Journal of Cancer Research and Practice

journal homepage: www.ejcrp.org

Original Article

Anti-tumor Efficacy of a Bevacizumab Preconditioning followed by Etoposide and Cisplatin Regimen in Human Epidermal Growth Factor Receptor-2-Positive Breast Cancer Brain Metastasis Refractory to Whole Brain Radiotherapy

Tom Wei-Wu Chen[1,2,3], Ching-Hung Lin[1,4], Dah-Cherng Yeh[5], Ling-Ming Tseng[6], Kun-Ming Rau[7], Bang-Bin Chen[8], Ta-Chung Chao[9], Shu-Min Huang[1], Dwan-Ying Chang[1,2], I-Chun Chen[1,2,3], Ann-Lii Cheng[1,2,4], Yen-Shen Lu, [1,4]* For the Taiwan Breast Cancer Consortium

[1]Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan
[2]Department of Medical Oncology, National Taiwan University Cancer Center, Taipei, Taiwan
[3]Graduate Institute of Oncology, National Taiwan University College of Medicine, Taipei, Taiwan
[4]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan
[5]Breast Medical Center, Cheng Ching Hospital, Taichung, Taiwan
[6]Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan
[7]Department of Hematology Oncology, E-Da Cancer Hospital, Kaohsiung, Taiwan
[8]Department of Medical Imaging, National Taiwan University Hospital, Taipei, Taiwan
[9]Department of Oncology, Taipei Veterans General Hospital, Taipei, Taiwan

Abstract

Background: For human epidermal growth factor receptor-2 (HER2)-positive metastatic breast cancer (MBC), treating brain metastasis (BM) remains challenging. We have previously demonstrated that administering bevacizumab 1 day before etoposide and cisplatin (BEEP) can significantly improve antitumor efficacy in cases of breast cancer with BM. Herein, we report the antimetastatic brain tumor efficacy of BEEP in an HER2-positive subpopulation. **Materials and Methods:** Thirty-five MBC patients with BM were enrolled from January 2011 to January 2013. BEEP was given in 21 day cycles: bevacizumab 15 mg/kg on day 1, etoposide 70 mg/m^2/day from days 2 to 4, and cisplatin 70 mg/m^2 on day 2. The primary endpoint was composite central nervous system (CNS) volumetric objective response rate (ORR). Anti-HER2 treatments were not permitted during the clinical trial. **Results:** A total of 23 patients were HER2-positive, 9 ER-positive, and 14 ER-negative. All had been exposed to trastuzumab; 11 (47.8%) had received lapatinib treatment, and 6 (26.1%) of them had received both lapatinib and capecitabine treatment. Of these, 16 patients (69.6%, 95% confidence interval [CI] 47.1–86.8) achieved CNS-ORR, including 7 (30.4%) with ≥80% and 9 (39.1%) with 50%–80% CNS volumetric reduction. A further 5 patients (21.7%) had 20%–50% CNS volumetric reduction. Median CNS-specific progression-free survival and overall survival were 7.4 (95% CI 5.8–9.0) and 11.8 (95% CI 8.7–14.9) months,

Address for correspondence: Prof. Yen-Shen Lu, Department of Oncology, National Taiwan University Hospital, 7, Chung-Shan South Road, Taipei, Taiwan.
E-mail: yslu@ntu.edu.tw

Submitted: 31-Dec-2022 **Revised:** 15-Jan-2023
Accepted: 17-Jan-2023 **Published:** 10-Mar-2023

Access this article online

Quick Response Code:

Website: www.ejcrp.org

DOI: 10.4103/ejcrp.eJCRP-D-23-00001

How to cite this article: Chen TW, Lin CH, Yeh DC, Tseng LM, Rau KM, Chen BB, *et al*. Anti-tumor efficacy of a bevacizumab preconditioning followed by etoposide and cisplatin regimen in human epidermal growth factor receptor-2-positive breast cancer brain metastasis refractory to whole brain radiotherapy. J Cancer Res Pract 2023;10:11-8.

respectively. Toxicities were tolerated with granulocyte-colony stimulating factor support. **Conclusion:** The BEEP regimen had a significant antitumor effect in cases of BM of HER2-positive breast cancer that progressed following whole brain radiotherapy.

Keywords: Bevacizumab, brain metastases, breast cancer, human epidermal growth factor receptor-2, vascular normalization

Introduction

With improved control of systemic disease in cases of metastatic breast cancer (MBC), the incidence of brain metastasis (BM) is increasing. Although all subtypes of MBC may subsequently develop BM, the phenomenon is most commonly recorded in human epidermal growth factor receptor-2 (HER2)-positive subtype cases, and up to 30%–50% of HER2-positive MBC patients may develop BM.[1,2] Multiple possible explanations exist for this HER2-predominant phenomenon such as improving extracranial control, the predilection of HER2-positive disease to deposit tumors in the brain, and the most prominent anti-HER2 drug to date – trastuzumab – not being able to penetrate the sealed blood–brain barrier, which provides a drug sanctuary in which metastatic tumors can develop.[3,4]

Once BM develops, the standard first-line treatment is surgery, stereotactic surgery, or whole brain radiotherapy (WBRT).[2] However, BM commonly recurs afterward. For HER2-positive MBC patients with BM, various systemic drugs targeting HER2 have been evaluated either singly or in combination with cytotoxic chemotherapy for the treatment of BM. The most commonly applied drug is the dual anti-HER2 and epidermal growth factor receptor small molecular inhibitor lapatinib, which is commonly used in combination with capecitabine, yielding a central nervous system (CNS) objective response rate (ORR) ranging from 10% to 65% depending on when the combination is initiated.[5-7] Other anti-HER2 small molecular inhibitors have also been tested but the results remain unsatisfactory. For example, neratinib in combination with capecitabine has demonstrated a CNS-ORR of 8% in HER2-positive BM patients after failed one or more lines of CNS-directed treatment.[8] Notably, some retrospective studies have suggested that an antibody-drug conjugate, trastuzumab emtansine (T-DM1), could also have some clinical activity in cases of BM.[9,10]

At present, the focus of improvements to the systemic treatment efficacy of HER2-positive BM is on blocking the HER2-pathway activation; however, this direction has had limited success.[11] Other methods, such as improving the delivery of drugs to BM tumors, are also being explored for the treatment of MBC BM patients. Bevacizumab, an antivascular endothelial growth factor (VEGF) monoclonal antibody, has been demonstrated to improve the vascular permeability or "normalize" the peritumoral pressure in metastatic tumors, including BM.[12-15] To maximize the benefit of vascular normalization, a window period between bevacizumab and chemotherapy is crucial to increase delivery of the chemotherapy drug into the tumor.[16] In our previous clinical trial, we demonstrated that the before etoposide and cisplatin (BEEP) regimen (a combination of etoposide and cisplatin preceded by a preconditioning bevacizumab with a 1-day window period) is highly efficacious in all subtypes of MBC BM patients that have progressed or have been refractory to WBRT.[17] In a single cohort of 35 patients, the ORR in brain metastatic tumors was 77%.[17]

Twenty-three patients in the BEEP study were HER2-positive, and the ORR in the HER2-positive cohort was similar to the whole study cohort. All but one of the HER2-positive patients did not receive trastuzumab, lapatinib, or any other HER2-directed therapy during the clinical trial period, further solidifying the notion that HER2-positive BM may still be responsive to a non-HER2-directed combination. Herein, we provide the efficacy and safety details of HER2-positive patients in a BEEP trial to provide a non–HER2-based treatment option for HER2-positive MBC patients with BM.

Materials and Methods

Patients

Between January 2011 and January 2013, MBC patients with progressive BM following WBRT, as proven by either computed tomography (CT) or magnetic resonance imaging (MRI), were enrolled. Patients were required have had at least one measurable lesion ≥10 mm at the longest diameter, as measured using contrast-enhanced CT or T1-weighted gadolinium-enhanced MRI. Additional criteria were as follows: Age between 18 and 75, adequate end-organ function, and Eastern Cooperative Oncology Group performance status ≤3. Being HER2-positive was defined as either having an immunohistochemical score of 3+ or evidence of gene amplification in accordance with the American Society of Clinical Oncology guidelines. At the time of the clinical trial, lapatinib was not reimbursed by Taiwan's National Health Insurance, but patients were informed about treatment with lapatinib single agent or in combination with capecitabine if they had not received lapatinib before enrollment. Concurrent anti-HER2 or other antineoplastic treatments were not allowed during the clinical trial (one patient had a protocol violation and received additional trastuzumab on cycles 3 and 4 and lapatinib on cycle 5; this patient had a best response of PR and was included in the intention-to-treat analysis). Those who were receiving nonescalating steroids with a daily dose of 10 mg prednisone or equivalent were acceptable. 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 phase II study conducted to demonstrate the efficacy of the BEEP regimen (bevacizumab, etoposide, and cisplatin) in the BM of MBC patients, including HER2-positive subtype. The primary endpoint was the CNS-responsive rate according to the composite volumetric criteria. Other secondary endpoints included progression-free survival (PFS), CNS-specific PFS, overall survival (OS), and safety.

Treatment plan

The BEEP regimen was given as bevacizumab (15 mg/kg on day 1), etoposide (75 mg/m^2 from day 2 to day 4), and cisplatin (70 mg/m^2 on day 2). BEEP was given for a maximum of 6 cycles with every cycle lasting 21 days. If the patient indicated disease progression or intolerable toxicity, the treatment was discontinued. After an amendment of protocol, patients whose disease had not progressed after 6 cycles of BEEP were allowed to continue with bevacizumab single agent or bevacizumab in combination with other systemic treatments as maintenance therapy until evidence of disease progression or intolerable toxicity.

Safety assessment was based on the Common Terminology Criteria for Adverse Events 3.0. If the patient experienced hematological Grade 3 or higher toxicities, the doses of etoposide and cisplatin were de-escalated to 60 mg/m^2. If nonhematological Grade 3 or higher toxicities were determined to be correlated with bevacizumab, bevacizumab was temporarily withheld from administration until the toxicity returned to Grade 1 or 2. On study commencement, prophylactic granulocyte-colony stimulating factor (G-CSF) was not recommended, but later, the protocol was amended to mandatory use of G-CSF because of a higher-than-expected incidence of Grade 3 or 4 neutropenia; incidence decreased after mandatory prophylactic administration of G-CSF.

Efficacy assessment

Brain tumor assessment was performed with either contrast-enhanced CT or T1-weighted gadolinium-enhanced MRI every 9 weeks. T2 fluid-attenuated inversion recovery images from MRI were not selected as the tumor assessment method because of diffuse white matter changes after WBRT. For tumor volume assessment, commercially available software (MIStar, Apollo Medical Imaging Technology) was used and all images were reviewed by 2 independent radiologists who were blinded to the clinical status. The total tumor volume was the sum of the largest (up to 3) tumors noted in the brain parenchyma. The efficacy was assessed by composite volumetric criteria as used by Lin *et al*. and Lu *et al*.[17,18] In short, a CNS-ORR was defined as a volume decrease ≥50% in all measurable lesions along with no progression of neurological symptoms, no increase in the dose of steroids, and no extra-CNS progression. CNS disease progression was defined as a ≥40% increase in the targeted CNS volume or new brain metastatic tumor measuring more than 6 mm at its longest diameter. Any deterioration of neurological signs or increase in required dose of steroids because of CNS tumor was also considered as CNS disease progression. In the amended protocol, RECIST 1.1 was also included as one of the measurement methods to assess tumor response. Neurological examinations were conducted and recorded within 1 day of each BEEP cycle.

Statistical analysis

The statistical assumption for the sample size of the whole study has been reported in Lu *et al*.[17] In the HER2-positive section, 95% confidence interval (CI) was used to report CNS-ORR, CNS-PFS, PFS, and OS. PFS was defined as the interval from the date of the first protocol treatment to the date of the first radiologically documented CNS, extra-CNS disease progression, or death from any cause. CNS-PFS was defined as the interval from the date of first protocol treatment to the date of the first radiologically documented CNS disease progression or death from any cause.

Results

A total of 23 of the 35 patients (66%) enrolled into the BEEP clinical trial were HER2-positive. The median age of the HER2-positive cohort was 55 (range 33–75) years old; 9 (39%) patients were estrogen receptor (ER) positive and 14 (61%) were ER negative. The most common metastatic sites in addition to BM in the HER2-positive cohort were lung (56.5%), bone (56.5%), and liver (47.8%). The HER2 amplification status was determined by primary breast ($n = 33$) or axillary lymph node ($n = 2$) for all patients. All (100%) HER2-positive patients had received trastuzumab either in the adjuvant or metastatic setting before enrolling in this study; 11 (48%) had received lapatinib and 6 had received the combination of lapatinib and capecitabine. The median lines of chemotherapy received in the metastatic setting before enrollment was 3 (range 1–8). HER2-positive patients received more lines of treatment before enrolling into the study as compared with the HER2-negative group (median 2 (range 1–8) lines). Two (8.6%) patients received metastatic brain tumor resection and 6 (26.1%) patients (2 also had metastatic brain tumor resection) received stereotactic radiosurgery before the CNS tumor progression before enrolment into this study. Table 1 lists the clinical characteristics and treatment history (including CNS-directed therapy) for the HER2-positive patients enrolled into the BEEP study.

A total of 110 cycles of BEEP regimen were administered in the HER2-positive cohort and a median of 6 (range 1–6) cycles per patient. The cycles of BEEP regimen received in the HER2-positive cohort were not different from the HER2-negative patients (HER2-positive vs. HER2-negative 6 ([range 1–6] vs. 5.5 [range 3–6]). There were 9 patients who discontinued protocol treatment due to the following reasons: 4 patients discontinued the treatment because of disease progression, of which 2 patients had extra-CNS progression but with CNS lesions that remained under control; 3 patients discontinued due to adverse events, and 2 of them received hospice care.

Efficacy of the before etoposide and cisplatin regimen in human epidermal growth factor receptor-2-positive metastatic breast cancer patients

A total of 16 of the 23 patients (69.6%, 95% CI 47.1–86.8) had an objective volumetric response in the CNS tumors (≥50% decrease in target CNS tumor volume). Among them, 7 (30.4%) and 9 (39.1%) patients had ≥80% and 50%–80% CNS volumetric tumor reduction, respectively. Five patients (21.7%) had 20%–50% tumor volumetric decrease [Figure 1]. Of those who had a history of refractory to lapatinib treatment, 7 of 11 had CNS tumor response; of those who were lapatinib naïve, 9 of 12 (75.0%) had CNS volumetric tumor response. When RECIST 1.1 was used to evaluate the CNS tumor response, none of the patients exhibited a complete response but 11 patients had a partial response; therefore, 47.8% of patients had ORR according to RECIST. A total of 10 patients had stable disease, and none of the patients had tumor progression; 2 patients had nonassessable tumor. The ORR between patients evaluated by CT and MRI was not significantly different (P = 0.33). Two patients with a prominent brain metastatic tumor reduction are shown in Figure 2.

Table 1: Clinical characteristics and previous treatments of patients (n=23)

Characteristics	n (%)
Age (years), median (range)	55.1 (33.4-75.0)
<50	6 (26.1)
50-60	11 (47.8)
>60	6 (26.1)
Hormone receptor status	
ER negative and PR negative	14 (60.9)
ER positive and/or PR positive	9 (39.1)
ECOG performance status	
0-1	12 (52.2)
2	3 (13.0)
3	8 (34.8)
Number of extra-CNS metastatic sites, median (range)	2 (0–3)
0	2 (8.7)
1	7 (30.4)
>2	14 (60.9)
Extra-CNS metastatic sites	
Lung	13 (56.5)
Liver	11 (47.8)
Bone	13 (56.5)
LNs/soft tissue	6 (26.1)
Previous CNS-directed therapy	
WBRT	23 (100.0)
SRS	6 (26.1)
Surgery	2 (8.7)
Intrathecal treatment	1 (4.4)
Chemotherapy line in metastatic setting, median (range)	3 (1–8)
1-2	6 (26.1)
>3	17 (73.9)
Previous hormonal therapy	8 (88.9[a])
Previous HER2-targeted therapy	
Trastuzumab	23 (100.0)
Lapatinib	11 (47.8)
Lapatinib plus capecitabine	6 (26.1)
Others	5 (21.7)

[a]Percentage of ER positive patients. WBRT: Whole brain radiotherapy, SRS: Stereotactic radiosurgery, HER2: Human epidermal growth factor receptor-2, CNS: Central nervous system, LN: Lymph node, ECOG: Eastern cooperative oncology group, ER: Estrogen, PR: Progesterone

With a median follow-up time of 14.5 months, the median PFS for the HER2-positive group was 7.3 months (95% CI 5.4–9.2 months); the median CNS-specific PFS was 7.4 months (95% CI 5.8–9.0 months); and the median OS was 11.8 months (95% CI 8.7–14.9) [Figure 3]. No patients had CNS progression during the protocol treatment while 2 patients (8.7%) had extra-CNS disease progression during the protocol treatment. Four patients received bevacizumab either single or combined therapy as maintenance treatment in the HER2 cohort. One patient died of pneumonia not related to disease progression, the other three patients' disease did not have event at the last time of follow-up. Thus, a statistical comparison or summary was not available.

Safety

The most common Grade 3 or 4 toxicities included neutropenia (30.9%), leukopenia (13.6%), thrombocytopenia (9.1%), and infection (20.0%). A total of 10 (43.5%) patients required a dose reduction of etoposide and cisplatin to 60 mg/m^2 due to hematological toxicity; 2 patients discontinued treatment due to toxicities (one was Grade 4 neutropenia, and another was acute renal failure). Mandatory G-CSF support (filgrastim 300 μg/day, starting 3 days after completion of BEEP per cycle) was added due to the high risk of Grade 3 or 4 neutropenia. With regards to notable adverse events, 2 (1.8%) episodes of Grade 1/2 epistaxis and 1 (0.9%) episode of Grade 3/4 cerebellum infarction occurred in a total of 110 BEEP treatment cycles. No fatal intracranial hemorrhage was noted during the treatment period. Table 2 shows the details of the adverse events.

Discussion

In this prospective clinical trial, we demonstrated that a bevacizumab-preconditioned chemotherapy

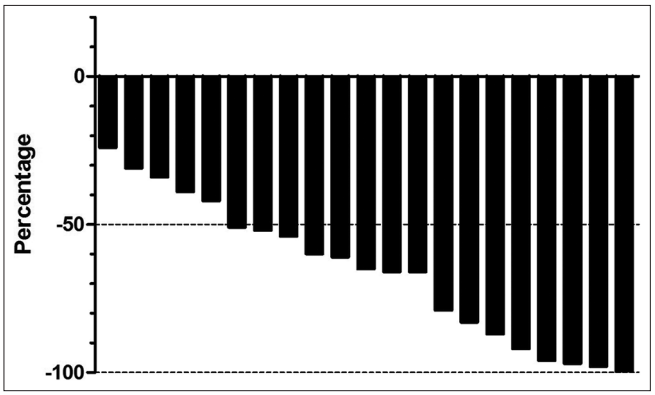

Figure 1: Waterfall plot of maximum CNS tumor volume change from 21 evaluable patients. CNS: Central nervous system

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

Adverse events	Cycles (n=110)	
	Grade ½, *n* (%)	Grade ¾, *n* (%)
Hematological toxicity		
Neutropenia	33 (30.0)	34 (30.9)
Leukopenia	49 (44.5)	15 (13.6)
Anemia	51 (46.4)	6 (5.5)
Thrombocytopenia	25 (22.7)	10 (9.1)
Nonhematological toxicity		
Hypertension*	47 (42.7)	1 (0.9)
Nausea	29 (26.4)	0
ALT/AST elevated	24 (21.8)	2 (1.8)
Infection with normal ANC or Grade 1 or 2 neutropenia	7 (6.4)	15 (13.6)
Vomiting	19 (17.3)	0
Proteinuria*	19 (17.3)	0
Fatigue	12 (10.9)	0
BUN elevated	11 (10.0)	1 (0.9)
Voice changes*	12 (10.9)	0
Infection with Grade 3 or 4 neutropenia*	0	7 (6.4)
Creatinine elevated*	3 (2.7)	0
Febrile neutropenia*	0	2 (1.8)
Epistaxis*	2 (1.8)	0
Cerebellum infarction*	0	1 (0.9)
Dose reduction due to toxicity		10 (43.5)
Treatment discontinued due to toxicity		2 (8.7)

*Adverse event of interest. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ANC: Absolute neutrophil count, BUN: Blood urea nitrogen

regimen – BEEP – has significant activity in HER2-positive MBC patients with refractory brain metastatic tumors following WBRT. The volumetric ORR was 70% and the CNS-specific PFS was 7.4 months. The treatment was well tolerated and the risk of neutropenia decreased substantially with prophylactic G-CSF support. Other nonhematological toxicities were well tolerated, and intracranial vascular events were rare after BEEP treatment. To the best of our knowledge, the BEEP regimen is the first non-HER2 directed systemic treatment with such a notable ORR in BM lesions.

As most HER2-positive MBC BM tumors retained the high expression of HER2, continually targeting the HER2 pathway remains a dominant doctrine in treating HER2-positive BM.[4,19] Although this paradigm has provided some positive results, most remain unsatisfactory. The combination of lapatinib and capecitabine is the most accepted choice but with ORR from only 10% to 38%.[18,20] Only in a cohort of 45 treatment-naïve patients did this combination yield a 63% ORR.[6] Other anti-HER2 targeted small molecule inhibitors such as neratinib,[8] afatinib,[21] and tucatinib[22] all demonstrated some activity in treating BM, but the optimal administration or combination has yet to be explored.

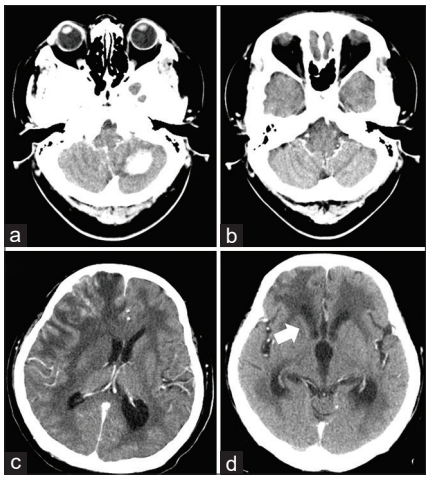

Figure 2: (a and b) Patient 01017 has a complete response of the left cerebellum metastatic brain tumor after BEEP treatment (c and d) patient 01015 had a 97% volume decrease of the brain metastatic tumor including the right parietal mass after BEEP treatment. White arrow indicates the normalized right ventricle after tumor volume reduction. BEEP: bevacizumab, etoposide, and cisplatin

One of the reasons that BM is refractory to systemic treatments may be related to the poor penetration rate of the blood–brain barrier. Antiangiogenic therapy has been associated with the phenomenon of vascular normalization as one of the mechanisms that could improve the treatment efficacy of chemotherapeutic drugs.[14,23,24] However, a window period may be necessary for the vascular normalizing effect to function properly before chemotherapeutic agents can be administered.[17] In the BEEP trial, a 24-h window period between the infusion of bevacizumab and chemotherapy led to a more pronounced change in vascular permeability as compared with just 1 h after bevacizumab infusion.[25] In another of our group's studies, we analyzed the efficacy of BEEP in MBC patients with leptomeningeal metastasis. In 5 evaluable patients, the CNS-response rate was 60% with a CNS-PFS of 4.7 months.[26] In 2013, Lin *et al*. also published an abstract regarding the clinical efficacy of the combination of bevacizumab and carboplatin in CNS metastatic MBC patients.[27] The study reported an impressive ORR of 63%; however, the efficacy of CNS control in the HER2-positive subgroup was not available. Interestingly, although bevacizumab and carboplatin were mostly administered on the same day, the first cycle was specifically designed with a window gap of 1 week between bevacizumab and carboplatin to study the pharmacokinetics in the CSF. The full paper may elucidate the full effect of the 1-week window period between bevacizumab and carboplatin in the first cycle. Overall, these examples further addressed the importance of bevacizumab and the timing of administration in relation to chemotherapy to obtain the optimal efficacy in treating MBC patients with CNS metastasis.

Other reasons exist that may explain the high ORR noted in BM using the BEEP regimen. Clinical studies examining paired brain metastatic tumors and primary

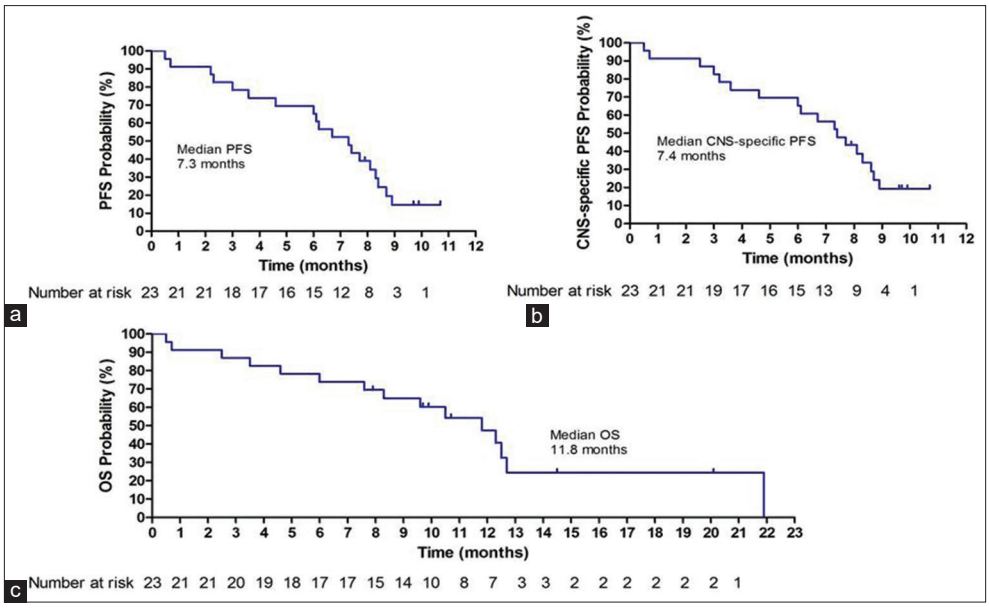

Figure 3: (a) PFS. Median PFS time was 7.3 months (95% CI, 5.4–9.2 months), (b) CNS-specific PFS. Median CNS-specific PFS time was 7.4 months (95% CI, 5.8–9.0 months), (c) OS. Median OS time was 11.8 months (95% CI, 8.7–14.9 months). CNS: Central nervous system, PFS: Progression-free survival, CI: Confidence interval, OS: Overall survival

breast tumors have demonstrated that metastatic brain tumors have a decreased number of tumor-infiltrating lymphocytes (TIL).[28] However, in many neoadjuvant breast cancer studies, both for HER2-positive and triple-negative disease, an increased TIL has been correlated with increased rate of pathological complete response (pCR).[29,30] VEGF has demonstrated in preclinical studies that it does not only provide the substrate for angiogenesis but also blocks the trafficking of T-cells into the tumor microenvironment though endothelial cells.[31-33] Bevacizumab, alongside other VEGF receptor inhibitors, has been shown to enhance the trafficking of T-cells in the tumor and many clinical trials are designed to see if some combination of antiangiogenic drugs with immune checkpoint inhibitors has a measurable effect.[34] Moreover, bevacizumab has also been demonstrated to activate dendritic cells to prime naïve T-cells against the tumor, facilitating the cytotoxicity of immune cells.[35,36] Whether bevacizumab orchestrated T-cells further increase the cytotoxic activity of chemotherapy is a question worth exploration in the treatment of BM.

Despite the promising result, our study has several limitations. First, the small number of patients limited the extrapolation of our study to the general population. Second, although BEEP has shown impressive CNS-ORR without the addition of trastuzumab or other anti-HER2 treatment, the addition of such treatment during the BEEP cycles may further improve the response, especially in terms of the systemic disease control. In an elegant mouse model designed Kodack *et al*., the addition of both antiangiogenic and anti-HER2 molecules had the most favorable tumor control ability,[37] and the combination of both bevacizumab and trastuzumab with neoadjuvant chemotherapy has previously reported a high pCR rate in HER2-positive inflammatory breast cancer.[38] This concept of combining bevacizumab and anti-HER2 requires further exploration. Last, it remains to be tested whether the BEEP has equivalent efficacy in the CNS as well as in the extra-CNS tumors. Although in our study all patients initially demonstrated tumor control in both brain and extra-CNS lesions, two patients had extra-CNS tumors as the first-site of tumor progression while the BM tumors were still under control.[17] When other anti-HER2 antibodies including pertuzumab and T-DM1 are available, a question will exist as to the choice of treatment when a patient has both CNS and extra-CNS progression.

Conclusion

The BEEP regimen demonstrated an impressive activity in HER2-positive BM patients who are refractory or progressed following WBRT. Further investigation of the utility of the BEEP regimen in CNS metastasis of MBC should be undertaken.

Acknowledgment

The authors would like to thank the Taiwan Breast Cancer Consortium, which is supported by the National Science Council, Executive Yuan, Taiwan, helped the data collection. We thank Roche-Taiwan for supporting the free bevacizumab. We thank all the patients who participated in the study, as well as the physician co-investigators, Chen-Ting Liu (KS-CGMH) and Yi-Fang Tsai (TP-VGH), and medical teams of all participating centers for their dedicated efforts. The authors also acknowledge the dedicated work of the research nurses and study coordinators and patients who participated in the trial. We also acknowledge Wallace Academic Editing for editing this manuscript.

Financial support and sponsorship

This study was supported by Ministry of Health and Welfare of Taiwan, R.O.C (Grant Numbers: DOH100-TD-B-111-001 DOH101-TD-B-111-001 DOH102-TD-B-111-001).

Conflicts of interest

There are no conflicts of interest.

References

1. Ramakrishna N, Temin S, Chandarlapaty S, Crews JR, Davidson NE, Esteva FJ, *et al.* Recommendations on disease management for patients with advanced human epidermal growth factor receptor 2-positive breast cancer and brain metastases: American society of clinical oncology clinical practice guideline. J Clin Oncol 2014;32:2100-8.
2. Fontanella C, De Carlo E, Cinausero M, Pelizzari G, Venuti I, Puglisi F. Central nervous system involvement in breast cancer patients: Is the therapeutic landscape changing too slowly? Cancer Treat Rev 2016;46:80-8.
3. Arvold ND, Oh KS, Niemierko A, Taghian AG, Lin NU, Abi-Raad RF, *et al.* Brain metastases after breast-conserving therapy and systemic therapy: Incidence and characteristics by biologic subtype. Breast Cancer Res Treat 2012;136:153-60.
4. Thomson AH, McGrane J, Mathew J, Palmer J, Hilton DA, Purvis G, *et al.* Changing molecular profile of brain metastases compared with matched breast primary cancers and impact on clinical outcomes. Br J Cancer 2016;114:793-800.
5. Lin NU, Eierman W, Greil R, Campone M, Kaufman B, Steplewski 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.
6. Bachelot T, Romieu G, Campone M, Diéras 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.
7. Moasser MM, Krop IE. The evolving landscape of HER2 targeting in breast cancer. JAMA Oncol 2015;1:1154-61.
8. Freedman RA, Gelman RS, Wefel JS, Melisko ME, Hess KR, Connolly RM, *et al.* Translational breast cancer research consortium (TBCRC) 022: A phase II trial of neratinib for patients with human epidermal growth factor receptor 2-positive breast cancer and brain metastases. J Clin Oncol 2016;34:945-52.
9. Bartsch R, Berghoff AS, Vogl U, Rudas M, Bergen E, Dubsky P, *et al.* Activity of T-DM1 in Her2-positive breast cancer brain metastases. Clin Exp Metastasis 2015;32:729-37.
10. Jacot W, Pons E, Frenel JS, Guiu S, Levy C, Heudel PE, *et al.* Efficacy and safety of trastuzumab emtansine (T-DM1) in patients with HER2-positive breast cancer with brain metastases. Breast Cancer Res Treat 2016;157:307-18.
11. Chien AJ, Rugo HS. Tyrosine kinase inhibitors for human epidermal growth factor receptor 2-positive metastatic breast cancer: Is personalizing therapy within reach? J Clin Oncol 2017;35:3089-91.
12. Jain RK. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. Science 2005;307:58-62.
13. Kamoun WS, Ley CD, Farrar CT, Duyverman AM, Lahdenranta J, Lacorre DA, *et al.* Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. J Clin Oncol 2009;27:2542-52.
14. Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 2011;10:417-27.
15. Chauhan VP, Stylianopoulos T, Martin JD, Popović Z, Chen O, Kamoun WS, *et al.* Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. Nat Nanotechnol 2012;7:383-8.
16. Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, *et al.* Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004;10:145-7.
17. Lu YS, Chen TW, Lin CH, Yeh DC, Tseng LM, Wu PF, *et al.* Bevacizumab preconditioning followed by etoposide and cisplatin is highly effective in treating brain metastases of breast cancer progressing from whole-brain radiotherapy. Clin Cancer Res 2015;21:1851-8.
18. Lin NU, Diéras 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.
19. Priedigkeit N, Hartmaier RJ, Chen Y, Vareslija D, Basudan A, Watters RJ, *et al.* Intrinsic subtype switching and acquired ERBB2/HER2 amplifications and mutations in breast cancer brain metastases. JAMA Oncol 2017;3:666-71.
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.
21. Cortés J, Dieras V, Ro J, Barriere J, Bachelot T, Hurvitz S, *et al.* Afatinib alone or afatinib plus vinorelbine versus investigator's choice of treatment for HER2-positive breast cancer with progressive brain metastases after trastuzumab, lapatinib, or both (LUX-Breast 3): A randomised, open-label, multicentre, phase 2 trial. Lancet Oncol 2015;16:1700-10.
22. Borges VF, Ferrario C, Aucoin N, Falkson CI, Khan QJ, Krop IE, *et al.* Efficacy results of a phase 1b study of ONT-380, a CNS-penetrant TKI, in combination with T-DM1 in HER2+ metastatic breast cancer (MBC), including patients (pts) with brain metastases. J Clin Oncol 2016;34:513.
23. Goel S, Wong AH, Jain RK. Vascular normalization as a therapeutic strategy for malignant and nonmalignant disease. Cold Spring Harb Perspect Med 2012;2:a006486.
24. Jain RK. Normalizing tumor microenvironment to treat cancer: Bench to bedside to biomarkers. J Clin Oncol 2013;31:2205-18.
25. Chen BB, Lu YS, Lin CH, Chen WW, Wu PF, Hsu CY, *et al.* A pilot study to determine the timing and effect of bevacizumab on vascular normalization of metastatic brain tumors in breast cancer. BMC Cancer 2016;16:466.
26. Wu PF, Lin CH, Kuo CH, Chen WW, Yeh DC, Liao HW, *et al.* A pilot study of bevacizumab combined with etoposide and cisplatin in breast cancer patients with leptomeningeal carcinomatosis. BMC Cancer 2015;15:299.
27. Leone JP, Emblem KE, Weitz M, Gelman RS, Schneider BP, Freedman RA, *et al.* Phase II trial of carboplatin and bevacizumab in patients with breast cancer brain metastases. Breast Cancer Res 2020;22:131.
28. Ogiya R, Niikura N, Kumaki N, Yasojima H, Iwasa T, Kanbayashi C, *et al.* Comparison of immune microenvironments between primary tumors and brain metastases in patients with breast cancer. Oncotarget 2017;8:103671-81.
29. Luen SJ, Salgado R, Fox S, Savas P, Eng-Wong J, Clark E, *et al.* Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: A retrospective analysis of the CLEOPATRA study. Lancet Oncol 2017;18:52-62.
30. Wein L, Savas P, Luen SJ, Virassamy B, Salgado R, Loi S. Clinical validity and utility of tumor-infiltrating lymphocytes in routine clinical practice for breast cancer patients: Current and future directions. Front Oncol 2017;7:156.
31. Alfaro C, Suarez N, Gonzalez A, Solano S, Erro L, Dubrot J, *et al.* Influence of bevacizumab, sunitinib and sorafenib as single agents or in combination on the inhibitory effects of VEGF on human dendritic cell differentiation from monocytes. Br J Cancer 2009;100:1111-9.
32. Ziogas AC, Gavalas NG, Tsiatas M, Tsitsilonis O, Politi E, Terpos E, *et al.* VEGF directly suppresses activation of T cells from ovarian cancer patients and healthy individuals via VEGF receptor type 2. Int J Cancer 2012;130:857-64.
33. Du Four S, Maenhout SK, De Pierre K, Renmans D, Niclou SP, Thielemans K, *et al.* Axitinib increases the infiltration of immune cells and reduces the suppressive capacity of monocytic MDSCs in an intracranial mouse melanoma model. Oncoimmunology 2015;4:e998107.

34. Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. Immunity 2013;39:1-10.
35. Manzoni M, Rovati B, Ronzoni M, Loupakis F, Mariucci S, Ricci V, *et al.* Immunological effects of bevacizumab-based treatment in metastatic colorectal cancer. Oncology 2010;79:187-96.
36. Hodi FS, Lawrence D, Lezcano C, Wu X, Zhou J, Sasada T, *et al.* Bevacizumab plus ipilimumab in patients with metastatic melanoma. Cancer Immunol Res 2014;2:632-42.
37. 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.
38. Pierga JY, Petit T, Delozier T, Ferrero JM, Campone M, Gligorov J, *et al.* Neoadjuvant bevacizumab, trastuzumab, and chemotherapy for primary inflammatory HER2-positive breast cancer (BEVERLY-2): An open-label, single-arm phase 2 study. Lancet Oncol 2012;13:375-84.