methods, interpreted by same two pathologists (test independently scored with consensus)	Yes	NR
Santinelli [37] (2008); 2001–2006 [P]	Consecutive cases with metachronous breast cancer metastases in a pathology institute prospectively identified and matched with primary tumour; and women with IBC and synchronous node metastases for same timeframe	$N = 65$ (distant metastases or LR), and $N = 54$ (nodes); no	50.4–53.4 for various groups (26–76)	Synchronous lymph nodes (45), distant metastases (35), LR (breast, skin, chest wall) (30), metachronous regional nodes (9)	Metachronous nodes 72.9; LR 62.6; DM 66.7 (12–228)	Yes, tested using same assays and methods, and interpreted by the same pathologists (independent test scoring by two pathologists, with consensus)	Yes	NR
Guarneri [36] (2008); 2004–2007 [R]	Biopsy-diagnosed metastases in women with primary IBC identified in pathology archives, and had available specimens from both tumors	$N = 75$; no	53 (27–67)	Locoregional/soft tissue (30), liver (20), lung or pleura (7), CNS (5), bone (5), distant soft tissue (3), abdomen (3), bone marrow (2)	53 (7–308); DM 54/LR 43	Yes, tested using same assays and methods and pathologist (all tests were re-evaluated), discordance reviewed by another pathologist	Yes	NR
Cho [40] (2008); NR [R]	Women with IBC and paired lymph node metastases identified via pathology records, with available clinical data and tissue specimens	$N = 73$; no	47.8 (28–70)	Ipsilateral regional lymph nodes (73)	Synchronous	Yes, sampled and tested at same time using same methods and pathologists	Yes	NR
Tapia [39] (2007); 1999–2006 [R]	Cases with cytology-tested metastases from three pathology institutes with matched tissue from primary breast cancer	$N = 105$; no	57.5 (26–85)	Distant metastases: pleura (74), lung (9), CNS (8), liver (4), ascites (3), distant nodes (3), skin/soft tissue (3), pericardium (1)	Metachronous 66 (0-254); 8 synchronous metastases	Mostly yes except for one pre-analytic component: same test/methods for paired samples, but differences in sampling of primary (tissue) and metastasis (needle biopsy)	Yes	No

Table 1 continued

Study (year) Timeframe [design][a]	Eligible subjects	Number of subjects (*N*); were subjects from a well-defined study base?	Median or mean age (range), years	Type of regional or distant metastasis (number[b] where reported)	Median time (range) to metastasis, months	Were the same tests and methods used for determining HER2 status of primary and metastasis?	Were paired tests performed at same time/ laboratory?	Was test interpretation masked?[c]
Gong [38] (2005); 1996–2003 [R]	Women with known HER2 status for both primary breast cancer and paired (synchronous or metachronous) regional or distant metastases	$N = 60$; no	52 (26–79)	Axillary or SC nodes (38), soft tissue/ chest (5), lung/ pleura (10), liver (4), bone (3)	16 (0–103)	Mostly yes except for one component—same methods used for paired tissue or needle samples, however, differences in sampling of primary or metastasis	Yes	NR
Zidan [30] (2005); 1990–2002 [R]	Women with metastatic breast cancer who had tumor samples from both primary and corresponding metastasis suitable for IHC testing	$N = 58$; yes, 58 from 209 with metastases for study timeframe	56 (29–82)	Bone (39), liver (21), skin or soft tissue/LR (20), lungs (19), pleura (11)	54 (12–144)	Yes, sampled and tested using same methods and pathologists	Yes	NR
Carlsson [29] (2004); NR [R]	Women with good quality tissue samples from both primary tumor and corresponding node metastases obtained from the Scandinavian Breast Cancer Group study [59]	$N = 47$; yes, 47 from 283 cases enrolled in a clinical trial	52.2 (NR)	Lymph nodes only (47)	NR; synchronous + metachronous	Yes, same tests and methods performed on archival material, and interpreted by the same pathologists	Yes	Yes
Regitnig [28] (2004); 1984–2001 [R]	Women with tissue available from both primary breast cancers and distant metastases, identified through pathology department	$N = 31$; no	53.7 (33–78)	Distant metastases: bone or bone marrow (8), distant soft tissue/skin (6), brain (5), lung or pleura (4), liver (3), other sites (4)	Mean 34.5 (−16[e] to 103)	Probably yes, same tests and methods performed on archival material, but insufficient details on pathologists	Yes	NR
Edgerton [27] (2003); NR [R]	Women with IBC and with respective metastases or local recurrence, identified via a pathology department	$N = 113$; no	50 (DM), 61 (LR)	DM (93), LR/ chest wall or in-breast (20)	36 for DM, 28 for LR	Yes, tested using same methods and pathologists (double-review for HercepTest)	Yes	NR
Sekido [26] (2003); 1997–2000 [R]	Women with primary IBC and corresponding breast cancer metastases or recurrence	$N = 44$; no	50.7 (28–74)	Regional nodes, distant metastases, or LR (breast, skin, chest wall) (numbers not given for all cases)	59.5 (14–127) in subset of 14 cases only	Probably yes, same tests and methods but insufficient details on pathologists	Yes	NR

Table 1 continued

Study (year) Timeframe [design][a]	Eligible subjects	Number of subjects (*N*); were subjects from a well-defined study base?	Median or mean age (range), years	Type of regional or distant metastasis (number[b] where reported)	Median time (range) to metastasis, months	Were the same tests and methods used for determining HER2 status of primary and metastasis?	Were paired tests performed at same time/ laboratory?	Was test interpretation masked?[c]
Gancberg [23] (2002); 1981–1999 [R]	Women with primary IBC with at least 1 distant metastatic site, and available tissue blocks.	$N = 107$ (data for 100 paired tests); no	NR (NR)	Distant metastases: bone (38), distant soft tissue (32), liver (26), lung/ pleura (13), brain (2), gastrointestinal (9), ovary (6),	NR (1–216)	Yes, tested using same assays and methods, interpreted by the same pathologist(s) (one observer for IHC; two observers for FISH)	Yes	Yes[f]
Tsutsui [24] (2002); 1992–1998 [R]	Women who had surgical treatment at a cancer centre and had available both the primary breast lesion and matching metastatic regional lymph nodes	$N = 76$ with HER2 data (from 107); no	52 (25–83)	Regional lymph node metastases (76)	Synchronous nodes	Yes, tested using same assay and methods and interpreted by the same pathologist	Yes	NR
Vincent-Salomon [25] (2002); NR [R]	Women with IBC who presented during follow-up with distant metastases proven on surgical biopsy	$N = 44$; no	49 (31-74)	Lung (27), liver (17)	78 (12-228)	Probably yes, same test on archival material, but insufficient details on pathologists	Yes	NR
Xu [42] (2002); 1996–1999 [R]	Women with IBC selected on the basis of initial HER2 status using IHC: one group HER2-positive, and one group HER2-negative; re-tested subset that had paired metastases using FISH	$N = 47$ (data for 33 paired tests); no	57.3 (estimated from group data)	Lymph node metastases (33)	Synchronous nodes	Probably yes, same tests and methods, but insufficient details on pathologists	Yes	NR
Cardoso [16] (2001); 1988–1996 [R]	Women with specimens suitable for IHC testing, from node-positive breast cancers recruited into a multicentre trial of adjuvant chemotherapy [60].	$N = 370$; yes 370 (from 777 women in a clinical trial) [60]	49	Ipsilateral axillary lymph nodes (370)	Synchronous	Yes, sampled and tested at same time using same methods and pathologist.	Yes	Yes
Simon [35] (2001); 1985–1995 [R]	Two subject groups: one group each of node-positive ($\geq$3 metastatic nodes) or node-negative, randomly selected from >3000 women with IBC. Group with node metastases included in review[g]	$N = 125$ with paired data from 196; yes, sampled from defined cohort	NR (NR)	Lymph node metastases (125)	Synchronous nodes	Probably yes, same tests and methods performed on archival material, but insufficient details on pathologists	Yes	NR

Table 1 continued

Study (year) Timeframe [design][a]	Eligible subjects	Number of subjects (*N*); were subjects from a well-defined study base?	Median or mean age (range), years	Type of regional or distant metastasis (number[b] where reported)	Median time (range) to metastasis, months	Were the same tests and methods used for determining HER2 status of primary and metastasis?	Were paired tests performed at same time/ laboratory?	Was test interpretation masked?[c]
Tanner [15] (2001); NR [R]	Women with tumor samples available from primary IBC and subsequent metastatic tumors, identified in a breast cancer specimen database[h]	$N = 46$; no	NR	Loco-regional recurrence or metastases (33), distant metastases (12)	NR (1–228)	Probably yes, same tests and methods but insufficient details on pathologists	Yes	NR
Shimizu [21] (2000); 1996–1998 [R]	Women who had surgical resection of metastatic tumors, and had tissue available from both primary breast cancer and associated metastasis	$N = 21$; no	50 (35–75)	LR/skin (14), lung (3), SC nodes (3), liver (1)	19 (5–104)	Mostly yes except for one component—same assay and methods for *paired* testing, but different assays across subjects; interpreted by same pathologist	Yes	NR
Masood and Bui [22] (2000); NR [R]	Women with breast cancer and with respective metastases identified through pathology department	$N = 56$; no	50.5 (30–72)	Nodes (56), distant metastases (11)	NR	Yes, sampled and tested at same time using same methods and pathologist, and consensus between two observers	Yes	No

HER2 human epidermal growth factor receptor 2, *IBC* invasive breast cancer, *IHC* immunohistochemistry, *FISH* fluorescence in situ hybridization, *CISH* chromogenic in situ hybridization, *NR* not reported, *NA* not applicable, *DM* distant metastases, *LR* local recurrence, *SC* supraclavicular, *CNS* central nervous system

[a] Study design: P (observational, prospective), R (observational, retrospective)

[b] Number of subjects for each metastatic site may exceed total subject numbers due to some subjects having two or more sites of metastases; any reported site of metastases is included in table, however, where data are given for sequential multiple metastases, the first site (providing complete data in all cases) was used for analytic purposes

[c] "Was test interpretation masked?"—refers to whether HER2 testing of each primary tumor and corresponding metastasis was interpreted independently of each other?

[d] From the initial study-base defined in Simmons et al. [41], losses were due to subjects with metastatic sites not suitable for biopsy or had inadequate samples, or due to subjects found to have benign or other pathology on biopsy of "suspected" metastases

[e] Regitnig et al. [28]: data on time to metastasis reflect two subjects with distant metastases diagnosed prior to primary breast cancer

[f] Gancberg et al. [23]: Interpretation of tests was masked for primary versus metastasis *and* between IHC and FISH

[g] Data from Simon et al. [35] included in our review were based on 125 subjects (lymph node-positive for metastatic cancer) in whom 16 arrayed tissue samples (four samples each of the primary and of three different node metastases) yielded HER2 results. Because each of primary tumour and three corresponding node metastases had four samples tested, a small number of cases were reported as having heterogeneous HER2 status (HER2-positive and HER2-negative tissue samples for each tumor)—we counted heterogeneous expression as positive in pooled analysis

[h] Study by Tanner et al. [15] performed both IHC and CISH with complete concordance: paired HER2 data for primary and metastasis (in pooled analysis) are for CISH

and interpretation criteria, in the same laboratory and under the same conditions. Testing of both primary and metastatic tumor was performed *at the same time* (predominantly by testing representative tissue sections from formalin-fixed and paraffin-embedded primary and metastatic breast cancer); one exception was the study from Lower et al. [17] which included subjects attending a physician's practice hence HER2 data did not involve testing under the same conditions (Table 1). All other studies tested primary and metastasis using generally consistent methods and were examined in two groups in preliminary analysis to judge *within-subject* consistency of HER2 testing: (1) 13 studies which maintained *all* conditions of testing for primary and metastatic tumor [16, 22–24, 27, 29, 30, 32, 33, 36, 37, 40] and (2) 13 studies using the same test and assay, and maintaining testing methods on the whole but either *omitted information* on one testing aspect [15, 20, 21, 25, 26, 28, 31, 34, 35, 42] or were inconsistent in only one testing element [38, 39, 41]. For the latter group, this was omission of information on test interpreters rather than actual testing differences, with only three studies showing inconsistency relating only to type of samples used [38, 39, 41] (Table 1). Pooled estimates for the proportion of HER2 discordance were *not* significantly different between group 1 studies (HER2 discordant 5.9%; 95% CI 3.5–9.9%) and group 2 studies (HER2 discordant 4.6%; 95% CI 2.6–8.1%),

$P = 0.53$. Therefore, based on pre-defined quality appraisal criteria for consistency of paired testing (see "Methods" section), only the study from Lower [17] sufficiently differed on this criterion to warrant sensitivity analysis.

Pooled proportion of HER2 discordance between primary and metastasis

Forest plots of the studies included in this review, stratified by test used (study-specific data ordered by increasing HER2 discordance within test strata), are displayed in Fig. 1: pooled estimates of the proportion of HER2 discordance between primary and metastasis (*HER2 discordant%*) were 5.5% (95% CI 3.6–8.5%) or 5.2% (96% CI 3.5–7.8%) excluding the study from Lower [17]. Modelled estimates of HER2 discordant% were not associated with the proportion of HER2 positivity in primary cancers ($P = 0.42$), median age ($P = 0.78$), or study time-frame ($P = 0.22$). In the subset of studies reporting time to metachronous metastases (13 studies), median time to metastasis was not associated with HER2 discordant% ($P = 0.48$).

Table 2 summarises overall and *covariate-specific* estimates for HER2 discordant% for categorical variables and association of covariates in modelled estimates (study-level covariates examine *between-study* variability in HER2 discordance). Sensitivity analysis reports each estimate excluding the study from Lower et al. [17]. We used this approach because the study from Lower [17] was an "outlier" for maintaining testing consistency *within* subjects, and not due to the quality of this study which focused on survival (and reported relatively better survival in subjects with HER2-negative primary cancer and HER2-positive metastasis) [17].

Type of test used was not associated with HER2 discordant% across studies ($P = 0.27$). The small group of studies indicated by IHC/FISH represents studies reporting "overall" HER2 status based on IHC or FISH: although these studies had relatively higher summary HER2 discordant%, this estimate had a wide confidence interval. Within the subset of IHC studies, scoring and interpretation criteria, and type of antibody, were not significantly associated with HER2 discordant%. There were too few studies primarily using ISH-based testing to allow comparative analysis, however, modelled estimates for HER2 discordant% were very similar for ISH scoring criteria categories, and for type of probe (Table 2).

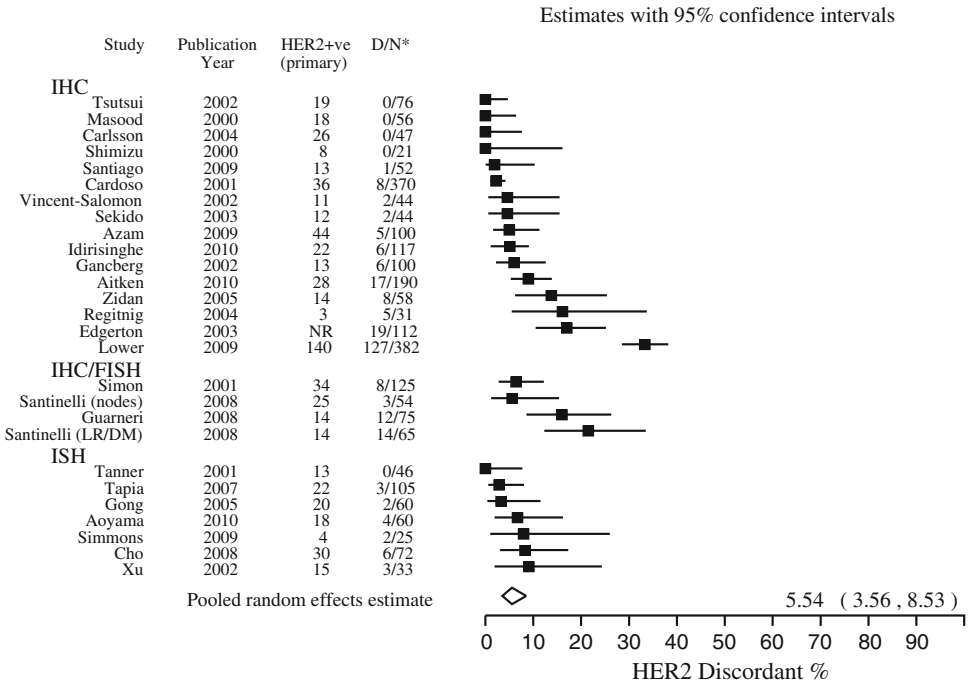

Fig. 1 Study-specific estimates and pooled estimate for HER2 discordant % in studies reporting HER2 status in primary breast cancer and paired metastasis. D/N* = number of HER2 discordant cases/total number of paired primary breast cancer and metastasis. Estimates for HER2 discordant % for each group of studies according to the test used are shown in Table 2 (details of test scoring and interpretation criteria are described in "Methods" section). The group of studies indicated by IHC/FISH represents studies that assigned an overall HER2 status based on IHC and FISH using the following algorithms: Guarneri [36] considered HER2 status as positive where IHC was 3+, or IHC was 2+ and FISH was amplified *or* FISH was amplified; Santinelli [37] considered HER2 as positive in cases where IHC was 3+, or IHC was 2+ and FISH was amplified; Simon [35] considered HER2 as positive in cases where IHC was 2+ or 3+ *or* FISH was amplified and reported 94% concordance between IHC and FISH in their data. The work of Santinelli [37] has been included as two series (nodes; LR/DM = local recurrence or distant metastases)

Table 2 Modelled estimates of *HER2 discordant proportion* in studies of HER2 status in primary breast cancer and its paired metastasis

Study-level covariate used to examine between-study variability in HER2 discordant proportion (no. of studies providing data for covariate, no. of subjects)	Estimates for separate random-effects logistic regression models		Sensitivity analysis[a]	
	HER2 discordant % (95% CI)	*P* value	HER2 discordant % (95% CI)	*P* value
Overall pooled estimate	5.54 (3.56, 8.53)	–	5.23 (3.48, 7.77)	–
Test used to determine HER2 status (27 studies, 2520 subjects)		0.27		0.14
Immunohistochemistry (IHC)	5.15 (2.94, 8.87)		4.57 (2.78, 7.42)	
Immunohistochemistry/In situ hybridization (IHC/FISH)[b]	10.70 (4.13, 24.97)		10.91 (5.06, 21.97)	
In situ hybridization (ISH: FISH or CISH)[c]	4.34 (1.83, 9.93)		4.52 (2.17, 9.18)	
Type of metastasis included in paired testing of primary cancer and metastasis (27 studies, 2520 subjects)		0.0017		0.0006
Studies of nodes only	4.18 (2.45, 7.06)		4.46 (2.92, 6.74)	
Studies of nodes or local recurrence or distant metastases[d]	1.42 (0.40, 4.86)		1.59 (0.51, 4.82)	
Studies of distant metastases or local recurrence[e]	13.42 (7.16, 23.73)		13.62 (8.60, 20.90)	
Studies of distant metastases only	9.55 (4.94, 17.67)		6.03 (3.16, 11.19)	
Type of metastasis included in paired testing of primary cancer and metastasis (26 studies, 2337 subjects)[f]		0.0011		0.0038
Studies of nodes only *and* studies of nodes or local recurrence or distant metastases[d]	3.32 (1.96, 5.58)		3.48 (2.14, 5.61)	
Studies of distant metastases only (studies of distant metastases *and* subjects with distant metastases from studies of distant metastases or local recurrence)	11.49 (6.84, 18.65)		9.69 (5.84, 15.66)	
Type of metastasis included in paired testing of primary cancer and metastasis: regional nodes versus distant metastases *(21 studies, 2110 subjects)*[f]		0.0082		0.019
Studies of lymph nodes only	4.14 (2.35, 7.19)		4.28 (2.58, 7.01)	
Studies of distant metastases only (studies of distant metastases *and* subjects with distant metastases from studies of distant metastases or local recurrence)	11.54 (6.92, 18.64)		9.74 (5.94, 15.56)	
Metastasis synchronous or metachronous to primary (27 studies, 2520 subjects)		0.0024		0.0043
Studies of synchronous metastases	4.52 (2.54, 7.92)		4.64 (2.81, 7.58)	
Studies of synchronous or metachronous metastases	1.86 (0.69, 4.92)		1.98 (0.80, 4.86)	
Studies of metachronous metastases	10.57 (6.67, 16.35)		9.26 (6.03, 13.98)	
Test scoring/interpretation criteria[g] *for IHC studies, categorised from most to least consistent with current standards (15 studies, 1610 subjects)*		0.22		0.12
Standard scoring with 3+ counted as positive, equivocal results (2+) confirmed with ISH *or* standard scoring with most subjects tested or confirmed with ISH	5.40 (1.76, 15.36)		5.73 (2.16, 14.36)	
Standard scoring with ≥2+ counted as positive without ISH or ISH testing in minority of equivocal results *or* ISH in all subjects but IHC data based on IHC only	9.52 (2.93, 26.83)		5.44 (1.47, 18.19)	
Semi-quantitative scoring system (other than standard) without ISH testing, or other non-standard scoring	2.37 (0.79, 6.90)		2.52 (0.96, 6.45)	
IHC studies: type of antibody used in assay[g] *(15 studies, 1610 subjects)*				
Monoclonal antibody	5.71 (2.21, 13.97)	0.39	4.51 (1.86, 10.56)	0.63
Polyclonal antibody	2.97 (0.77, 10.73)		3.28 (1.01, 10.14)	
Test scoring/interpretation criteria[g] *for ISH studies (6 studies, 376 subjects)*		NC		NA
Amplified if FISH ratio >2.2 or *HER2* gene copy >6.0.	4.90 (1.40, 15.81)		–	
Amplified if FISH ratio 1.6–2.2 or *HER2* gene copy 4.0–6.0	4.65 (2.13, 9.88)		–	
ISH studies: Type of DNA probe for HER2 gene[g] *(6 studies, 376 subjects)*		NC		NA

Table 2 continued

Study-level covariate used to examine between-study variability in HER2 discordant proportion (no. of studies providing data for covariate, no. of subjects)	Estimates for separate random-effects logistic regression models		Sensitivity analysis[a]	
	HER2 discordant % (95% CI)	*P* value	HER2 discordant % (95% CI)	*P* value
Single DNA probe for *HER2* gene	4.90 (1.40, 15.81)			
Dual DNA probe for *HER2* gene	4.65 (2.13, 9.88)			

NC not calculated (due to few studies), *NA* not applicable (subgroup does not include outlier study)

[a] Sensitivity analysis for model estimates excluding the study from Lower et al. [17] (excludes 382 subjects from each model)—see "Methods" for details

[b] The group of studies indicated by IHC/FISH assigned an "overall" HER2 status based on IHC or FISH (fluorescence in situ hybridization)—see footnote of Fig. 1

[c] Only two of the studies (Tanner et al. [15]; Cho et al. [40]) reporting ISH-testing used CISH (chromogenic in situ hybridization), all other ISH studies used FISH

[d] The group of studies that included various metastases (metastatic nodes, local recurrence, or distant metastases) had ≤25% distant metastases

[e] The group of studies that included distant metastases or local recurrence had approximately 67% distant metastases and 33% local recurrences

[f] The category of studies of *distant metastases only* included in this model comprised data from studies pairing primary with distant metastases only *and* subjects with distant metastases from studies pairing primary with distant metastases or local recurrence (data for distant metastases not given separately to local recurrence in one study which was therefore not included in this analysis)

[g] Additional details of the classification of test scoring/interpretation criteria, and test technical parameters, are provided in text (see "Methods" section, testing parameters) and in online-only Table A

In contrast, type (site) of metastasis was significantly associated with modelled estimates of HER2 discordant% across studies ($P = 0.0017$); this association was unchanged in sensitivity analysis (Table 2). Figure 2 summarises study-specific estimates stratified by the type of metastasis (data ordered by increasing HER2 discordance), and pooled estimates for each metastasis-type grouping (see also Fig. 2 footnote). Models comparing different groupings for the type of metastasis paired with the primary are shown in Table 2: studies with *distant* metastases had higher HER2 discordant% (11.5%; 95% CI 6.9–18.6%) than those with nodal metastases only (4.1%; 95% CI 2.4–7.2%), $P = 0.0082$. Studies of primary paired with *distant* metastases also had higher HER2 discordant% (11.5%; 95% CI 6.8–18.6%) than those including nodal metastases only *or* various metastases (3.3%; 95% CI 2.0–5.6%), $P = 0.0011$. These associations persisted in sensitivity analysis. The group of studies pairing primary with various metastases (mostly nodal or loco-regional metastases, a minority with distant metastases) had the lowest HER2 discordant% (1.42%; 95% CI 0.40, 4.86) although the confidence interval for this estimate overlapped with that of the group of studies with nodes only. HER2 discordant% was also associated with whether the paired metastasis was synchronous or metachronous to primary cancer (Table 2) being higher for studies that included only metachronous metastases. This variable correlates with the type of metastasis, in that almost all distant metastases were metachronous, and the vast majority of lymph node metastases were synchronous.

Direction of HER2 discordance

Based on the McNemar test, there was no evidence of a systematic direction across studies for direction of discordance ($P = 0.13$): 14 studies had discordant pairs with overall change in HER2 from negative primary cancer to positive metastasis, 7 studies had discordant pairs with overall change in HER2 from positive primary to negative metastasis, 6 studies had neutral (equal) discordance direction or no discordance. However, exclusion of the outlier study [17] provided weak evidence ($P = 0.074$) that discordance in the direction of change from HER2-negative primary cancer to HER2-positive paired metastasis was more likely than the reverse. The results were very similar using the Wilcoxon signed rank test (data not shown) whereby weak but consistent evidence of a systematic difference for HER2 discordance direction (more studies with change to HER2-positive paired metastasis) was found across studies ($P = 0.067$) after excluding Lower et al. [17].

Discussion

Defining the potential for, and the implications of, discordance in HER2 status between primary breast cancer and its metastasis requires evaluation of two issues. First, quantifying the frequency of discordance and identifying associated factors (if any); second, determining whether discordance in HER2 status impacts clinical care, response

to treatment, or prognosis. Our work contributes towards addressing the first of these issues through evidence synthesis from observational studies. The latter issue requires prospective trials examining clinical end-points, particularly in the era of HER2-targeted adjuvant treatments. We examined HER2 discordance between paired primary and metastatic breast cancer in meta-analysis to explore factors associated with differences in discordant proportions *across* studies. The pooled proportion of HER2 discordance was modest (5.5%; 3.6–8.5%). However, there was a significant association between HER2 discordant proportions and the type of metastasis, in particular, studies pairing primary tumor with distant metastases showed higher proportions of HER2 discordance (9.6%; 4.9–17.7%) than those pairing primary with lymph node metastases only (4.2%; 2.5–7.1%).

There are many challenges to deriving clear answers from existing data on the stability of a HER2 status report between primary cancer and its metastasis, including the limitations of the relatively small size of studies, differences between studies in HER2 testing without centralised evaluation, and predominantly retrospective data sets. Notwithstanding these limitations and considering these studies provide opportunity to explore HER2 discordance in the pre-adjuvant trastuzumab era (without selection based on HER2 positivity), our meta-analysis revealed consistent themes that provide insight into underlying mechanisms. Discordance in HER2 status between primary and metastatic breast cancer may be due to various factors, including tumor progression (genetic drift or clonal selection for a particular HER2 phenotype), test-related technical and interpretation parameters including imperfect test reproducibility, and intra-tumoral heterogeneity of *HER2* gene amplification (clonal selection, or sampling differences causing ascertainment bias). Press et al. [53] have shown that the latter is rare, estimating that intra-tumoral HER2 heterogeneity occurred in <1% of women participating in clinical trials. Experts emphasize caution in interpretation of HER2 discordance between primary and metastatic tumor, because the contribution of the various factors outlined above is unknown [18] and we did not aim to quantify these factors in meta-analysis. Our work primarily explored patterns in HER2 discordance estimates across studies. In models examining test-related factors, we found that while HER2 testing varied between studies (but performed at the same time for paired tumors and largely maintained *within* subjects in all but one study [17]), this did not account for the diversity in HER2 discordance estimates. HER2 status discordance was, however, associated with the type of metastasis, suggesting that HER2 discordance may be a reflection of tumor-related factors, based on this

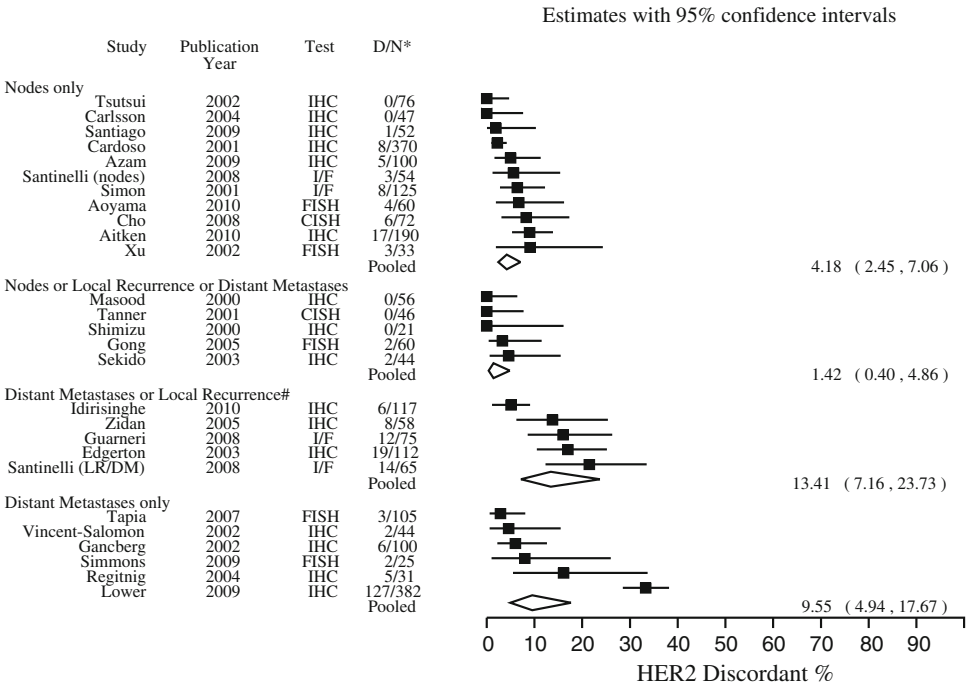

Fig. 2 Study-specific and pooled estimates for HER2 discordant % stratified by type of metastasis considered in each study, for studies reporting HER2 in primary breast cancer and paired metastasis. D/N* = number of HER2 discordant cases/total number of paired primary breast cancer and metastasis. Study-specific data ordered by increasing HER2 discordance within each metastasis-type stratum. The group of studies that included a mix of metastases (metastatic nodes, local recurrence, or distant metastases) had ≤25% distant metastases. # Group of studies that included distant metastases or local recurrence comprised approximately 67% distant metastases and 33% local recurrence. The work of Santinelli [37] has been included as two series (nodes; LR/DM = local recurrence or distant metastases

meta-analysis. Our finding of weak evidence in sensitivity analysis ($P = 0.074$) that discordance in the direction of change from HER2-negative primary cancer to HER2-positive paired metastasis was more likely than the reverse further supports tumor-related factors as possible underlying mechanisms of HER2 discordance.

Our findings *do not* discount variability in HER2 testing, interpretation, or reproducibility [18, 54–57], as possible or partial explanations for HER2 discordance between primary and metastatic tumor in individual cases or studies; because HER2 testing has imperfect reproducibility it is possible that the *absolute* proportion of discordance is overestimated. However, the effect of testing variability between studies was not significant in our *pooled* analysis, and the absence of association in our models for test-related factors (based on study-level measures which included test type, and scoring and interpretation criteria) means that these test-related factors did not account for differences across studies in HER2 discordant proportions. Our modelled estimates indicate a statistically significant association for type of metastasis, as outlined earlier, with higher HER2 discordance from studies pairing primary with distant metastases (or that included a majority with distant metastases) than those pairing primary with regional lymph node metastases only or various metastases (Table 2). While we acknowledge that HER2 status reports, due to inherent limitations of test reproducibility, would be expected to give some discordant results for repeated measures due to chance alone [18], this cannot account for the associations shown in our meta-analysis because discordance caused by imperfect test reproducibility would *not* have a differential effect by type of metastasis (rather, this would be expected to occur randomly across studies) unless HER2 testing of primary and metastasis was separated in time. Importantly, as shown in Table 1, all studies performed same-time HER2 testing with consistency in testing conditions for paired tumors (except for the one outlier study [17]).

One potential limitation, where a test is associated with variability in technique and reproducibility, is whether it is appropriate to pool data. We dealt with this in several ways: the effect of test-related parameters on discordance estimates was examined through analysis; we used random-effects logistic regression models to allow for study heterogeneity; and study-specific descriptive information on HER2 testing (sampling, assays, and scoring) was summarized in evidence tables. Furthermore, our meta-analysis is agnostic with respect to *absolute* accuracy of HER2 testing: the focus of our work is the *relative* HER2 status between primary and paired metastatic tumor within subjects compared analytically *across* (between) studies. As such, the first issue for valid comparisons is whether testing conditions were maintained *within* subjects (see "Consistency of testing conditions"). This has been carefully considered in quality appraisal (Table 1) and has been examined in our models, and formed the basis for excluding the outlier study [17] in sensitivity analysis. Once within-subject testing consistency was ascertained, our models considered covariates (Table 2) to investigate potential explanatory factors for *between-study* variability in HER2 discordance. Of note, pre-defined categories for HER2 test interpretation criteria were used in our models to investigate differences in study-specific interpretation relative to current standards and not to judge the interpretive quality of testing—the latter cannot be determined in study-level meta-analysis. It is also possible that our test-related parameters, while capturing information on HER2 testing for pre-analytic (sampling), analytic (test, assay), and interpretation (scoring, positive/amplified threshold) variables, have not measured all sources of testing variability. For example, pre-analytic factors, such as the age and processing of archived tissue, may have contributed to some of the observed discordance in paired testing and between studies. Study-level and retrospective individual person data meta-analysis cannot *completely* allow for all technical variability in HER2 testing between studies, and the optimal strategy requires *prospectively implemented* individual person data meta-analysis with centralised paired HER2 testing.

Guidelines recommend HER2 testing of primary IBC (at diagnosis) or at the time of metastatic relapse [12], and some recommend testing metastases if HER2 status of the primary is unknown [58]. Several estimates for HER2 discordance between primary and paired *distant* metastases are reported in our models, for various comparisons: the estimate with the largest number of cases of primary paired with *distant* metastases was 11.5% (6.8–18.7%). Our findings should *not* be taken as grounds to change practice in HER2 testing of distant metastases. However, they support review of recommendations for consideration of *selective* HER2 re-testing of metastases where biopsy is justifiable on clinical grounds, *if* such results would affect clinical decision-making. Two prospective studies in our analysis indicated that HER2 re-testing of metastases led to change to trastuzumab therapy or to participation in a clinical trial [41], or to therapeutically relevant HER2 discordance [37], in some subjects. Any change in the current approach for testing metastases should factor the possibility that re-testing may cause false-negative HER2 status [18]. Where feasible, re-testing should include simultaneous testing of primary and metastasis using the same methodology [18].

We report evidence that the type of metastasis was the main association in meta-analysis of diverse estimates for HER2 discordance between primary breast cancer and its paired metastasis. In support of this conclusion are the observations that HER2 discordance was significantly more likely in studies pairing primary with distant metastasis than those with regional nodal metastasis; evidence suggesting systematic directionality in discordance; association of

HER2 discordant proportion with whether metastases were synchronous or metachronous to primary cancer; and lack of association between HER2 discordant proportion and test-related parameters. Further research will be necessary to delineate mechanistic insight as to whether observed associations in this meta-analysis are due to biological factors related to type of metastasis, such as acquired changes in molecular phenotype during the course of tumor evolution, or reflect other variables associated with type of metastasis.

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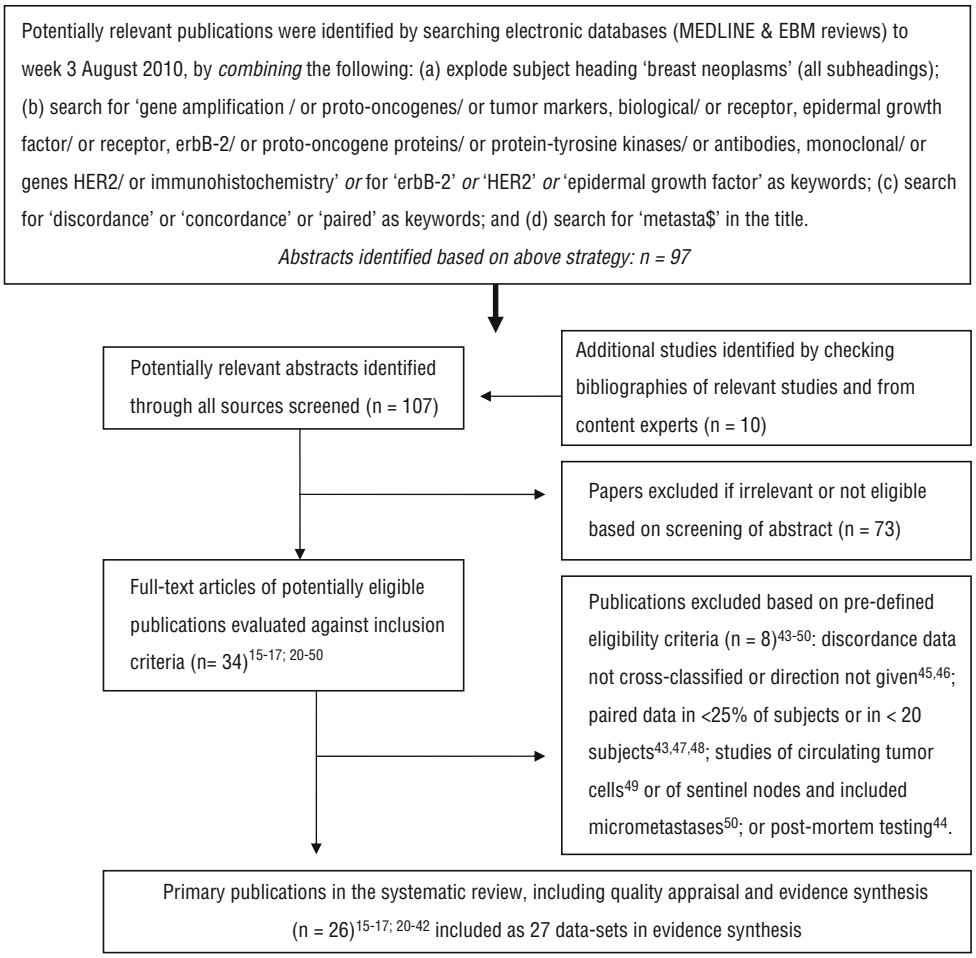

Appendix 1 Flow diagram summarising the literature search strategy. Flow diagram adapted from PRISMA recommendations (accessed January 2011 via www.prisma-satement.org)

Conflict of interest The authors Houssami, Macaskil, Balleine, Pegram have no conflict of interest to declare, and the author Bilous is a member of Roche HER2 testing Advisory Board.

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