Predictive value of *BRCA1* expression on the efficacy of chemotherapy based on anti-microtubule agents: a pooled analysis across different malignancies and agents

Qihua He[1,2*], Mingzhe Zhang[3*], Jianrong Zhang[1,2*], Shengyi Zhong[1,2], Yang Liu[1,2], Jianfei Shen[1,2], Jiaxi He[1,2], Long Jiang[1,2], Chenglin Yang[1,2], Yuan Zeng[1,2], Minzhang Guo[1,2], Xuewei Chen[1,2], Jianxing He[1,2], Wenhua Liang[1,2]

[1]Department of Thoracic Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510000, China; [2]Guangzhou Institute of Respiratory Disease & China State Key Laboratory of Respiratory Disease&National Clinical Research Center for Respiratory Disease, Guangzhou 510000, China; [3]Department of Cardiology, Xiamen Cardiovascular Hospital, Xiamen 361000, China

Contributions: (I) Conception and design: Q He, J He, W Liang; (II) Administrative support: None; (III) Provision of study materials or patients: M Zhang, J Zhang, S Zhong, Y Liu, J Shen, J He; (IV) Collection and assembly of data: M Zhang, J Zhang, S Zhong, Y Liu, L Jiang, C Yang, Y Zeng, M Guo, X Chen; (V) Data analysis and interpretation: Q He, X Chen, W Liang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work.

Correspondence to: Wenhua Liang, MD. No. 151, Yanjiang Rd, Guangzhou 510120, China. Email: liangwh1987@163.com; Jianxing He, MD, PhD, FACS. No. 151, Yanjiang Rd, Guangzhou 510120, China. Email: drjianxing.he@gmail.com.

Background: Breast cancer susceptibility gene 1 (*BRCA1*) expression has been suggested as a predictor in anti-neoplastic treatment with anti-microtubule agents. However, the existing evidence is conflicting. Consulting the literature, we sought to examine the true impact of *BRCA1* expression on the efficacy of anti-microtubule agents.

Methods: Medline by PubMed and Embase databases were searched for eligible studies. The primary endpoints were objective response rate (ORR) and progression free survival (PFS). Additional subgroup analyses stratified for detection methods, regimen, and patient origin were also performed.

Results: A total of 13 relevant studies involving a total of 1,490 cases were enrolled. Involved agents included paclitaxel, docetaxel and vinorelbine; Malignancies included non-small cell lung cancer, gastric cancer, esophageal carcinoma, ovarian carcinoma, malignant pleural mesothelioma, breast cancer, and small cell lung cancer. Through meta-analyses, we observed a potentially greater ORR in the population with high *BRCA1* expression *vs.* low *BRCA1* expression (OR 1.63, 95% CI: 0.92 to 2.88, P=0.09) but the heterogeneity is severe (P=0.01; I^2=61%). Similar results were observed in PFS (high *vs.* low expression, HR 0.93, 95% CI: 0.75 to 1.15, P=0.49; heterogeneity, P<0.01, I^2=75%). After stratification by testing methods, a significantly higher ORR in the population with high *BRCA1* expression was shown in the subgroup using mRNA as a quantitative method (OR 2.90, 95% CI: 1.92 to 4.39, P<0.01; I^2=0) whereas the difference in the subgroup using immunohistochemistry (IHC) was not significant (OR 0.60, 95% CI: 0.33 to 1.10, P=0.10; I^2=0). Stratification by regimen (platinum-based *vs.* non platinum-based) and patient origin (Asian *vs.* Caucasian) did not reduce the heterogeneity.

Conclusions: Although the predictive value of *BRCA1* expression on the anti-microtubule chemotherapy remained uncertain based on overall results, our exploratory analyses suggested that detection using mRNA might be a preferred technique, however, further validation is required to substantiate our findings.

Keywords: Breast cancer susceptibility gene 1 (*BRCA1*); anti-microtubule agents; meta-analysis

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Introduction

Despite the development of monoclonal antibodies and small molecule pathway inhibitors, chemotherapy remains the go-to treatment for patients with cancer, including neoadjuvant chemotherapy, adjuvant chemotherapy and palliative chemotherapy. Excitingly, an improvement in the efficacy of chemotherapy has been observed in recent years. However, its sensitivity varies from one patient to another. With the advances of molecular biological techniques, we have gained a deeper understanding of the pathogenesis and proliferation of the tumor at the molecular level. Thus, focus on the molecular characteristics of the disease to guide treatment choice has increased; one example is the trending use of molecular markers to predict activity of chemotherapeutic agents.

Anti-microtubule agents act by binding to soluble and/or polymerized tubulin in the microtubules ultimately affecting microtubule function. Vinca alkaloids and taxanes are two families of anti-microtubule agents wildly used in clinics including solid tumors and hematological malignancies, such as non-small cell lung cancer, breast cancer and ovarian cancer (1). Taxanes, a class of diterpenes derived from the plants of the genus Taxus (yews), are mitotic inhibitors that stabilize and protect the microtubule polymer from disassembly, causing chromosomes to be unable to forma metaphase spindle conformation, blocking progress of mitosis, and triggering cell death (2,3). Vinca alkaloids, such as vinorelbine, restrain mitosis and apoptosis by binding to tublin and preventing its assembly into microtubules (4).

Breast cancer susceptibility gene 1 (*BRCA1*), a scaffold protein, was first identified as an early-onset breast and ovarian cancer susceptibility gene (5). It has multiple roles not only in DNA damage repair but also in cell cycle regulation and apoptosis through association with other proteins (6). It has been reported that *BRCA1* correlated positively with taxanes sensitivity, which functions as a sensitizer to apoptosis induced by anti-microtubule agents (7). A number of investigations have found that *BRCA1* may be used as a predictive biomarker of response to anti-microtubule agents (5). Yang *et al*. (8) reported the potential role of *BRCA1* in predicting sensitivity of NSCLC, and found that patients with high/positive *BRCA1* had better ORR. However, existing evidence is conflicting. We conducted a systematic review to evaluate the associations of expression of *BRCA1* and the efficacy of anti-microtubule agents on cancer patients.

Materials and methods

Literature search

Literature search was conducted using PubMed and Embase from their dates of inception to Oct 23, 2014. The search strategy employed was a combination of: *BRCA1* or "Breast cancer susceptibility gene 1" or "Breast cancer 1" and chemotherapy or paclitaxel or docetaxel or vinorelbine. Language was limited to English and Chinese.

Inclusion criteria and exclusion criteria

Articles retrieved from the search were independently reviewed by two reviewers (Mingzhe Zhang & Jianrong Zhang), and any discrepancies were resolved by discussion with the third reviewer (Jianfei Shen). The following criteria was used to select publications: (I) cancer patients, regardless of tumor type, should be included; (II) only studies that detected *BRCA1* expression by immunohistochemistry (IHC) or reverse transcriptase polymerase chain reaction (QPCR) were included; (III) original papers must contain enough data to calculate the objective response rate (ORR); studies that failed to meet all of the above criteria were excluded from analyses. Reviews, animal or cell line studies were also excluded.

Data collection and quality assessment

Publication characteristics including first author's name, publication year, patients' original country, middle/mean age of study sample, first-line chemotherapeutic agents with doses and sample type, detection method of *BRCA1*, sample size, and disease stage were extracted from each eligible publication. End points of interest were ORR, overall survival (OS), and progression-free survival (PFS). Each included study was scored by two independent reviewers (Shengyi Zhong and Yang Liu).

Statistical analysis

To estimate ORR, patients were divided into two groups: patients that responded to treatment (responders) and patients that did not respond to treatment (non-responders). Responders were defined as complete response (CR) or partial response (PR). Non-responders included stable disease (SD) and progressive disease (PD). Disease control ratio (DCR), was defined as CR, PR and SD. The pooled

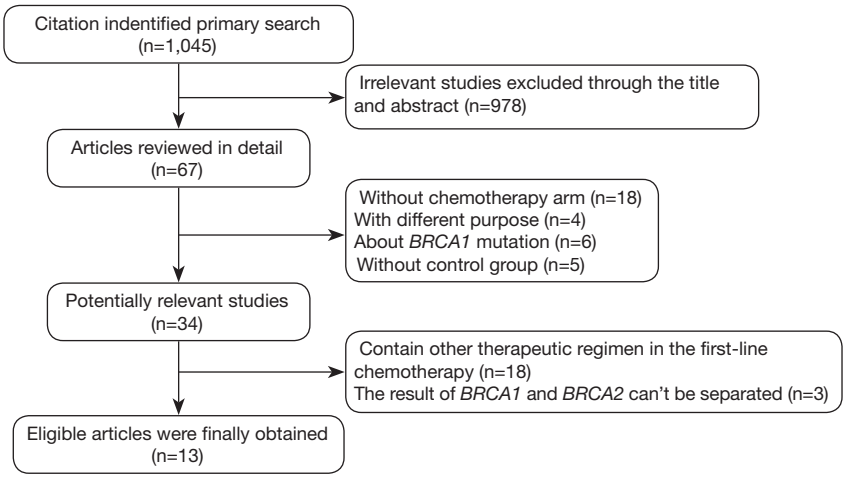

Figure 1 Profile summarizing the trial flow. *BRCA1*, breast cancer susceptibility gene 1.

odds ratio (OR) and its 95% confidence intervals (CIs) were calculated by the methods proposed by Mantel and Haenszel (9), or DerSimonian R and Laird N (10). Time-to-event data OS and PFS, hazard ratios (HRs) and associated 95% CI were estimated using the methods reported by Parmar (11). Heterogeneity between the studies was determined by Qtestand I^2 metric (I^2=0–25%: no heterogeneity; I^2=25–50%: moderate heterogeneity; I^2=50–75%: large heterogeneity; I^2=75–100%: extreme heterogeneity) (12). The fixed-effect model was applied in the initial analysis, and if significant heterogeneity existed, the random-effect model was used. Begg's test was used to evaluate the publication bias. P<0.05 indicated significant publication bias (13). All P values were two-tailed, REVIEW MANAGER (version 5.3 for Windows; the Cochrane Collaboration, Oxford, UK) and STATA version 11.1 (Stata Corporation, USA) were used to perform most data analyses.

Results

Eligible studies

Our search of PubMed database revealed 1,045 potentially relevant articles, 976 studies were immediately excluded upon review of their title and abstract. A total of 69 full text articles were carefully screened, 33 of which were excluded due to lack of sufficient data for extraction, another 20 articles were then excluded due to containing other therapeutic and unable to separate the results of *BRCA1* and *BRCA2*. Finally a total of 13 studies were selected for analysis. *Figure 1* summarizes the flow chart. Among these studies, the object response rate (ORR) was provided in 9 studies (5,7,14-20), the remaining 4 studies provided only OS or PFS (21-24). Characteristics of all involved studies are summarized in *Table S1*.

Characteristics of eligible studies

Our meta-analysis contained 13 studies involving a total of 1,490 cancer patients who had been treated with anti-microtubule agents as first- or second-line chemotherapy treatment. In all included studies, the major components of the chemotherapy regimen were anti-microtubule agents (including taxanes, paclitaxel, docetaxel and vinorelbine). Of the 13 included studies, 4 were for non-small-cell lung cancer, 3 were for breast cancer, and 2 were for ovarian cancer; the remaining four were for malignant pleural mesothelioma, esophageal squamous cell carcinoma, small cell lung cancer and gastric cancer. Of the 13 studies, 7 were from an East-Asian population (14,16-20,24), the other 6 studies were from a European population (5,7,15,21-23). Characteristics of included studies are summarized in *Table S1*.

BRCA1 level and the clinical outcome of chemotherapy

The ORR was reported in 9 of the included studies consisting of a total of 729 patients. By synthesis, we observed greater ORR in population with high *BRCA1* expression *vs.* low expression (OR 1.63, 95% CI: 0.92 to 2.88, P=0.09) but the heterogeneity was severe (P=0.01; I^2=61%) (*Figure 2*). No significant difference was observed

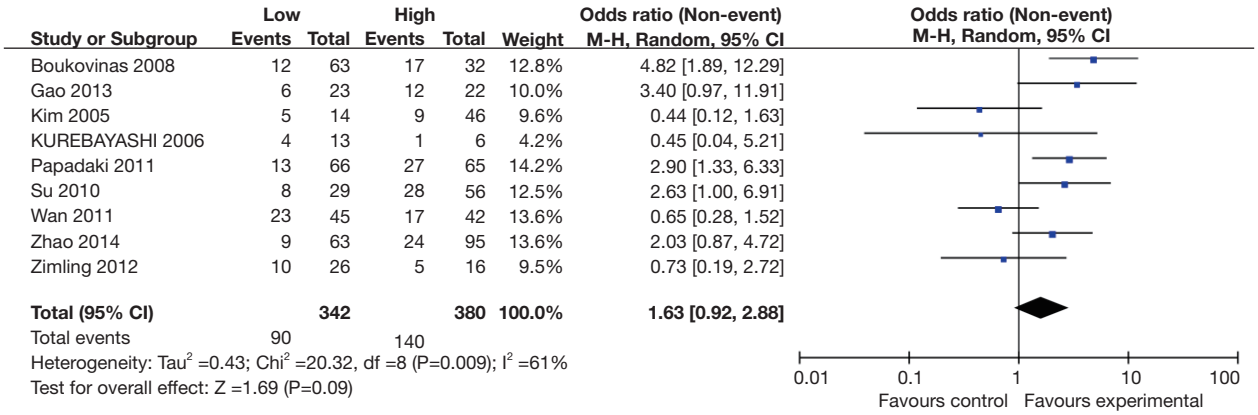

Figure2 Meta-analysis on objective response rate among neoplastic patients who received anti-microtubule agents according to *BRCA1* expression. CI, confidence interval; I^2, inconsistency statistic. *BRCA1*, breast cancer susceptibility gene 1.

Figure 3 Forest plots for the association between *BRCA1* level and PFS and OS in patients who received anti-microtubule. (A) Hazard ratio of PFS in patients who received anti-microtubule agents with high *BRCA1* expression *vs*. low *BRCA1* expression; (B) hazard ratio of OS in patients who received anti-microtubule agents with high *BRCA1* expression *vs*. low *BRCA1* expression. PFS, progression free survival; OS, overall survival; *BRCA1*, breast cancer susceptibility gene 1.

in PFS (high *vs*. low expression, HR 0.93, 95% CI: 0.75 to 1.15, P=0.49; heterogeneity, P<0.01, I^2=75%) and OS (high *vs*. low expression, HR 0.77, 95% CI: 0.57 to 1.04, P=0.09; heterogeneity, P=0, I^2=74%) (*Figure 3*). When analyzing the DCR, 4 studies consisting of 233 patients were included for comparison. No significant difference between the two groups was found (high *vs*. low expression, OR=0.83, 95% CI: 0.38 to 1.80, P=0.63; I^2= 17%, P=0.63 for heterogeneity).

Subgroup analyses

After stratification by testing methods, a significantly higher ORR in the population with high *BRCA1* expression was shown in the subgroup using mRNA as a measure approach (OR 2.90, 95% CI: 1.92 to 4.39, Chi^2 =0.39, P<0.01) whereas the difference in the subgroup using IHC was not significant (OR 0.60, 95% CI: 0.33 to 1.10, Chi^2 =1.92, P=0.10). The interaction between the two subgroups was significant (Chi^2 =17.61, P<0.01). However, results stratified by therapeutic regimens revealed a similar tendency between subgroups (for platinum-based studies, high *vs*. low expression, OR 1.62, 95% CI: 1.10 to 2.39, Chi^2 =9.26, P=0.01; for non-platinum-based studies, high *vs*. Low expression, OR 1.79, 95% CI: 0.41 to 7.70, Chi^2 =11.90, P=0.44); but there was no significant interaction between stratifications (Chi^2 =0.7, P=0.4). A potential association between *BRCA1* and efficacy was found in the non-Asian

Table 1 Subgroup analysis on objective response rate among cancer patients receiving anti-microtube agents to *BRCA1* expression

Categories of included studies	Number of included studies	ORR (event/total)		Test of heterogeneity			Test of effect size		Test for subgroup differences		
		Low/negative *BRCA1* expression	High/positive *BRCA1* expression	Chi^2	P value	I^2 (%)	OR (95% CI)	P value	Chi^2	P value	I^2 (%)
Total	9	90/342	140/380	20.32	0.01	61	1.63 (0.92, 2.88)	0.09			
BRCA1 detection method											
Immunohistochemical	4	42/98	32/110	0.39	0.94	0	0.6 (0.33, 1.10)	0.1	17.61	<0.01	94
Non-immunohistochemical	5	48/244	108/270	1.92	0.75	0	2.90 (1.92, 4.39)	<0.01			
Therapeutic regimen											
Platinum-based	6	65/236	104/283	9.26	0.1	46	1.62 (1.10, 2.39)	0.01	0.03	0.86	0
Non platinum-based	4	25/106	36/97	11.90	0.008	75	1.79 (0.41, 7.70)	0.44			
Patient origin											
Asia	6	55/187	91/267	11.23	0.05	55	1.31 (0.65, 2.62)	0.45	1.08	0.30	7.4
Non-Asia area	3	35/155	49/113	5.30	0.07	62	2.42 (0.95, 6.13)	0.06			

BRCA1, breast cancer susceptibility gene 1; ORR, objective response rate.

Figure 4 Subgroup analyses regarding objective response rate in patients who received anti-microtubule agents with high *BRCA1* expression *vs*. low *BRCA1* expression. *BRCA1*, breast cancer susceptibility gene 1.

population but not in the Asian population (for non-Asian population studies, high *vs*. low expression, OR 2.42, 95% CI: 0.95 to 6.13, Chi^2 =11.23, P=0.06; for Asian studies, high *vs*. low expression, OR 1.31, 95% CI: 0.65 to 2.62, Chi^2 =5.3, P=0.45) but the interaction was not significant (Chi^2 =1.08, P=0.3). Details about the results on subgroup analysis are shown in *Table 1* and *Figure 4*.

Discussion

Due to its ubiquitous presence and importance in all cells, microtubules are one of the most validated intracellular targets in oncology (25). Because of this, the mechanism of resistance to anti-microtubule agents earn widespread concerns and many studies have reported on the subject. Several mechanisms explain the resistance, including decrease of the cellular accumulation mediated by P-glycoprotein (26) exportation and altered expression or post-translational modification of tubulin or other microtubule regulatory proteins (27).

Recently, some studies have reported on the relationship and mechanism between *BRCA1* expression and chemotherapy outcomes for carcinoma, but the results were controversial. Therefore a meta-analysis is needed to incorporate all available results to give further insight on this conflicting issue. After combining the available data of the included studies, our results were in concordance with our initial hypothesis that increased *BRCA1* expression might be associated with higher sensitivity of anti-

microtubule agents and longer PFS/OS. However, the effect sizes of all syntheses were not statistically significant.

Since significant heterogeneity was observed in the overall analyses, we carried out additional subgroup analyses. Interestingly, our results show that using non-immunohistochemical (PCR and Relative cDNA quantification) detection methods offer the notable result that high *BRCA1* expression was associated with higher sensitivity of anti-microtubule agents whereas the difference in the subgroup using IHC was not significant (for non-immunohistochemical study, high *vs*. low expression, OR 2.90, 95% CI: 1.92 to 4.39, P<0.01; I^2=0; for immunohistochemical studies, high *vs*. low expression, OR 0.60, 95% CI: 0.33 to 1.10, P=0.10; I^2=0), and the heterogeneity of the subgroup was extreme(P<0.01, I^2=94%). This result implies that PCR and Relative cDNA quantification maybe a more accurate evaluation method compared to IHC in determining the expression of *BRCA1*.

IHCis a process that exploits the principle of antibodies binding specifically to antigens in biological tissues to detect antigens (e.g., proteins) (28). The detection target of IHC is the proteins which are at the last destination to take a leading role in biological effects, so it has clinical significance but it also carries obvious limitations. Firstly, an antibody may not be specific to the object protein since one antibody may combine with a variety of proteins. Secondly, many factors can cause a false positive or a false negative result. For example, the concentration and the effect of an antibody, whether reagent covers the tissue, and the incubation time of the antibody can cause a false negative result, whereas improper selection of antigen retrieval method, antibody titer deduced or failure can result in false negative results (29). Another limitation is that IHC only carries out semi-quantitative assessment of the protein expression, and the judgments of the pathologist are inevitably subjective. By contrast, q-PCR based mRNA level detection is a quantitative determination. It has the advantages of: (I) accurate quantification; (II) reliable sensitivity and specificity; (III) reducing pollution and automation, etc. Because of this, we believe that detection based on mRNA might be a preferred technique over IHC. We are looking forward to future research to further prove our conclusion and explore the cause.

Another issue that captivated our attention is the confounding effect of cisplatin on the predictive value of *BRCA1*. Several cell studies, based on clinical trials, demonstrated high/positive *BRCA1* expression could resist platinum-based chemotherapy. But cisplatin and anti-microtubule agents are often combined in cancer therapy due to their differing mechanisms of action. The question now is how we might determine whether an anti-microtubule agent the proper choice according to *BRCA1* expression. In subgroup analysis based on therapeutic regimen, no subgroup difference was found between the platinum-based population and then on platinum-based population. According to this result, we believe the existence of platinum in chemotherapy regimen did not offer confounding effect to *BRCA1* expression. The predictive value of *BRCA1* for anti-microtubule agents is valid.

This is the first study to address the interaction between *BRCA1* expression and the outcome of anti-microtubule agents in cancer patients. However, there are several limitations. First, it was based on retrospective analysis; prospective analysis is needed to further clarify these issues. Second, although our purpose is the prediction of *BRCA1* for paclitaxel, we cannot eliminate the effects of the combination of the chemotherapeutic agents. In addition, we are unable to study the effects on each cancer separately and are therefore unable to distinguish the individual role of *BRCA1*. Further studies are necessary to validate our results.

In conclusion, although the predictive value of *BRCA1* expression on the anti-microtubule chemotherapy remained uncertain based on overall results, our exploratory analyses suggested that detection using mRNA might be a preferred technique over IHC, however, further validation is required to substantiate our findings.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Supplementary

Table S1 Characteristics of eligible studies evaluating *BRCA1* level and clinical outcome

Lead author [Y] (ref.)	Tumor type	No. of patients	Median age	Patient	Stage	Chemotherapyregimen	*BRCA1* detection	Antibody	Assessment	Evaluation	Cut off	*BRCA1*	CR + PR	SD + PD
Zimling [2012] (6)	Malignant pleural Mesothelioma	49	64	Europe	I-IV	Vinorelbine 25 mg m^{-2} i.v. weekly and cisplatin100 mg i.v. every 4 weeks	IHC	Mousemonoclonal anti-human *BRCA1*	H-score	Positive: H-score ≥ upper quartile; Negative: H-score < upper quartile		Negative	10	16
												Positive	5	11
Gao [2013] (14)	Esophageal Squamous cell Carcinoma	45	62	Asian	II-IV	Docetaxel (60–75 mg/m^2) plus 5-fluorouracil (500 mg/m^2, D1, D5)	qPCR				Using a cut off value of 11.96	Low	6	17
												High	12	10
Papadaki [2011] (15)	NSCLC	131	60	Europe	IIIB-IV	Docetaxel cisplatin; docetaxel gemcitabine	cDNA quantification				Median value	Low	13	53
												High	27	38
Su [2011] (16)	NSCLC	85	60	Asian	IIIB-IV	Cisplatin (75 mg/m^2) or carboplatin (AUC =5) pius vinorelbine (25 mg/m^2, D1, D8) orpaclitaxe (175 mg/m^2)	Real-time PCR				The median expression levels (10.11×10^{-2})	Low	8	21
												High	28	28
Kim [2005] (17)	Breast cancer	60	NA	Asian		Docetaxel (60mg/m^2, q3w), four cycles unless progressive disease	IHC	Ab-1 (MS110) provider: oncogene (Cambridge, MA)			According to the previous reports (10%)	Low	5	9
												High	9	37
Kurebayashi [2006] (18)	Breast cancer	19	58	Asian	Primary	13 patients received taxane (docetaxel or paclitaxel) alone, 3 patients taxane with medroxyprogesterone Acetate, 2 patients taxane with pamidronate and one taxane with trastuzumab	IHC	Rabbit polyclonal, Ab-1,Oncogene, Boston, MA, USA			10% of the tumor cells	Absence	4	9
												Present	1	5
Zhao [2014] (19)	SCLC	158	59	Asian	IIIB-IV	Cisplatin (75 mg/m^2, D1) or carboplatin (AUC =5, D1) plus gemcitabine (1,000 mg/m^2, D1, D8), vinorelbine (30 mg/m^2, D1, D8) or paclitaxel (175 mg/m^2, D1)	Fluorescence-based, real-time detection method				The median expression levels (4.3)	Low	9	54
												High	24	71
Wan [2011] (20)	Breast cancer	87	NA	Asian	IIIB-IV	Taxanes (150 mg/ m^2, D1) plus cisplatin (25mg/m^2, D1-3)	IHC	Mouse anti-*BRCA1*; monoclonal antibody (ZHGB BIO, China)		According to the previous studies, positive≥10% of the tumor cells negative<10% of the tumor cells		Low	23	22
												High	17	25
Boukovinas [2008] (7)	NSCLC	95	60	Europe	IIIB-IV	Gemcitabine (1,000 mg/m^2, D1, D8) plus docetaxel (100 mg/m^2 D8)	PCR				Median mRNA expression levels (3.64)	Low	12	51
												High	17	15
Lesnock [2013] (21)	Ovarian cancer	393	NA	White black and other	I-III	Intravenous paclitaxel and cisplatin or combination of intravenous paclitaxel and intraperitoneal cisplatin and paclitaxel	IHC	MS110 clone; monoclonal antibody Ab-1 (Oncotech Inc., Tustin, CA, USA)		Low *BRCA1* expression: <10% staining; normal:>10% staining				
Weberpals [2011] (22)	Ovarian carcinoma	116	57	Europe	II-IV	Cisplatin plus topotecan followed by paclitaxel plus carboplatin or carboplatin plus paclitaxel	IHC	Mouse monoclonal *BRCA1* antibody (MS110, Calbiochem, Darmstadt, Germany)			*BRCA1* was 2.5			
Papadaki [2012] (23)	NSCLC	100	63	Europe	IV	Docetaxel/gemcitabine or vinorelbine/gemcitabine	Relative cDNA quantification				Median mRNA expression levels (4.28)			
Wei [2011] (24)	Gastric cancer	152	58	Asian	III-IV	Docetaxel-based	q-PCR				Cut-off point for *BRCA1* was 4.6			

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; IHC, Immunohistochemistry; qPCR, real-time polymerase chain reaction; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; *BRCA1*, breast cancer susceptibility gene 1.