

Bevacizumab Preconditioning Followed by Etoposide and Cisplatin Is Highly Effective in Treating Brain Metastases of Breast Cancer Progressing from Whole-Brain Radiotherapy

Yen-Shen Lu^{1,2}, Tom Wei-Wu Chen¹, Ching-Hung Lin^{1,2}, Dah-Cherng Yeh³, Ling-Ming Tseng⁴, Pei-Fang Wu¹, Kun-Ming Rau⁵, Bang-Bin Chen⁶, Ta-Chung Chao⁷, Shu-Min Huang¹, Chiun-Sheng Huang⁸, Tiffany Ting-Fang Shih⁶, and Ann-Lii Cheng^{1,2} for the Taiwan Breast Cancer Consortium

Abstract

Purpose: We hypothesized that a window period between bevacizumab and cytotoxic agents may enhance drug delivery into tumor tissue through bevacizumab-induced vascular normalization in patients with brain metastases of breast cancer (BMBC).

Experimental Design: A single-arm phase II study was conducted in which BMBC patients refractory to whole-brain radiotherapy (WBRT) were enrolled. In a 21-day cycle, patients received bevacizumab (15 mg/kg) on day 1, which, with a 1-day window period, was followed by etoposide (70 mg/m²/day; days 2–4) and cisplatin (70 mg/m²; day 2; BEEP regimen). The BEEP regimen was administered for a maximum of 6 cycles. The primary endpoint was the central nervous system (CNS)–objective response rate according to volumetric response criteria.

Results: A total of 35 patients were enrolled between January 2011 and January 2013. The median age was 54.3 years (range,

33–75); 19 patients (54.3%) had an Eastern Cooperative Oncology Group performance status of 2 or 3. Twenty-seven patients [77.1%; 95% confidence interval (CI), 59.9–89.6] achieved a CNS-objective response, including 13 patients (37.1%) with a ≥80% volumetric reduction of CNS lesions. With a median follow-up of 16.1 months, the median CNS progression-free survival and overall survival times were 7.3 months (95% CI, 6.5–8.1) and 10.5 months (95% CI, 7.8–13.2), respectively. Common grade 3 or 4 toxicities included neutropenia (30.8%) and infection (21.3%).

Conclusions: By administering bevacizumab 1 day before etoposide and cisplatin, the BEEP regimen appeared highly effective in BMBC refractory to WBRT. Further study of vascular normalization window concept is warranted. *Clin Cancer Res*; 21(8): 1851–8. ©2015 AACR.

Introduction

With a better improved control of systemic disease, brain metastasis has become a common complication in breast cancer. However, management of brain metastases remains a significant challenge (1, 2). First-line local treatment modalities for brain metastases include whole-brain radiotherapy (WBRT), stereotac-

tic surgery, and surgical operation. The median overall survival (OS) times are approximately 5, 9, and 5 months for hormone-receptor–positive, HER2/neu–positive, and triple-negative brain metastases of breast cancer patients who receive WBRT, respectively (3). For brain metastases of breast cancer patients refractory to WBRT, second-line treatment options remain limited.

Chemotherapy is not considered suitable for central nervous system (CNS) tumors because of the blood–brain barrier (BBB), which limits the entrance of systemically administered chemotherapeutic drugs into the brain tumor lesion. However, several studies have suggested that the BBB can be partially disrupted during the growth of a metastatic brain tumor, thus allowing a certain amount of chemotherapeutic drugs to be delivered to the brain tumor lesion (4). Chemotherapy combining etoposide and cisplatin demonstrated an overall response rate of 38% to 55% in patients with brain metastasis of breast cancer who had not received WBRT (5, 6). Prospective clinical studies that have focused on treating breast cancer with progressive brain metastases after WBRT are limited to HER2-positive subtype. A treatment combining capecitabine with lapatinib, a small-molecule anti-HER2-targeted agent, resulted in a response rate of 18% to 38% and a time-to-progression (TTP), or progression-free survival (PFS), of 2.8 to 5.1 months in HER2-positive patients with brain metastases who had been predominantly (86%–100%) pretreated with WBRT (7–9).

¹Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan. ²Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan. ³Department of Surgery, Taichung Veterans General Hospital, Taichung, Taiwan. ⁴Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan. ⁵Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan. ⁶Department of Medical Imaging, National Taiwan University Hospital, Taipei, Taiwan. ⁷Division of Hematology and Oncology, Department of Internal Medicine, Taipei Veterans General Hospital, Taipei, Taiwan. ⁸Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan.

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Corresponding Author: Ann-Lii Cheng, National Taiwan University Hospital, No. 7, Chung-Shan South Rd., Taipei 10002, Taiwan. Phone: 886-2-23123456, ext. 67251; Fax: 886-2-23711174; E-mail: alcheng@ntu.edu.tw

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Translational Relevance

Management of brain metastases of breast cancer that progresses after whole-brain radiotherapy (WBRT) remains a challenge. Bevacizumab, an antivascular endothelial growth factor antibody, has been shown to have an ability to create a normalization window of peritumoral vessels in preclinical brain metastases models, thus enhancing the delivery of cytotoxic agents to the tumor. A window period between the administration of bevacizumab and cytotoxic agents may optimize the treatment effect. This phase II study demonstrated that administration of a combination of etoposide and cisplatin preceded by bevacizumab with a 1-day window period (BEEP regimen) is highly effective in brain metastases of breast cancer refractory to WBRT. The central nervous system (CNS)-objective response rate was 77.1% according to volumetric criteria and 54.3% according to RECIST. The CNS-specific progression-free survival was 7.3 months, even though 54.3% of patients had an Eastern Cooperative Oncology Group performance status of 2 or 3 before treatment. On the basis of these data, further study of this concept is warranted.

Studies have suggested that leaky peritumoral vessels leading to increased interstitial pressure and decreased tumor perfusion may be an explanation for poor chemotherapy efficacy in metastatic brain tumors (10). Bevacizumab, an antivascular endothelial growth factor (anti-VEGF) antibody, has exhibited an ability to normalize peritumoral vessels in preclinical metastatic brain models (11), leading to an enhanced delivery of cytotoxic agents to the tumor. The combination of bevacizumab with chemotherapeutic agents has proven effective for treating breast, lung, and colon cancers; however, its role in the treatment of brain metastases remains unclear because of the exclusion of these patients from clinical trials. For improving the delivery and efficacy of chemotherapy, the administration of chemotherapeutic agents during an optimal period of bevacizumab-mediated tumor vessel normalization window is crucial. Thus, a careful decision should be made regarding the latency period between the administration of bevacizumab and cytotoxic agents. In an *in vivo* xenograft study, the administration of chemotherapy to tumor-bearing mice 1 to 3 days after the administration of bevacizumab resulted in a higher intratumoral chemotherapy penetration and tumor-growth inhibition compared with the concomitant administration of the 2 drugs (12).

We conducted a phase II study on the basis of the hypothesis that the administration of bevacizumab 1 day before chemotherapy can enhance the activity of etoposide and cisplatin by increasing the drug delivery into tumor tissue through bevacizumab-induced vascular normalization. In our proof-of-concept exploratory study, 8 patients underwent serial dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) of the brain to determine the vascular normalization effects of bevacizumab in metastatic brain tumor lesions. Although tumor vascular normalization was observed 1 hour after bevacizumab was infused (2.5 hours from the time it started), the vascular normalization process became markedly noticeable 24 hours after the administration of bevacizumab (13). This report presents the clinical outcomes of the complete phase II clinical trial.

Materials and Methods

Patients

Patients were enrolled between January 2011 and January 2013 from 4 medical centers in Taiwan. Female patients with breast cancer who had developed progressive brain metastases after WBRT, as proven through an image study (CT or MRI), and had at least one measurable lesion (≥ 10 mm at the longest diameter, as measured using contrast-enhanced CT or T1-weighted, gadolinium-enhanced MRI), were enrolled in the study. Additional major inclusion criteria were an age of 18 to 75 years; adequate hematologic, renal, and hepatic functions; and an Eastern Cooperative Oncology Group performance status (ECOG PS) ≤ 3 . Concurrent antineoplastic agents and radiotherapy were not permitted, but those patients who required nonescalating steroids with a daily total dose of 10 mg of prednisone or equivalent were considered to be eligible. HER2-positive patients, defined as having a immunohistochemistry score of 3+ or evidence of gene amplification according to FISH, were informed about the treatment option with a combination of lapatinib and capecitabine; however, treatment with lapatinib before enrolling in the study was not mandatory. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki. The Institutional Review Board for each participating institution individually approved the study protocol. This trial is registered at ClinicalTrials.gov, identifier NCT01281696.

Study design

This was a single-arm, open-label, phase II study, with the aim of demonstrating the efficacy of bevacizumab, etoposide, and cisplatin (BEEP regimen) in treating the metastatic brain tumors of breast cancer patients. The primary endpoint was the CNS-objective response rate (ORR) according to the volumetric criteria (7). Secondary endpoints included PFS, CNS-specific PFS, and OS.

Treatment plan

Patients received bevacizumab (15 mg/kg; day 1), etoposide (70 mg/m²/day; days 2–4), and cisplatin (70 mg/m²; day 2). Treatment was based on a 21-day cycle, for a maximum of 6 cycles. However, it was discontinued if a patient exhibited disease progression, showed intolerable toxicities, or died.

Adverse events were assessed according to Common Terminology Criteria for Adverse Events (CTCAE) 3.0. If the patients experienced grade 3 or 4 hematologic toxicities, the doses of etoposide and cisplatin were deescalated to 60 mg/m². If it was determined that grade 3 or 4 toxicities were related to bevacizumab, then bevacizumab was discontinued until the toxicity resolved to grade 1 or 2. Prophylactic use of the G-CSF was not suggested initially, but was amended in the protocol to mandatory use because of a higher-than-expected grade 3 or 4 neutropenia incidence during the protocol treatment.

Efficacy assessment

Brain tumor assessment was conducted using contrast-enhanced CT or T1-weighted gadolinium-enhanced MRI every 9 weeks. MRI T2 fluid-attenuated inversion recovery (FLAIR) images were not selected as the tumor assessment method because of diffuse white matter changes after WBRT (14, 15). Images were obtained at 5-mm slice thickness without gaps in the axial dimension. For each patient, the same imaging modality was used throughout the trial.

All brain images were centrally reviewed by two radiologists in our institution, who were blinded to the clinical status of the patients. The total tumor volume was the sum of the target lesions (a maximum of 3) selected within the brain parenchyma. The measurement of tumor volume was performed using a commercial software MIStar (Apollo Medical Imaging). The efficacy was evaluated according to volumetric response criteria modified from those of the study by Lin and colleagues (7). A CNS-objective response was defined as either a complete response or partial response ($\geq 50\%$ reduction in the volumetric sum of all measurable target CNS lesions), if no increase in the dosage of steroids was noted, no deterioration of neurologic symptoms and signs occurred, and no progressive extra-CNS disease developed. CNS disease progression was defined as one of the following: a $\geq 40\%$ increase in the volumetric sum of all measurable target CNS lesions, a new brain parenchyma lesion measuring ≥ 6 mm at its longest diameter (in original version of volumetric criteria, they did not define the size criteria of new lesion), deterioration of neurologic symptoms and signs, or increased steroid dosage because of the CNS tumor. In the protocol amendment, RECIST 1.1 was also added as a method for assessing CNS tumor response (16). The evaluation of the extra-CNS tumor using contrast-enhanced CT images was conducted every 3 months, and its response was evaluated according to RECIST 1.1 (16).

Neurologic examinations were conducted within 1 day of each BEEP cycle. The results were recorded in a neurologic examination worksheet adapted from the study by Lin and colleagues (7), which contained 7 categories of neurologic assessments that were measured according to CTCAE 3.0.

Statistical analysis

The sample size was calculated using a Simon 2-stage design (17). The efficacy of BEEP was considered of little and high interest for CNS-ORRs of 15% (H0) and 30% (H1), respectively. With an alpha of 0.15 and a power of 80%, the BEEP treatment was rejected as an efficacious regimen if the CNS tumor response rate was $\leq 1/11$ at the first stage or $\leq 6/31$ at the second stage.

All statistical analyses were performed using SPSS Version 17.0 (SPSS) in the intent-to-treat population. The brain and extra-CNS response rates were reported with 95% confidence intervals (CI). Time-to-event endpoints were summarized using the Kaplan-Meier method. PFS is defined as the interval from the date of the first protocol chemotherapy to the date of the first radiological-documented CNS, extra-CNS disease progression, or death from any cause. CNS PFS is defined as the interval from the date of first protocol chemotherapy to the date of the first radiological-documented CNS disease progression or death from any cause.

Results

Patient characteristics and prior therapies

A total of 35 patients were enrolled between January 2011 and January 2013. Of them, 33 patients (94.3%) exhibited extra-CNS metastases at the baseline (Table 1). The most common metastatic sites were bone (62.9%), lung (60.0%), and liver (40.0%). In total, 6 patients were estrogen receptor (ER)-positive/HER2-negative, 23 patients were HER2-positive, and 6 patients were triple-negative. The diagnosis for these subtypes was determined on the basis of primary tumor or axillary lymph node in 33 and 2 patients, respectively. Two patients had both brain and primary tumors enabling a comparison of the subtype status. No subtype

Table 1. Demography and clinical characteristics of patients ($n = 35$)

Characteristics	n (%)
Age	
Median (range)	54.3 (33.4–75.0)
<50 years	9 (25.7)
50–60 years	17 (48.6)
>60 years	9 (25.7)
ER ^a and HER2 status	
ER-positive/HER2-negative	6 (17.1)
ER-positive/HER2-positive	9 (25.7)
ER-negative/HER2-positive	14 (40.0)
ER-negative/HER2-negative	6 (17.1)
ECOG PS	
0–1	16 (45.7)
2	8 (22.9)
3	11 (31.4)
Number of extra-CNS metastatic sites	
Median (range)	2 (0–3)
0	2 (5.7)
1	10 (28.6)
2	12 (34.3)
3	11 (31.4)
CNS-directed therapy	
WBRT	35 (100.0)
Stereotactic radiosurgery	9 (25.7)
Surgery	7 (20.0)
Intrathecal treatment	1 (2.9)
Previous chemotherapy	
Chemotherapy line in metastatic setting	
Median (range)	3 (1–8)
1	5 (14.3)
2	8 (22.9)
3	7 (20.0)
More than 3	15 (42.9)
Previous Her2-targeted therapy	($n = 23$)
Trastuzumab	22 (95.7)
Lapatinib	11 (47.8)
Others ^b	2 (8.7)

^aThere is no patient with ER⁺PR⁺ in this study.

^bIncluding afatinib and TDM-1.

discordance was observed between the brain and primary tumors of both the patients: 1 patient was ER-positive/HER2-negative and the other was ER-positive/HER2-positive. Table 1 presents a summary of the average number of lines of chemotherapy before the BEEP regimen, history of CNS-directed therapy, and anti-HER2-targeted therapy. Cisplatin and etoposide were administered to 6 and 4 patients, respectively, for metastatic disease systemic treatment before they were enrolled in this study.

A total of 169 cycles of the BEEP regimen were administered, with a median of 6 cycles per patient at the time of the data cutoff (February 28, 2014). A total of 20 patients (57.1%) completed 6 cycles of the BEEP treatment. Two patients decided to receive hospice care after the first cycle of treatment, but died 16 and 20 days after the study treatment began. Eight patients discontinued the protocol treatment because of disease progression, of which 7 patients exhibiting extra-CNS disease progression with the CNS lesions remained under control. Eighteen patients (51.4%) received dose reductions for etoposide (60 mg/m²) and cisplatin (60 mg/m²), and 3 patients discontinued protocol treatment because of toxicity. All 35 patients were included in the safety and efficacy analysis as intent-to-treat analysis.

Safety and toxicity

Table 2 presents a summary of the toxicity profile. Hematologic toxicity was a primary concern, but was generally manageable.

Table 2. Incidence of common adverse events and adverse events of interest

Adverse events	Cycles (N = 169)	
	Grade 1/grade 2 n (%)	Grade 3/grade 4 n (%)
Hematologic toxicity		
Neutropenia	48 (28.4)	52 (30.8)
Leukopenia	68 (40.2)	27 (16.0)
Anemia	68 (40.2)	12 (7.1)
Thrombocytopenia	51 (30.2)	13 (7.7)
Nonhematologic toxicity		
Hypertension ^a	63 (37.3)	1 (0.6)
Nausea	46 (27.2)	0 (0.0)
ALT/AST Increased	33 (19.5)	2 (1.2)
Infection with normal ANC or grade 1/2 neutropenia	5 (3.0)	26 (15.4)
Hyponatremia	21 (12.4)	7 (4.1)
Vomiting	26 (15.4)	1 (0.6)
Proteinuria ^a	25 (14.8)	0 (0.0)
Hypokalemia	17 (10.1)	7 (4.1)
Fatigue	21 (12.4)	0 (0.0)
Epigastralgia	18 (10.7)	0 (0.0)
Infection with grade 3/4 neutropenia ^a	0 (0.0)	10 (5.9)
Creatinine increased ^a	8 (4.7)	0 (0.0)
Febrile neutropenia ^a	0 (0.0)	3 (1.8)
Hemorrhage ^a	3 (1.8)	0 (0.0)
Cerebellum infarction ^a	0 (0.0)	1 (0.6)
Tracheoesophageal fistula ^a	0 (0.0)	1 (0.6)
Dose reduction due to toxicity		18 (51.4)
Treatment discontinued due to toxicity		3 (8.6)

Abbreviation: ALT, alanine transaminase, ANC, absolute neutrophil count, AST, aspartate transaminase.

^aAdverse events of interest.

Nonhematologic toxicity was generally mild. Two patients died: one of infection and one of a tracheoesophageal fistula. The patient with tracheoesophageal fistula presented with extensive mediastinal lymph node metastases between the esophagus and trachea at the baseline, but the CNS tumors completely resolved after 3 cycles of the BEEP treatment. Because of the high incidence of grade 3 or 4 neutropenia, we amended the protocol to mandate prophylactic filgrastim (300 µg/day) treatment starting 3 days after the administration of the BEEP. After the amendment, the incidence of grade 3 or 4 neutropenia decreased from 37% to 12% per cycle.

Clinical efficacy of the BEEP

Twenty-seven patients (77.1%; 95% CI, 59.9–89.6) achieved an objective CNS response. Thirteen patients (37.1%) showed a CNS volumetric reduction of ≥80% (Table 3); 2 patients (5.7%) had a complete response. Overall, 33 patients (94.3%) exhibited tumor volume reduction from the baseline (Table 3 and Fig. 1). The trend of brain tumor volume change according to time is depicted in Fig. 1B (partial response patients) and C (stable disease patients). Tumor response could not be assessed for 2

Table 3. CNS response evaluation (n = 35)

Evaluation	n (%)
Best volumetric CNS response	
≥80% reduction	13 (37.1)
50%–<80% reduction	14 (40.0)
20%–<50% reduction	6 (17.1)
0%–<20% reduction	0 (0.0)
Progression	0 (0.0)
Nonevaluable ^a	2 (5.7)

^aPatients received hospice care after 1st cycle of treatment.

patients because they withdrew from the study to receive hospice care after the first cycle of treatment. Both patients died within 21 days of the first date of protocol treatment, and the possibility of disease progression in their case cannot be excluded. No difference was seen in the CNS-ORR between patients examined using CT (13 of 17 patients, 76.5%) and MRI (14 of 18 patients, 77.8%) as the image evaluation modality. Even if we lowered the threshold of tumor progression to 25% increase in total CNS tumor volume [closer to World Health Organization (WHO) two-dimensional criteria; ref. 18], all ORR results remained unchanged.

All patients were also evaluated for optimal CNS response according to RECIST 1.1 (16). Two patients (5.7%) exhibited a confirmed complete response and 19 (54.3%) exhibited a confirmed partial response; therefore, 21 (60.0%) exhibited an objective CNS response. Twelve (34.3%) patients had a stable disease as the best response and 5 (14.3%) of them had a stable disease for at least 18 weeks. Two patients (5.7%) had a non-assessable CNS tumor status. CNS responders were observed among all subtypes (Table 4); the 2 patients who had CNS complete response were HER2-positive/ER-negative and triple-negative.

Impact on survival

With a median follow-up of 16.1 months, the median PFS and OS were 6.1 months (95% CI, 5.0–7.2) and 10.5 months (95% CI, 7.8–13.2), respectively. The median CNS-specific PFS was 7.3 months (95% CI, 6.5–8.1; Fig. 2). The analysis of clinical efficacy according to the ECOG PS, subtype, and previous lapatinib treatment in the HER2-positive subgroup is summarized in Table 4. In comparison with patients with an ECOG PS of 0 to 2, patients with an ECOG PS of 3 exhibited a lower response rate, PFS, and OS. For HER2-positive patients, prior exposure to lapatinib treatment did not affect the clinical efficacy. All triple-negative patients responded to the BEEP treatment, but the CNS-specific PFS, PFS, and OS were the shortest among the 4 subtypes. No significant differences in efficacy were observed according to the maximum diameter of metastatic brain tumor (>3 cm vs. ≤3 cm). The results of CNS-specific PFS and PFS did not change if we lowered the threshold of tumor progression to 25% increase in total CNS tumor volume from baseline.

Post-BEEP regimen course

Of the 20 patients who completed 6 cycles of the BEEP regimen, all but one received additional treatments, including hormonal agents, chemotherapy, or CNS-localized radiotherapy. Six patients received bevacizumab-based regimen with either BEEP regimen or a combination of bevacizumab and a platinum as maintenance treatment, and 15 were retreated with bevacizumab-based regimen. At the final follow-up, 4 patients showed no evidence of disease progression, both in the CNS or extra-CNS regions.

Of the 35 evaluable patients, 11 (31%), 10 (29%), and 3 (9%) had first progression event in CNS, extra-CNS, or both sites, respectively. In 4 (11%) patients, no disease progression was noticed at the last follow-up, and in 7 (20%), no first site of progression was observed (not mandatory as per protocol). A *post hoc* analysis of survival between patients who received maintenance versus no bevacizumab-based regimen after 6 cycles of protocol treatment was performed. The result showed that

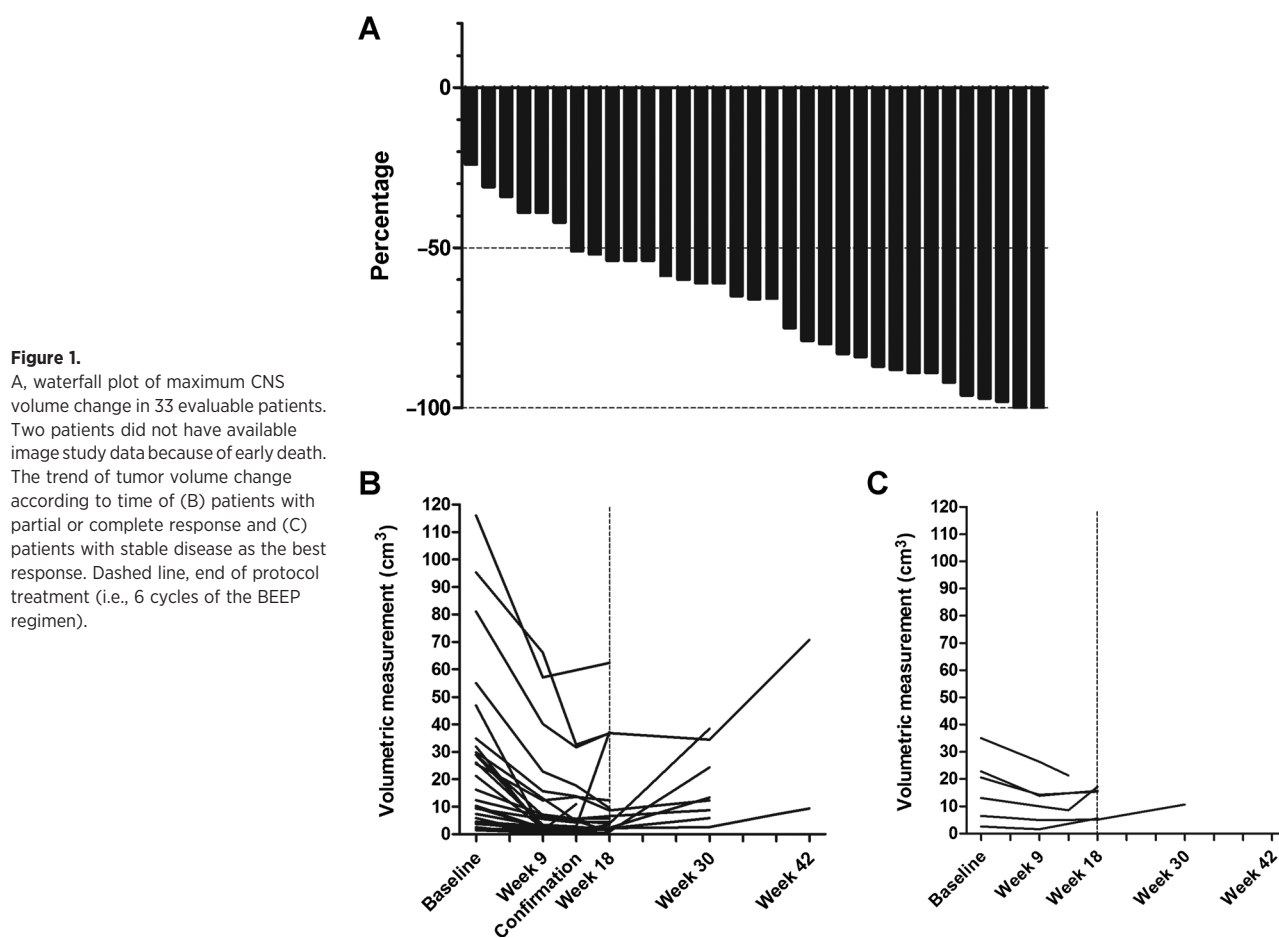


Figure 1.

A, waterfall plot of maximum CNS volume change in 33 evaluable patients. Two patients did not have available image study data because of early death. The trend of tumor volume change according to time of (B) patients with partial or complete response and (C) patients with stable disease as the best response. Dashed line, end of protocol treatment (i.e., 6 cycles of the BEEP regimen).

patients who received maintenance bevacizumab-based regimen experienced significantly longer PFS ($P = 0.009$) and CNS-specific PFS ($P = 0.026$). The median OS was not significantly different ($P = 0.632$; Supplementary Fig. S1).

Discussion

The results suggested that the administration of bevacizumab a day before etoposide and cisplatin (BEEP) regimen is highly efficacious against brain metastases of breast cancer. According to a thorough review of relevant studies, no other systemic treatment modalities have demonstrated an objective CNS response as high as the BEEP regimen in progressive brain metastases of breast cancer patients following WBRT.

We aimed for a 30% CNS-ORR of the BEEP regimen based on previous studies of brain metastasis of breast cancer patients (7–9), as well as the traditional view that the efficacy of salvage chemotherapy is expected to be lower in later lines of chemotherapy, which is widely observed in patients who have undergone WBRT. The results demonstrated that the BEEP regimen had a CNS-ORR of 77%, which was considerably higher than expected. Previous studies using the combination of etoposide and cisplatin have indicated an overall response rate of 38% to 55% according to WHO criteria for brain metastasis of breast cancer patients; however, only 27% to 45% of enrolled patients received prior systemic chemotherapy for metastatic disease and

0% to 9% of patients underwent WBRT before participating in these studies (5, 6). The high response rate in our study indicates that a preconditioning treatment of bevacizumab enhanced the antitumor effect of etoposide and cisplatin, even in metastatic brain tumors. In a study that recruited breast cancer patients with non-CNS metastatic diseases who had undergone prior chemotherapy in a metastatic setting, the administration of bevacizumab on the same day as other chemotherapy drugs, such as taxanes, anthracyclines, capecitabine, gemcitabine, and vinorelbine, indicated only a moderately increased response rate (from the average of 29.6% to 39.5%; ref. 19). The evidence from this and another study (20) suggested that providing a window between bevacizumab and systemic chemotherapy might be a practical method to improve the efficacy of systemic chemotherapy. This is in accordance with the preliminary results of DCE-MRI demonstrating that vascular permeability and diffusion markedly improved in 24 hours after the administration of bevacizumab (13). This evidence supports our hypothesis that a preconditioned tumor vasculature leads to improved drug penetration and efficacy.

Another possible explanation for the high efficacy of the BEEP regimen is a probable synergistic relationship between bevacizumab and platinum drugs for brain metastases of breast cancer patients. In a recent report by Lin and colleagues (21), a therapy combining bevacizumab and carboplatin showed a 64% response rate using volumetric criteria and a 5.7-month PFS in brain metastases of breast cancer patients. However, a preconditioning

Table 4. Clinical efficacy by ECOG PS, previous lapatinib-based treatment, and subtype (*n* = 35)

	<i>N</i>	CNS-ORR		CNS-specific PFS		PFS		OS	
		<i>N</i>	%	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
ECOG PS									
0-1	16	13	81.3	1		1		1	
2	8	7	87.5	1.3 (0.5-3.4)	0.545	2.0 (0.8-5.1)	0.141	5.7 (1.8-18.7)	0.004
3	11	7	63.6	2.2 (0.9-5.1)	0.068	4.0 (1.7-9.6)	0.001	6.9 (2.5-19.2)	<0.001
Previous lapatinib-based treatment ^a									
No	12	9	75.0	1		1		1	
Yes	11	7	63.6	0.5 (0.2-1.3)	0.151	0.6 (0.3-1.5)	0.291	0.8 (0.3-2.2)	0.652
Subtype									
ER ⁺ /HER2 ⁺	9	4	44.4	1		1		1	
ER ⁻ /HER2 ⁺	14	12	85.7	1.7 (0.7-4.6)	0.274	1.3 (0.5-3.3)	0.596	1.5 (0.5-4.4)	0.460
ER ⁺ /HER2 ⁻	6	5	83.3	1.4 (0.4-4.2)	0.593	3.7 (1.2-12.1)	0.029	1.7 (0.5-5.8)	0.428
Triple-negative	6	6	100.0	2.9 (0.9-9.3)	0.070	3.9 (1.2-12.7)	0.025	4.8 (1.4-17.3)	0.015
Maximum brain tumor diameter									
≤3 cm	24	17	70.8	1		1		1	
>3 cm	11	10	90.9	0.6 (0.3-1.4)	0.254	0.6 (0.3-1.4)	0.244	1.1 (0.5-2.5)	0.795

NOTE: Bold numbers signify that the *P* value is less than 0.05.

^aIn HER2 overexpress/amplification patients, *N* = 23.

effect of bevacizumab could still partly explain the high response rate in this study. Although the bevacizumab and carboplatin were administered on the same day for most of the treatment cycles, there was a 1-week window between the administration of bevacizumab and carboplatin in the first cycle of every patient. The retention half-life of carboplatin is approximately 30 hours, whereas that of cisplatin is 1.5 to 3.6 hours. The long half-life of carboplatin may cause most of the drug to remain in the circulation even 24 hours after bevacizumab is injected. The characteristics of the study population in the study by Lin and colleagues (21) differed from those of our study population. First, Lin and

colleagues recruited only patients with an ECOG PS of ≤2 and 87% were ECOG PS of 0 to 1, whereas 31% of the patients in our study had an ECOG PS of 3 and 23% were ECOG 2. Second, 22.6% of the patients in the study by Lin and colleagues did not receive WBRT. Finally, all HER2-positive patients received trastuzumab in addition to bevacizumab and carboplatin in the study by Lin and colleagues, whereas none of the HER2-positive patients in our study received trastuzumab during the 6 cycles of the BEEP regimen. The high response rates and long PFS of bevacizumab combined with a platinum-based regimen in brain metastasis of breast cancer patients warrant further exploration.

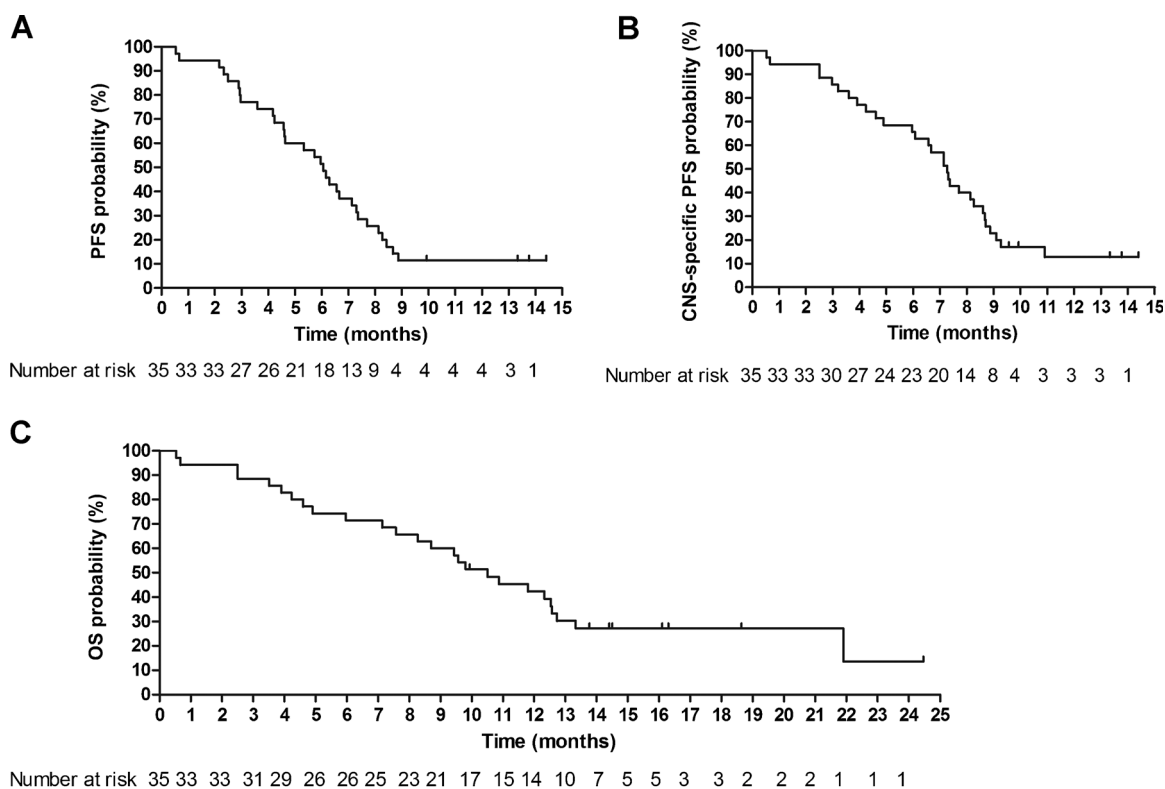


Figure 2.

A, PFS. Median PFS was 6.1 months (95% CI, 5.0-7.2 months); 4 patients (11.4%) were censored. B, CNS-specific PFS. Median CNS-specific PFS was 7.3 months (95% CI, 6.5-8.1 months); 5 patients (14.3%) were censored. C, OS. Median OS was 10.5 months (95% CI, 7.8-13.2 months); 9 patients (25.7%) were censored.

Seven patients in our study discontinued protocol treatment because of extra-CNS systemic disease progression, whereas the CNS lesions remained under control, indicating possible specific efficacy of this regimen for metastatic brain lesions from breast cancer.

The optimal assessment method for metastatic brain lesions of solid tumors after the administration of bevacizumab remains debatable (22). We used contrast-enhanced volumetric response of the metastatic brain tumors as a demonstration of the BEEP regimen efficacy for 2 reasons. First, although the Response Assessment in Neuro-Oncology criteria suggested including T2/FLAIR images to further delineate the response of primary brain tumor after the administration of bevacizumab or other antiangiogenic agents (23), the observation of post-WBRT diffuse white matter changes in T2/FLAIR images did not facilitate but rather hindered the accurate measurement of metastatic brain lesions, which is a phenomenon also observed in other studies (14, 15). Thus, we were constrained to use contrast-enhanced images for efficacy assessment instead of T2/FLAIR images. Second, in recent medical literature, volumetric response criteria and RECIST have been used as primary endpoints to evaluate agents in brain metastasis breast cancer patients (7, 24–26). Because this was a single-arm study, it was critical to use similar assessment criteria to be able to compare with previous study results. In addition, volumetric response criteria were considered more likely to observe subtle changes in metastatic brain tumor size compared with RECIST in metastatic brain lesions (7, 25). After thorough discussion, we decided to use volumetric response criteria as a primary endpoint and RECIST as an exploratory endpoint with contrast-enhanced images as our efficacy endpoints of the BEEP regimen. Nevertheless, the best cutoff value of tumor volumetric change as to determine the CNS tumor status should prompt more detailed investigations and discussions.

Brain metastasis occurs more commonly in patients with HER2-positive and triple-negative breast cancers than in patients with ER-positive breast cancers (27, 28). Treatment with a combination of lapatinib and capecitabine is the only proven active regimen for treating brain metastases of HER2-positive breast cancer. In our study, 23 patients were HER2-positive, and 11 (48%) of them had received lapatinib treatment before enrolling in this trial. The clinical efficacy of the BEEP regimen for the HER2-positive subgroup in our study was higher than that observed in clinical trials in which lapatinib and capecitabine were administered to patients who were previously treated with WBRT (7–9). In a recent study that was restricted to HER2-positive patients who had not been previously treated with WBRT (24), the clinical efficacy, according to the objective CNS response rate (65.9% according to RECIST) and the median TTP (5.5 months), was similar to that observed in our study. Most patients (30 of 38) recruited in the previous bevacizumab and carboplatin study were also HER2-positive. The specific activity of anti-vascular endothelial growth factor receptor-2 (VEGFR2)-targeted therapy in HER2-positive breast tumors in a mouse model with brain metastases was recently reported (29). A clinical trial directly comparing the regimen of bevacizumab and platinum with lapatinib and capecitabine in HER2-positive brain metastasis breast cancer patients is warranted.

The tolerability of the BEEP regimen in a heavily pretreated patient is a primary concern. In this study, nonhematologic toxicities were generally mild, but many patients developed severe hematologic toxicities. Two patients withdrew and 3 discontinued

protocol treatment because of hematologic toxicity. However, the incidence of grades 3 and 4 neutropenia decreased after a protocol amendment that mandated G-CSF prophylaxis.

The short response duration in this study is another concern. In contrast with the high response rate, the PFS was insubstantial. Only 6 cycles (4.5 months) of the BEEP regimen treatment were planned because of a limited budget. Many patients experienced disease progression approximately 3 months after stopping the BEEP treatment. Whether longer BEEP treatment cycles or maintenance bevacizumab can prolong tumor control warrants further exploration.

Our study had several limitations. Firstly, because of the lack of a control arm in our study, selection bias may have confounded the interpretation of the efficacy comparison between the combination of BEEP with other therapeutic regimens. However, with 54.3% of the patients having an ECOG PS of 2 or 3, it was unlikely that the high response rate observed in this study was due to the selection of patients with low tumor burden. Secondly, it would be difficult to determine whether single-agent bevacizumab is also active in brain metastasis in breast cancer as shown in glioblastoma in a single-arm combination regimen study. However, based on the result of a previous study that demonstrated no major activity from single-agent bevacizumab in metastatic breast cancer (30), single-agent bevacizumab is less likely to have been the cause of the high CNS response rate in our study. Finally, except for DCE-MRI imaging, other direct proof-of-concept evidence was not available. If the metastatic brain lesions were available before and after the BEEP treatment, biomarkers such as VEGF or the drug concentration of etoposide and cisplatin could be measured to further understand or prove the theory of vascular normalization.

In conclusion, the administration of bevacizumab 1 day before etoposide and cisplatin (BEEP regimen) was highly effective in brain metastases of breast cancer patients refractory to previous WBRT. Additional studies to confirm and define the optimal combination and sequence of bevacizumab and chemotherapeutic agents are warranted.

Disclosure of Potential Conflicts of Interest

A.-L. Cheng is a consultant/advisory board member for Daiichi Sankyo, Eisai, and Exelixis Inc. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Y.-S. Lu, T.W.-W. Chen, C.-H. Lin, P.-F. Wu, T.-C. Chao, A.-L. Cheng

Development of methodology: Y.-S. Lu, T.W.-W. Chen, T.-C. Chao, A.-L. Cheng

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y.-S. Lu, T.W.-W. Chen, C.-H. Lin, D.-C. Yeh, L.-M. Tseng, P.-F. Wu, K.-M. Rau, T.-C. Chao, C.-S. Huang, T.T.-F. Shih, A.-L. Cheng

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y.-S. Lu, T.W.-W. Chen, B.-B. Chen, A.-L. Cheng

Writing, review, and/or revision of the manuscript: Y.-S. Lu, T.W.-W. Chen, C.-H. Lin, L.-M. Tseng, C.-S. Huang, A.-L. Cheng

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y.-S. Lu, T.W.-W. Chen, C.-H. Lin, K.-M. Rau, S.-M. Huang, C.-S. Huang

Study supervision: Y.-S. Lu, T.W.-W. Chen, L.-M. Tseng, A.-L. Cheng

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References

- Linskey ME, Andrews DW, Asher AL, Burri SH, Kondziolka D, Robinson PD, et al. The role of stereotactic radiosurgery in the management of patients with newly diagnosed brain metastases: a systematic review and evidence-based clinical practice guideline. *J Neurooncol* 2010;96:45–68.
- Tosoni A, Franceschi E, Brandes AA. Chemotherapy in breast cancer patients with brain metastases: have new chemotherapeutic agents changed the clinical outcome? *Crit Rev Oncol Hematol* 2008;68:212–21.
- Dawood S, Gonzalez-Angulo AM, Albarracin C, Yu TK, Hortobagyi GN, Buchholz TA, et al. Prognostic factors of survival in the trastuzumab era among women with breast cancer and brain metastases who receive whole brain radiotherapy: a single-institution review. *Cancer* 2010;116:3084–92.
- Fidler IJ. The role of the organ microenvironment in brain metastasis. *Semin Cancer Biol* 2011;21:107–12.
- Cocconi G, Lottici R, Bisagni G, Bacchi M, Tonato M, Passalacqua R, et al. Combination therapy with platinum and etoposide of brain metastases from breast carcinoma. *Cancer Invest* 1990;8:327–34.
- Franciosi V, Cocconi G, Michiara M, Di Costanzo F, Fossier V, Tonato M, et al. Front-line chemotherapy with cisplatin and etoposide for patients with brain metastases from breast carcinoma, nonsmall cell lung carcinoma, or malignant melanoma: a prospective study. *Cancer* 1999;85:1599–605.
- Lin NU, Dieras V, Paul D, Lossignol D, Christodoulou C, Stemmler HJ, et al. Multicenter phase II study of lapatinib in patients with brain metastases from HER2-positive breast cancer. *Clin Cancer Res* 2009;15:1452–9.
- Lin NU, Eierman W, Greil R, Campone M, Kaufman B, Stepkowski K, et al. Randomized phase II study of lapatinib plus capecitabine or lapatinib plus topotecan for patients with HER2-positive breast cancer brain metastases. *J Neurooncol* 2011;105:613–20.
- Metro G, Foglietta J, Russillo M, Stocchi L, Vidiri A, Giannarelli D, et al. Clinical outcome of patients with brain metastases from HER2-positive breast cancer treated with lapatinib and capecitabine. *Ann Oncol* 2011;22:625–30.
- Jain R. Measurements of tumor vascular leakiness using DCE in brain tumors: clinical applications. *NMR Biomed* 2013;26:1042–9.
- Eichler AF, Chung E, Kodack DP, Loeffler JS, Fukumura D, Jain RK. The biology of brain metastases—translation to new therapies. *Nat Rev Clin Oncol* 2011;8:344–56.
- Dickson PV, Hamner JB, Sims TL, Fraga CH, Ng CY, Rajasekaran S, et al. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. *Clin Cancer Res* 2007;13:3942–50.
- Lu Y-S, Chen B-B, Lin C-H, Chen W-W, Wu P-F, Cheng A-L, et al. Abstract 2984: Normalization of tumor vasculature by anti-angiogenesis therapy in metastatic tumor: a clinical study to determine the timing and effect. *Cancer Res* 2014;74:2984.
- Fujii O, Tsujino K, Soejima T, Yoden E, Ichimiya Y, Sugimura K. White matter changes on magnetic resonance imaging following whole-brain radiotherapy for brain metastases. *Radiat Med* 2006;24:345–50.
- Monaco EA 3rd, Faraji AH, Berkowitz O, Parry PV, Hadelberg U, Kano H, et al. Leukoencephalopathy after whole-brain radiation therapy plus radiosurgery versus radiosurgery alone for metastatic lung cancer. *Cancer* 2013;119:226–32.
- Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer (Oxford, England: 1990)* 2009;45:228–47.
- Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1–10.
- Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;47:207–14.
- Brufsky AM, Hurvitz S, Perez E, Swamy R, Valero V, O'Neill V, et al. RIBBON-2: a randomized, double-blind, placebo-controlled, phase III trial evaluating the efficacy and safety of bevacizumab in combination with chemotherapy for second-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol* 2011;29:4286–93.
- Chang TY, Dong YH, Lin C-H, Lin HH, Kuo SH, Lai C-L, et al. Does chemotherapy schedule matter when combining with bevacizumab? A stratified meta-analysis of randomized controlled trials. *ASCO Meeting Abstracts* 2014;32:1076.
- Lin NU, Gelman RS, Younger WJ, Sohl J, Freedman RA, Sorensen AG, et al. Phase II trial of carboplatin (C) and bevacizumab (BEV) in patients (pts) with breast cancer brain metastases (BCBM). *ASCO Meeting Abstracts* 2013;31:513.
- Lin NU, Lee EQ, Aoyama H, Barani IJ, Baumert BG, Brown PD, et al. Challenges relating to solid tumour brain metastases in clinical trials, part 1: patient population, response, and progression. A report from the RANO group. *Lancet Oncol* 2013;14:e396–406.
- Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol* 2010;28:1963–72.
- Bachelot T, Romieu G, Campone M, Dieras V, Cropet C, Dalenc F, et al. Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. *Lancet Oncol* 2013;14:64–71.
- Lin NU, Carey LA, Liu MC, Younger J, Come SE, Ewend M, et al. Phase II trial of lapatinib for brain metastases in patients with human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol* 2008;26:1993–9.
- Sutherland S, Ashley S, Miles D, Chan S, Wardley A, Davidson N, et al. Treatment of HER2-positive metastatic breast cancer with lapatinib and capecitabine in the lapatinib expanded access programme, including efficacy in brain metastases—the UK experience. *Br J Cancer* 2010;102:995–1002.
- Lin NU, Claus E, Sohl J, Razzak AR, Arnaout A, Winer EP. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. *Cancer* 2008;113:2638–45.
- Lin NU, Winer EP. Brain metastases: the HER2 paradigm. *Clin Cancer Res* 2007;13:1648–55.
- Kodack DP, Chung E, Yamashita H, Incio J, Duyverman AM, Song Y, et al. Combined targeting of HER2 and VEGFR2 for effective treatment of HER2-amplified breast cancer brain metastases. *Proc Natl Acad Sci U S A* 2012;109:E3119–27.
- Cobleigh MA, Langmuir VK, Sledge GW, Miller KD, Haney L, Novotny WF, et al. A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. *Semin Oncol* 2003;30:117–24.