The treatment of brain metastasis from breast cancer, role of blood–brain barrier disruption and early experience with trastuzumab

Rose Marie Tyson, Dale F Kraemer, Matthew A Hunt, Leslie L Muldoon, Peter Orbay, Leeza Maron, Kristoph Jahnke & Edward A Neuwelt†

†Author for correspondence Oregan Health & Science University, Department of Neurology, Portland, OR, 97239, USA Tel.: +1 503 494 5626 Fax: + 1 503 494 5627 neuwelte@ohsu.edu

Background: Therapeutic approaches in the treatment of metastatic systemic brain tumors from breast cancer have improved. As patients live longer, the potential for CNS sanctuary disease increases. Metastases to the brain are diagnosed in breast cancer patients at a rate of 10 to 20%. Median survival is only 3 to 12 months with current standard therapies of whole-brain radiotherapy, surgery and stereotactic radiosurgery, and can vary with the type of treatment given. Chemotherapy and immunotherapy using trastuzumab may be more effective against brain metastases if delivery to the tumor can be improved. **Results:** The treatment was well tolerated with acceptable bone marrow toxicity. Median overall survival was 45.4 weeks and the majority of patients achieved symptomatic relief with reduction of steroids. **Methods & objectives:** We evaluated the use of osmotic blood–brain barrier disruption chemotherapy, with or without monoclonal antibody, for the treatment of brain metastases from breast cancer. We are interested in the prospective evaluation of the combination of a monoclonal antibody, trastuzumab, with enhanced delivery of carboplatin and methotrexate chemotherapy. **Discussion:** The use of carboplatin and methotrexate with blood–brain barrier disruption showed efficacy in the treatment of metastatic breast cancer to the CNS. This is comparable to other modalities, and without cognitive loss. Quality of life is improved with the withdrawal of steroids. **Conclusion:** The long-term goal is to use combined chemotherapy and immunotherapy with radiosurgery to increase survival and avoid complications from other treatments.

Keywords: blood–brain barrier disruption, carboplatin, intraarterial, metastasis, methotrexate, trastuzumab

Future Drugs Ltd

Background

Nature of disease & characteristics of diagnosis

Breast cancer is the most common malignancy in North American women and the second most common disease metastasizing to both brain parenchyma and leptomeninges [1]. The National Cancer Institute projected 211,240 new cases and 40,410 deaths in 2005. The lifetime risk of diagnosis with breast cancer in the USA is approximately 13% and the risk of breast cancer increases with age, doubling every 10 years up to menopause. Breast cancer is the second leading cause of cancer-related deaths in women and ranks fourth as a cause of death from cancer overall [2]. Almost all deaths from breast cancer are due to metastatic disease. A total of 10 to 16% of women with high-grade breast cancer have metastasis when diagnosed, and it is estimated that 20 to 30% of women with early stage disease will progress to metastatic disease. As the most common brain tumor in women, metastasis is found in 30% of patients at autopsy [1,3–5].

The spread of metastatic disease is not random, but requires methodic communication among breast cells, stroma and surrounding normal tissue at both primary and metastatic sites. In order for metastasis to develop, adhesion molecules, local mediators, hormones and growth factors must all contribute [6]. Neovascularization also assists in the metastatic process. The localization of a patient's metastatic disease, extent of the disease, biologic characteristics and the overall effect of treatment, determines the success of disease control and palliation. Survival time is also dependent on response to prior therapy and hormone receptor status. Marked progress has been made in the past 30 years in the understanding and treatment of breast cancer. As the efficacy of diagnostic and therapeutic approaches has improved, there is a decrease in mortality and also an improvement in palliation and quality of life (QoL) for women with metastatic disease where cure is not a possibility.

In women whose tumors overexpress the HER-2 oncogene, standard chemotherapy plus the monoclonal antibody (mAb) trastuzumab, as first-line treatment increases the time to disease progression (TTP), rates of objective response and overall survival compared with standard chemotherapy alone. Slamon and colleagues reported on a recent clinical trial of 469 women with HER-2 overexpression receiving

trastuzumab and chemotherapy versus chemotherapy alone. Combination trastuzumab and chemotherapy TTP (median 7.4 vs 4.6 months; $p < 0.001$), demonstrated an increase in objective response (50 vs 32%; $p < 0.001$), a longer duration of response (median 9.1 vs 6.1 months; $p < 0.001$), a lower rate of death at 1 year (22 vs 33%; $p = 0.008$), longer survival (median survival: 25.1 vs 20.3 months, $p = 0.046$) and a 20% decrease in death [7]. Adding a platinum drug to trastuzumab and docetaxel has proven to yield a significantly higher response rate and median TTP and is an important clinical option in the management of metastatic breast cancer patients [5]. An increase in QoL was also found to be significantly improved with trastuzumab and chemotherapy versus chemotherapy alone. Of the 469 women enrolled, 400 reported that combination therapy significantly improved global QoL. The rate of improvement was assessed with the European Organization for Research and Treatment Care QoL Questionnaire with a 51% improvement with combination therapy versus 36% with chemotherapy alone ($p < 0.05$) [8]. Trastuzumab with combination therapy was effective in reducing the relative risk of death by 20% at a median follow-up of 30 months [7]. Vogel and colleagues found that few studies have shown that adding a single agent improves survival to this degree [9].

With the introduction of trastuzumab for treatment of HER-2-positive breast cancer, the course of disease has changed. The incidence of CNS metastasis has increased, with 34% of women having brain metastasis at a median of 16 months after diagnosis of metastatic disease and 6 months from the start of trastuzumab therapy [10]. Dawson and colleagues published an abstract at the June 1 2005 American Society of Clinical Oncology (ASCO) meeting that reported the high rate of CNS metastases in women with HER-2-positive metastatic breast cancer who had received trastuzumab and chemotherapy [11]. In this retrospective study of 28 women, 11 (39%) were diagnosed with CNS metastasis and nine of the 11 (82%) had controlled systemic disease at the time of diagnosis. This study concluded that there is a higher rate of CNS metastasis in women whose cancer overexpressed HER-2 (39 vs 10–16%). Treatment with trastuzumab increased median survival to greater than 2 years versus historical data of median survival at less than 1 year without the mAb. Saito and colleagues also published an abstract that describes their retrospective study of 57 women with 27 patients positive for HER-2 [12]. Of the 27 women, 19 were treated with trastuzumab and their results correlate with Dawson [11]. Time from first recurrence to brain metastasis was 17.4 months with trastuzumab-treated women, compared with 8.3 months for patients who did not receive the antibody. Survival after diagnosis of brain metastasis also increased from 1.1 months for patients without trastuzumab treatment compared with 9.8 months with mAb treatment.

In order to improve outcomes in breast cancer, this change in the course of the disease must be met with new treatments for breast cancer metastasis to the brain.

Approaches to treating metastatic breast carcinoma to the CNS

The main goals of treating metastatic disease are palliation and extending survival. All treatment options that provide control of symptoms and prolong life with the least risk, least disruption of lifestyle and improvement in QoL, should be explored [6,13]. The median survival with metastatic disease is 2 to 3 years but is shorter in patients with disease of the liver, lung and brain. The median survival of untreated breast cancer patients with brain metastasis is only 4 to 6 months [13,14].

Metastasis is the most common brain tumor and according to autopsy studies, a quarter of cancer patients have intracranial metastases [3]. Approximately 100,000 patients have symptomatic intracranial metastases in the USA annually – it is five-times more common than the incidence rate for malignant primary brain tumors. The standard mode of treatment has been surgical resection (if a single lesion) and whole-brain radiotherapy (WBRT), which can extend survival to 6 to 10 months after diagnosis. Stereotactic radiosurgery (SRS) has been used as a salvage therapy to manage recurrent metastasis after an initial resection or after the failure of WBRT. The majority of patients respond briefly to treatments, if at all, and the recurrence rate after surgery and radiotherapy (RT) is high [1,15].

Surgery for brain metastasis can improve survival, especially in patients with single lesions. However, surgery may not be possible in the face of multiple lesions, surgically inaccessible lesions or patients with an inability to tolerate surgery. In a patient with a single metastasis, surgery is clearly beneficial in both survival and

function, when compared with WBRT alone [16,17]. Surgery and WBRT improves progression-free survival (PFS), but does not change survival or level of function [18]. Reports of median length of survival range from 10 to 21 months [16,17,19,20]; however, patients with progressive extracranial disease may not receive the same benefit as those with controlled extracranial disease [17]. With modern surgical techniques, image guidance and neuroanesthesia, extirpation of brain metastasis can be performed safely and should be considered the treatment of choice for single metastasis.

A patient with multiple brain metastases is much less likely to be a candidate for surgical resection of disease. In breast cancer, 50% of patients with brain metastasis have multiple metastases. Some of these patients are surgical candidates and in one series, 26% of the patients treated with surgery had multiple metastases [19]. Surgical removal of a single metastasis in a patient with multiple metastases may provide palliation and help improve neurologic deficits related to that single metastasis.

WBRT for brain metastasis

In patients who are not candidates for surgery or radiosurgery, WBRT may improve median survival over no treatment. As an adjuvant to surgery, it reduces the recurrence rate and chances of dying a neurologic death, but does not improve survival [18]. The long-term sequelae of WBRT may become more problematic in patients with better control of their extracranial disease, as their survival lengthens. With improved systemic therapies, the long-term sequelae of WBRT will become increasingly important [21,22].

Stereotactic radiosurgery

SRS provides a method of treating brain metastasis that may be surgically unresectable, either by location or patient condition. One direct comparison study showed that surgery was superior in survival to SRS [19]. SRS with WBRT has also been studied and showed a survival benefit in patients with a single metastasis. These patients also had reduced steroid use and improved functional scores [23]. Other studies have also shown a survival benefit to SRS and WBRT when compared with WBRT alone [23,24]. The Radiation Therapy Oncology Group (RTOG) 9508 Phase III trial provides the first level I data that supports the use of SRS in a select population with notably unresectable single metastasis. SRS provides an improvement in QoL but does not provide survival benefit, except in patients with a single metastasis [25].

Toxicity of therapies & the need for alternatives

Surgery may lead to complications such as neurologic deficits, bleeding and infection. However, these sequelae are generally limited to the perioperative period. The majority of patients with brain tumors have neurologic and neurocognitive impairment that can have a dramatic impact on QoL [26]. WBRT and SRS may lead to long-term complications that are more troublesome to the patient. Headaches, nausea and fatigue are a few of the acute toxicities associated with WBRT. Long-term toxic sequelae of WBRT include the risk of developing progressive dementia, ataxia and urinary incontinence [27]. Magnetic resonance imaging (MRI) and computed tomography (CT) images have identified cortical atrophy and hypodense white matter changes with the most probable cause being demyelination secondary to irradiation. In brain metastases resulting from breast cancer, the relative contribution of the tumor mass, surgery, chemotherapy and radiation to cognitive impairment remains unknown.

The use of sensitive, neurocognitive tests coupled with novel neuroimaging approaches holds promise for addressing some of these issues [28]. Correa reviewed a series of patients from the Sloan–Kettering Cancer Center (NY, USA) diagnosed with primary CNS lymphoma and treated with and without methotrexate (MTX) and WBRT [21]. The study examined cognitive functions and QoL of survivors. The conclusion acknowledged more cognitive dysfunction for patients treated using WBRT with or without chemotherapy, than in patients treated with chemotherapy alone. The damage to the CNS is often irreversible and progressive, and appears to increase with age and radiation dose. This is thought to be the result of vascular injury which causes ischemia of the surrounding tissue, demyelination of white matter and necrosis. These lesions may even be seen on MRI [29]. The QoL for these patients may also be reduced [22]. SRS may lead to radiation necrosis, which can lead to further diagnostic conundrums, although this problem may be reduced by magnetic resonance spectroscopy (MRS). However, given these toxicities and the limitations of each therapy, patients may have a preference that leads them to a particular treatment. For some, this may be a fear of surgery or of radiation. For others, ease of

treatment and length of anticipated hospital stay may also be factors. While there are unique factors to be considered in patients with metastatic disease, repeated cognitive testing may provide a better understanding of the mechanisms of neurotoxicity, the etiology of the cognitive impairment, its course over time and relationship to functional status. In patients with cognitive and short-term memory deficits, evaluation by a neuropsychologist is recommended.

The need to develop other treatment options for patients with brain metastasis led to the research of chemotherapy. The intact blood–brain barrier (BBB) precludes the passage of large molecular-weight compounds, thus restricting most chemotherapy agents [1]. Chemosensitive tumors often show complete systemic responses to chemotherapy concomitant with tumor progression in the brain. Early chemotherapy trials by Rosner and colleagues [30] and those reported by Lin [1], document response rates as high as 50% and median duration of response of 7 months. These results were repeated by Boogerd and colleagues [31] with a response rate of 59% in 22 patients [1]. Cisplatin levels have been measured in metastatic brain tissue at autopsy with the platinum concentration generally decreasing with increasing distance into the brain from the tumor [32]. These autopsy results lend credence to a dysfunctional BBB in patients with metastatic lesions. There are few chemotherapy agents that cross the BBB, and by virtue of this, most active agents used in treating systemic breast cancer poorly cross the BBB.

Chemotherapy

Chemotherapy is indicated as the initial intervention for breast carcinoma patients with sex hormone receptor-negative tumors and as the next intervention for patients with sex hormone-receptor-positive tumors that have not responded to hormone therapy. Combination chemotherapy yields a higher response rate and a more prolonged response than using single-agent chemotherapy. The overall response rate for chemotherapy is 50 to 60%; however, only 15 to 20% of women realize a complete remission. First- and second-line chemotherapy with taxanes and anthracyclines has proven to be effective and these substances are often used as single agents. Response rates range from 50 to 60%. Capecitabine, gemcitabine and vinorelbine are also frequently used, either as single agents or in combination, yielding response rates of between 20 and 25%. Gemcitabine has shown synergy with trastuzumab and platinum salts against breast cancer cell lines [33]. The loss of the *BRCA1* gene in p53 deficient cells is associated with increased sensitivity to the topoisomerase I and II agents, and to the platinum compounds, carboplatin and oxaliplatin. However, there is not the same sensitivity to antimetabolites and taxanes [34]. A recent Phase III study reported by Albain demonstrated the superiority of combination paclitaxel and gemcitabine over monotherapy [35,36].

Prior reluctance to use chemotherapy for brain metastasis came from concerns over the ability of chemotherapy drugs to cross the BBB and penetrate tumor cells as well as the intrinsic chemoresistance of metastatic disease. Metastatic breast carcinomas are somewhat chemosensitive and have been found to respond fairly well to chemotherapy drugs [3]. It has been found through animal studies that metastatic tumors that are strongly enhancing on imaging studies have a partially impaired BBB and therefore chemotherapy drugs can invariably enter into the tumor. This indicates that systemic resistance to chemotherapy drugs does not necessarily mean that metastatic brain lesions will be chemoresistant to the same drugs, for example MTX. Oncologists are beginning to use drugs as a first-line approach or in conjunction with RT. An early study by Cocconi and colleagues investigated metastatic disease from breast, small-cell lung carcinoma and malignant melanoma, and the response to intravenous cisplatin and etoposide [37]. In this study there were 56 patients with metastatic breast cancer with seven complete responses (CRs) and 14 partial responses (PRs). The overall objective response was 30% with a median TTP of 15 weeks and median survival of 27 weeks for the three tumor types. A historical study by Rosner and colleagues used upfront cyclophosphamide-based chemotherapy with breast metastases [30]. A total of 52 women were studied using cyclophosphamide, 5-fluorouracil (5-FU) and prednisone. Another 35 women received MTX, vincristine and 5-FU along with daily oral cyclophosphamide and prednisone. Objective responses were noted by CT or radionuclide brain scan in less than 50% of these patients with 10% CR and 40% PR. Median survival of patients was 39.5 months for patients with CR and 10.5 months for those with PR [30].

Toxicity of chemotherapy & immunotherapy

The potential for significant cardiac toxicity from doxorubicin can present with fatigue and dyspnea on exertion and arrhythmias which may be life

threatening. Dose-related delayed cardiomyopathy presents with congestive heart failure (CHF) symptoms and may be irreversible. Cardiotoxicity may take years to present itself. However, the use of liposomal doxorubicin formulations is associated with acceptable cardiac toxicity in conjunction with good overall response (OR) rates [36]. Trastuzumab can also result in ventricular dysfunction and CHF. This may be severe and has been associated with disabling cardiac failure, death and thrombosis leading to stroke. A decrease in left ventricular function warrants discontinuation of the agent. The incidence and severity of cardiac dysfunction is high in patients who have received trastuzumab in combination with anthracyclines and cyclophosphamide. This is also seen in combination with paclitaxel. Anthracyclines used in combination with cyclophosphamide have documented cardiotoxicity, although the addition of trastuzumab significantly increases the toxicity.

The ability of chemotherapy agents to cross the BBB is limited, yet neurologic toxicities can occur to healthy brain tissue when agents are delivered directly to the brain. For example, delivery of even a dose as low as 0.1 mg/kg adriamycin across the BBB, causes significant neurotoxicity in dogs [38]. Liposomal doxorubicin achieves 14-fold higher concentration in brain tumors and ten- to 30-fold higher concentrations in the cerebrospinal fluid (CSF) than free doxorubicin [1].

Chemotherapy may also lead to neuropsychologic dysfunction in a significant number of patients. Chemobrain is the popular term for the subtle cognitive dysfunction experienced by up to 25% of cancer patients who receive chemotherapy for non-CNS neoplasms. An unresolved question is whether cognitive impairment is attributable to specific chemotherapeutic agents or is a result of a combination of these, and if certain brain structures are predominantly affected. Mechanisms for chemobrain may involve chemotherapy or reactive oxygen species (ROS)-induced injury of the BBB, followed by leakage of neurotoxic agents into the brain and/or inflammatory response [39,40].

Methotrexate-based chemotherapy

MTX is an antimetabolite agent used in the treatment of solid tumors and in the treatment of leukemia and lymphoma. MTX has proven effective penetration and cytotoxic activity at brain levels that are only 3 to 5% of plasma levels. MTX is a mainstay of BBB disruption chemotherapy due to its lack of neurotoxicity when delivered across the BBB. Prior to the use of taxanes and anthracyclines, MTX was used as adjuvant therapy for breast carcinoma and given in combination with other antineoplastic agents (e.g., cyclophosphamide, 5-FU, vincristine and prednisone). Cyclophosphamide, MTX and 5-FU (CMF) was a commonly used regimen with a response rate of 50 to 60%; however, only 15 to 20% had evidence of complete remission [41]. This regimen provided substantial palliation with tolerable toxicity. Lassman and colleagues report on the Sloan–Kettering Cancer Center series of 24 women with parenchymal and/or leptomeningeal CNS metastases. This patient cohort confirmed the tolerability of high-dose MTX and although median survival was only 3.5 months, 75% of the patients initially have improved and remained stable [42].

Carboplatin-based chemotherapy

Platinum complexes are active in a wide range of solid tumors. Both cisplatin and carboplatin have shown activity in breast cancer. Carboplatin may be the more appropriate choice for treatment of metastatic disease, as it causes less severe nonhematologic toxicities. Two independent Phase II studies have shown that the combination of carboplatin and docetaxel is active in the first-line treatment of metastatic breast cancer. Brufsky and colleagues studied 40 women with advanced breast cancer in a Phase II trial evaluating 3-week dosing of docetaxel (75 mg/m^2) and carboplatin [43]. An OR rate of 59% was observed in 39 evaluable patients with six CR (15.4%), seven PR (43.6%) and nine stable disease (SD) (23%). The mean duration of response was 8.8 months and the mean TTP was 6.5 months. The primary toxicity was hematologic. The North Central Cancer Treatment Group (NCCTG) Phase II study evaluated the role of carboplatin and docetaxel as first-line therapy for metastatic breast cancer. This study enrolled 53 women irrespective of HER-2 status. The chemotherapy doses and dosing regimen was the same as the Brufsky Phase II trial [43]. The OR rate was 58% with three CR and 28 PR. The mean TTP was 9.8 months and the 1-year survival rate was 72% [7]. In four Phase II studies of previously untreated patients with metastatic breast cancer, single-agent carboplatin produced objective response rates of 20 to 35%. Incorporation of carboplatin as a standard agent in first-line treatment of metastatic cancer has support from several recent studies [5].

Clinical studies of chemotherapy delivery

Despite decades of experience with brain tumor chemotherapy, the pharmacology of the delivery of chemotherapeutics for the treatment of CNS breast metastases and other CNS neoplasms is not well characterized. The BBB consists of the tight junctions between endothelial cells that line the cerebrovasculature [44]. These tight junctions block access of blood-borne agents, such as chemotherapeutics, from the brain. Barrier permeability is determined by molecular weight and lipid solubility, and is particularly low to high molecular weight agents such as antibodies. A leaky blood–tumor barrier (BTB) develops in the neovasculature of tumors; however, barrier integrity in and around metastasis is highly inconsistent. Delivery across the BBB and BTB is complicated by inhomogeneous blood flow to the tumor and normal brain, efflux pumps such as p-glycoprotein, as well as the molecular weight, chemistry and toxicity of chemotherapeutics. MTX is thought to cross the BBB well, yet measurements indicate that tumor and CSF MTX concentrations are only approximately 3% of blood levels, indicating very low BBB permeability [44]. MTX is effective against brain tumors, as such large systemic doses can be administered that a therapeutic dose leaks across the BBB into the tumor. Few studies have been carried out to measure the brain delivery of other agents. Intrathecal administration is likely to be poorly effective against tumors in the brain parenchyma as diffusion will only reach a few cells in diameter, and the positive pressure in a tumor compared with a normal brain limits fluid flow into the tumor. Agents that require activation such as cyclophosphamide, etoposide phosphate or cytarabine, make studies investigating the delivery of an active drug after intravenous infusion difficult to assess using just radiolabelling. The taxanes (e.g., paclitaxel or docetaxel) are used for the treatment of CNS breast metastasis, as these lipid soluble agents are thought to pass the BBB and BTB easily. However, when delivery of taxanes is optimized with BBB disruption in a rat model there is significant neurotoxicity, and the maximum tolerated dose (MTD) is less than 10% of the normal clinical dose [unpublished data], suggesting that the taxanes are not in fact leaking into the brain. Altogether, these data point to the need for clinical trials to assess delivery of an active drug to evaluate chemotherapeutic pharmacology, and to provide a more solid basis for metastasized CNS breast cancer chemotherapy.

The impact of molecular weight on delivery across the BBB

The variable penetration of chemotherapeutic drugs into the brain and tumor is more dependent on lipid solubility than size. Greig reviewed the issue of parameters which determine CNS delivery and found that lipid solubility was the most important parameter, followed by molecular weight less than 500. For CNS penetration, appropriate solubility is key – water-soluble drugs penetrate poorly as do drugs that are too lipid soluble [45]. For instance, lapatinib [46,47] is an interesting new agent for metastatic breast cancer, which is both an inhibitor of epidermal growth factor receptor (EGFR) and HER-2, and is being pursued for CNS breast metastasis. However, its lipid solubility may be too high.

In contrast, the molecular weight of virus- and tumor-specific mAbs appears to limit uptake. In order to compare tumor uptake of an iodinated contrast agent, eight subjects with malignant brain tumors were evaluated by CT scanning, then for uptake of the low and high molecular weight-imaging agents ^{99m}Tc-glucoheptonate and ^{99m}Tc-albumin were measured by radionuclide brain scanning (**Figure 1**) [48]. The agent ^{99m}Tc-albumin was chosen for evaluation because its molecular weight (68,000) is similar to that of the most clinically promising mAb fragment, the immunoglobulin (Ig)G Fab monomeric fragment. The radionuclide brain scans in the eight subjects showed a highly variable permeability of brain tumors to these markers, with uptake of the high molecular-weight marker in the tumor being much less than that of the low molecular-weight radionuclide. A clinical implication of these studies is that the success of mAb therapy in the treatment of malignant brain tumors may require techniques to increase the permeability of the BBB and BTB to protein [48].

Blood–brain barrier disruption

The BBB is responsible for suboptimal drug delivery to CNS tumors and often negligible delivery to brain distant to tumor. The tight junctions between the endothelial cells selectively permit the passage of lipid-soluble drugs with weak protein binding or substances with low ionization. Efficacy of chemotherapy can be affected by the route of administration. Intra-arterial administration can increase both local plasma peak concentration and local area under the concentration–time curve versus intravenous administration. Intratumoral concentrations of

Table 2. Toxicity information for seven patients treated with trastuzumab and methotrexate or carboplatin-based chemotherapy.

Toxicity	Grade 3* events (patients)	Grade 4* events (patients)
Neutropenia	2 (2)	11 (5)
Febrile neutropenia	0	0
Thrombocytopenia	1 (1)	4 (2)
Anemia	1 (1)	0
Infection	0	0
Nausea and/or vomiting	0	0
Neurologic	0	0
Pulmonary edema	2 (1)	0
DVT/PE	0	0
Cardiac MI	0	2 (1)

National Cancer Institute common toxicity criteria.
DVT: Deep vein thrombosis; MI: Myocardial infarction; PE: Pulmonary embolism.

five grade 3–4 complications during 215 procedures. The procedural complication rate was 4%.

A total of 25 patients underwent 215 procedures. Median overall survival of the cohort was 45.4 weeks (95% confidence interval: 15.7 to 69.0 weeks). Of these patients evaluable for response, four had objective responses (CR or PR) for a response rate of 16%, 15 had SD (60%) while the other six had progressive disease (PD) (24%). Median TTP, as summarized in **Figure 4** was 4.13 months (95% confidence interval: 2.15 to 7.07 months). The 6-month PFS was 32% and the 12-month PFS was 12%. PFS was observed in one patient for 18 months.

Assessing the symptomatic relief from reduction of steroids, six had complete withdrawal (30%), five had a 50% withdrawal (25%) and three had partial withdrawal (15%). Three patients (15%) had no change in steroid levels and three (15%) had increased steroid levels. The majority of treatment-related toxicity was bone marrow toxicity. Grade 3–4 hematologic toxicity occurred in 21 patients and all of these patients had prior systemic therapy. In general, BBB disruption in conjunction with chemotherapy was well tolerated.

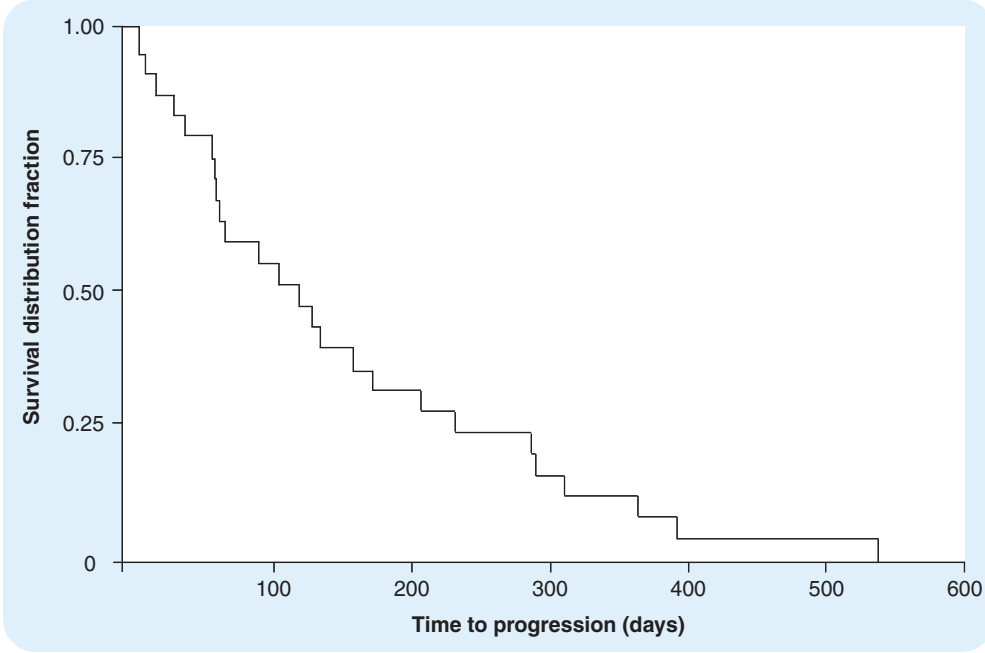
Discussion

The use of carboplatin and MTX-based chemotherapy with BBB disruption-enhanced delivery demonstrates efficacy against brain metastasis from breast cancer. The response rate, TTP and survival times calculated from the initiation of chemotherapy are comparable to other treatment modalities without the side effects of WBRT on cognitive function. The regimen is generally well tolerated and has an acceptable complication rate. The BBB disruption procedure, where patient condition allowed, proved to be safe and afforded symptomatic relief as measured by withdrawal of steroids. Among the adjuvant therapies, front-line sequencing of the chemotherapy for brain metastasis may prolong the survival time for these patients and allow us to better assess potential efficacy. Also, patients with favorable prognostic factors (e.g., young age and good functional status), or those who have sanctuary CNS metastases as a result of low drug penetration, may benefit from BBB disruption chemotherapy. Median survival of this patient cohort was greater than 10 months. The survival times with both carboplatin and MTX regimens support the evidence that the use of BBB disruption with chemotherapy provides longer survival time than WBRT without the neurologic sequelae. A total of 70% of patients received symptom relief from steroids, either with a complete or partial withdrawal, with resulting improvement in QoL. During the months of BBB disruption, seven patients were receiving intravenous trastuzumab, without toxicity and possible increased antibody delivery to the CNS due to the long plasma half-life. Trastuzumab is effective in maintaining stable systemic disease but has poor penetration to the CNS, although this may be improved by BBB disruption. Delivery to brain tumors of chemotherapeutic drugs may be improved, but at the cost of neurotoxicity. Based on the results of our pilot data, combining MTX and carboplatin with trastuzumab may enhance efficacy if delivery can be increased with BBB disruption. Conversely, increased delivery of other standard breast cancer drugs to the brain is unlikely to help due to neurotoxicity. This is exemplified by drugs such as taxanes and anthracyclines.

Expert commentary & conclusion

Brain metastases occur in approximately 15% of breast cancer patients. Risk factors for brain metastases include young age and estrogen receptor negative tumors [1,58,59]. The incidence appears to be increasing as a sanctuary site as systemic control improves, particularly for patients receiving trastuzumab with HER-2 amplified tumors [1,10,60]. Survival for women with metastatic breast cancer will not increase in response to systemic tumor control, until the issue of CNS sanctuary for breast cancer tumor cells is

Figure 4. Kaplan–Meier plot of time-to-progression from first day on study therapy.



Among 25 patients with breast cancer metastases, 14 received BBB disruption therapy, eight received only intra-arterial therapy and three received both intra-arterial and BBB disruption therapy. Intra-arterial therapy was used when functional status was low or risk factors were unacceptable. Ten patients received only carboplatin-based regimens, ten received only MTX-based regimens and five received a regimen based on either carboplatin or MTX and were switched to a regimen based on the other of these.
BBB: Blood–brain barrier; MTX: Methotrexate.

solved. Such CNS therapy must not only control CNS metastasis but do so in a fashion that does not injure the normal brain and compromise the QoL of these patients.

Several major avenues of research are underway which have the potential to discover the basic mechanisms underlying brain metastasis, develop rational targeted therapies and improve patient survival and QoL.

Outlook

Clinical trials in patients with metastatic breast cancer have for the first time shown not only responses but increases in survival time with the use of macromolecular biologic therapeutics such as trastuzumab in conjunction with chemotherapy [5,7]. Along with this increase in survival, an increase in the incidence of CNS metastasis has been observed in those HER-2 positive patients receiving trastuzumab. This suggests the importance of the BBB in preventing access to the therapeutic antibody [7,10,11]. A report from a National Cancer Institute (NCI) and National Institute of Neurological Disorders and Strokes (NINDS) consensus group of experts advising the National Institutes of Health (NIH) regarding brain tumor issues, pointed out that even though brain metastases are five times more common than primary brain tumors, the NIH portfolio of grants was nearly devoid of funded proposals dealing with CNS metastatic disease.

The current therapy for metastatic breast cancer to the CNS is inadequate. Patients with one to three accessible lesions undergo neurosurgical resection, particularly if the lesions are symptomatic and if the patient's overall condition warrants. Alternatively, SRS can be used either postoperatively and/or for inaccessible lesions. Even so, many patients have further CNS recurrences unless WBRT is employed, which carries with it unacceptable cognitive sequelae, particularly in older patients and patients with extended survival. Chemotherapy, despite a few intriguing reports, is not routinely used due to generally poor delivery across the BBB. Therefore, there is a critical need over the next 5 years to address delivery issues to the CNS and to develop novel biologic and targeted therapies based on translational studies. Several major

avenues of research are underway which have the potential to discover the basic mechanisms underlying brain metastasis, develop rational targeted therapies, and improve patient survival and QoL.

Directions for preclinical research include the development of animal model systems for brain metastasis which more closely resemble the clinical situation. It is important that models match human cancers in terms of clinical heterogeneity at the molecular level, but also that they target the brain to the same extent as that which occurs in patients. A mouse model of brain metastasis has been tested that may be useful for molecular and preclinical experiments. MDA-231 human breast carcinoma cells have been selected for brain metastatic propensity [61,62]. Imaging studies of these cells labeled with green florescence protein, luciferase or superparamagnetic iron oxide nanoparticles can demonstrate mechanisms of micrometastasis and therapy. This model was used to demonstrate a role for EGFR in the growth of human breast cancer brain metastasis [61]. Mutants of brain seeking metastatic breast cancer cell lines may be useful for determining the role of HER-2 or estrogen receptor mutant expression or overexpression in brain metastasis and therapeutic response. *In vitro* and *in vivo* model systems will be used to test the contribution of chemokines, cellular immune responses, phosphatase and tensin homology, histone deacetylase inhibitors and signaling pathways in metastatic aggression.

Mechanisms by which tumor cells cross the BBB, formation of a BTB, and the expression and function of the BBB, BTB efflux and nutrient uptake transporters should be investigated to characterize the nature of the BBB and BTB in breast cancer metastasis. Studies should address fundamental properties of the BBB and BTB, including its molecular characteristics, permeability and the pharmacokinetics of drug entry [63]. Molecular analyses of brain metastatic lesions may identify additional molecular pathways of translational interest. Translational approaches to the BBB and BTB include the development of mAbs or ligands to BBB and BTB proteins, the development of BBB and BTB permeabilization strategies, and the development of nanoparticle drug delivery devices [64]. Novel therapeutics and rational combination therapy approaches will be tested in tumor-based translational strategies. For example, therapies targeting HER-2, such as trastuzumab or herstatin, could be delivered to brain metastases by BBB disruption in combination with RT or conventional chemotherapy. Research is needed to identify and test additional molecular therapeutics to control or eliminate micrometastases in the brain. Pharmacokinetic studies will determine brain, metastasis, brain adjacent to tumor and plasma levels of drugs.

Clinical research over the next 5 years will address the molecular and cellular characteristics of breast cancer brain metastasis. Surgical material must be collected for gene array, proteomics, pathology and endothelial investigations. Formalin-fixed and paraffin-embedded tissue blocks must be collected for array and immunohistochemistry. Microarray analysis of resected brain metastasis will identify gene expression and proteomic signatures for histopathologic criteria, HER-2 amplified versus unamplified brain metastasis, and estrogen receptor positive versus negative brain metastasis. It will be essential to have series of matched EGFR brain metastasis and primary tumors as well as tumor samples from long-term (>5 years) survivors of brain metastasis for genomics and proteomics analysis.

Treatment strategies for the future will involve a combination of targeted therapeutics with conventional approaches, and innovative delivery mechanisms. The Oregon Health and Science University and National BBB Disruption consortium will apply osmotic BBB disruption to breast cancer brain metastasis for the delivery of conventional chemotherapeutics and for targeted therapies such as anti-HER-2 antibodies (trastuzumab) or peptide inhibitors (herstatin) [52,64]. We are developing a prospective evaluation of trastuzumab, with HER-2 positive tumors, with BBB disruption. A new protocol combining carboplatin, MTX and trastuzumab is being written with the long-term goal of combining chemotherapy and immunotherapy with SRS and avoiding WBRT to address the delivery problem across the BBB [65].

There have been three recent publications supporting the use of chemotherapy for CNS metastases. Peereboom discussed the sensitivity to chemotherapy and the importance of MTX in treating CNS breast cancer metastases, as well as the need for novel delivery techniques [66]. Lassman and colleagues reported on the Sloan–Kettering Cancer Center results using high dose MTX for CNS breast cancer metastases and tumor sensitivity [67]. Finally, Burstein

and colleagues presented two large studies on CNS progression in women with breast cancer and HER-2 overexpression [68]. It was concluded that patients with HER-2 overexpression who were also on trastuzumab are at greater risk of developing CNS disease. These three articles are consistent with our goal of using BBB disruption to enhance delivery of trastuzumab, MTX and carboplatin chemotherapy.

Conflict of interest

Drs Neuwelt and Muldoon are affiliated with the Oregon Health and Science University, Portland Veterans Affairs Medical Center (OR, USA) and the Department of Veterans Affairs and have significant financial interests in Adherex Technology, Inc., a company that may have a commercial interest in the results of this research and technology. The potential conflict of interest has been reviewed and a management plan has been approved by the Oregon Health and Science University and the Portland Veterans Affairs Medical Center Conflict of Interest in Research Committee.

Acknowledgement

This work was funded by the Veterans Administration and NIH grants NS44687, NS34608 and NS33618.

Highlights

- As metastatic breast carcinoma has become a chronic disease, brain metastases are increasing as a sanctuary site.
- Current treatment regimens include surgery, whole-brain radiotherapy, stereotactic radiosurgery and chemotherapy.
- The role of trastuzumab/herstatin.
- Current research and new avenues of research.
- Goal of treatment with chemotherapy: BBB disruption and trastuzumab and improvement in quality of life.

Bibliography

Papers of special note have been highlighted as of interest (•) or of considerable interest (••) to readers.

1. Lin NU, Bellon JR, Winer EP. CNS metastases in breast cancer. *J. Clin. Oncol.* 22(17), 3608–3617 (2004).
•• **Demonstrates increasing incidences of CNS metastasis in breast cancer patients.**
2. Jemal A, Murray T, Ward E, Samuels A *et al.* Cancer Statistics. *Cancer J. Clin.* 55(1), 10–30 (2005).
3. Newton HB, Slivka MA, Volpi C *et al.* Intra-arterial carboplatin and intravenous etoposide for the treatment of metastatic brain tumors. *J. Neurooncol.* 61, 35–44 (2003).
4. Zancan M. Metastatic breast cancer: current prognosis. *PeerView in Focus*, 4–5 (2003).
5. Perez EA. Carboplatin in combination therapy for metastatic breast cancer. *Oncologist* 9, 518–527 (2004).
6. Hortobagyi GN. Treatment of breast cancer. *N. Engl. J. Med.* 339(14), 974–984 (1998).
7. Slamon DJ, Leyland-Jones B, Shak S *et al.* Use of chemotherapy plus monoclonal antibody against *HER2* for metastatic breast cancer that over expresses *HER2*. *N. Engl. J. Med.* 344(11), 783–792 (2001).
•• **Demonstrates efficacy of herceptin.**
8. Osoba D, Slamon DJ, Burchmore M, Murphy M. Effects on quality of life of combined trastuzumab and chemotherapy in women with metastatic breast cancer. *J. Clin. Oncol.* 20(14), 3106–3113 (2002).
• **Demonstrates clinical impact of herceptin.**
9. Vogel CL, Cobleigh MA, Tripathy D *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of *HER2* over expressing metastatic breast cancer. *J. Clin. Oncol.* 20(3), 719–726 (2002).
10. Bendell J, Domchek S, Burstein H *et al.* Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer* 97, 2972–2977 (2003).
11. Dawson SJ, Raniere NF, Snyder RD *et al.* Trastuzumab (T) and CNS metastases in women with HER-2 positive metastatic breast cancer (MBC). Presented at: *The American Society of Oncology meeting, 1 June.* (2005).
12. Saito Y, Tokuda Y, Suzuki, Umemura S, Osamura RY. Clinicopathological study of brain metastases in breast cancer patients treated with or without trastuzumab. Presented at: *The American Society of Oncology meeting, 1 June* (2005).
13. Klos KJ, O'Neill BP. Brain metastases. *Neurologist* 10(1), 31–46 (2004).
14. Hall WA, Djalilian HR, Nussbaum ES, Cho KH. Long-term survival with metastatic cancer to the brain. *Med. Oncol.* 17, 279–286 (2000).
• **Demonstrates potential for long-term survival.**
15. Vecil GG, Suki D, Maldaun MV, Lang FF, Sawaya R. Resection of brain metastases previously treated with stereotactic radiosurgery. *J. Neurosurg.* 102(2), 209–215 (2005).
16. Patchell RA, Tibbs PA, Walsh JW *et al.* A randomized trial of surgery in the treatment of single metastases to the brain. *N. Engl. J Med.* 322(8), 494–500 (1990).
17. Vecht CJ, Haaxma-Reiche H, Noordijk EM *et al.* Treatment of single brain metastasis: radiotherapy alone or combined with neurosurgery? *Ann. Neurol.* 33(6), 583–590 (1993).
18. Patchell RA, Tibbs PA, Regine WF *et al.* Postoperative radiotherapy in the treatment of single metastases to the brain: a randomized trial. *JAMA* 280(17), 1485–1489 (1998).
19. Bindal AK, Bindal RK, Hess KR *et al.* Surgery versus radiosurgery in the treatment of brain metastasis. *J. Neurosurg.* 84(5), 748–754 (1996).
20. Yoshida S, Morii K. The role of surgery in the treatment of brain metastasis: a retrospective review. *Acta Neurochir (Wien)* 146(8), 767–770 (2004).

- **Demontrates role for surgery in CNS metastasis.**

21. Correa DD, DeAngelis LM, Shi W, Thaler H, Glass A, Abrey LE. Cognitive functions in survivors of primary central nervous system lymphoma. *Neurology* 62, 548–555 (2004).
22. Harder H, Holtel H, Bromberg JE *et al.* Cognitive status and quality of life after treatment for primary CNS lymphoma. *Neurology* 62(4), 544–547 (2004).
23. Andrews DW, Scott CB, Sperduto PW *et al.* Whole brain radiation therapy with or without stereotactic radiosurgery boost for patients with one to three brain metastases: Phase III results of the RTOG 9508 randomized trial. *Lancet* 363(9422), 1665–1672 (2004).

- **Demonstrates role for radiotherapy in CNS metastasis.**

24. Kondziolka D, Patel A, Lunsford LD, Kassam A, Flickinger JC. Stereotactic radiosurgery plus whole-brain radiotherapy versus radiotherapy alone for patients with multiple brain metastases. *Int. J. Radiat. Oncol. Biol. Phys.* 45(2), 427–434 (1999).
25. McElveen T, Andrews DW. Summary of the RTPG 95–08 Phase III randomized trial of whole brain radiation with or without stereotactic radiosurgery boost, including presentation of a clinical case study. *Am. J. Oncol. Rev.* 3(10), 592–600 (2004).
26. Mehta MP, Shapiro WR, Glantz MJ *et al.* Lead-in phase to randomized trial of motexafin gadolinium and whole-brain radiation for patients with brain metastases: centralized assessment of magnetic resonance imaging, neurocognitive and neurologic end points. *J. Clin. Oncol.* 20(16), 3445–3453 (2002).
27. Chang EL, Lo S. Diagnosis and management of central nervous system metastases from breast cancer. *Oncologist* 8(5), 398–410 (2003).
28. Wefel JS, Lenzi R, Theriault RL, Davis RN, Meyers CA. The cognitive sequelae of standard dose adjuvant chemotherapy in women with breast carcinoma: results of a prospective, randomized, longitudinal trial. *Cancer* 100(11), 2292–2299 (2004).

- **Demonstrates the issue of chemobrain.**

29. Neuwelt EA, Guastadisegni PE, Varallyay P, Doolittle ND. Imaging changes and cognitive outcome in primary central nervous system lymphoma after enhanced chemotherapy delivery. *Am. J. Neuroradiol.* 26(2), 258–265 (2005).

•• **Reviews cognitive outcome after blood–brain barrier disruption.**

30. Rosner D, Nemoto T, Lane WW. Chemotherapy induces regression of brain metastases in breast carcinoma. *Cancer* 58, 832–839 (1986).

- **Shows potential for chemotherapy in CNS metastasis.**

31. Boogerd W, Vos VW, Hart AA, Baris G, van der Sande JJ. Brain metastases in breast cancer; natural history, prognostic factors and outcome. *Neurooncol.* 15(2), 165–174 (1993).
32. Stewart DJ, Mikhael NZ, Nair RC *et al.* Platinum concentrations in human autopsy tumor samples. *Am J. Clin. Oncol.* 11(2), 152–158 (1988).
33. Konecny GE, Pegram MD. Gemcitabine in combination with trastuzumab and/or platinum salts in breast cancer cells with *HER2* overexpression. *Oncology* 18(14 Suppl. 12), 32–36 (2004).
34. Fedier A, Steiner RA, Schwarz VA, Lenherr L, Haller U, Fink D. The effect of loss of BRCA1 on the sensitivity to anticancer agents in p53-deficient cells. *Int. J. Oncology* 22, 1169–1173 (2003).
35. Albain KS. Adjuvant chemotherapy for lymph node-negative, estrogen receptor-negative breast cancer: a tale of three trials. *J. Natl Cancer Institute.* 96(24), 1801–1804 (2004).
36. Gralow JR. Optimizing the treatment of metastatic breast cancer. *Breast Cancer Res.* 89(Suppl.1), S9–S15 (2005).
37. Cocconi G, Lottici R, Bisagni G *et al.* Combination therapy with platinum and etoposide of brain metastases from breast carcinoma. *Cancer Invest.* 8(3–4), 327–334 (1990).
38. Neuwelt EA, Pagel M, Barnett P, Glasberg M, Frenkel EP. Pharmacology and toxicity of intracarotid adriamycin administration following osmotic blood–brain barrier modification. *Cancer Res.* 41, 4466–4470 (1981).

- **Demonstrates neurotoxicity of adriamycin.**

39. O'Shaughnessy J. Chemotherapy-related cognitive dysfunction in breast cancer. *Semin. Oncol. Nurs.* 19, 17–24 (2003).
40. Anderson-Hanley C, Sherman ML, Riggs R, Agocha VB, Eompas ED. Neuropsychological effects of treatments for adults with cancer: a meta-analysis and review of the literature. *J. Int. Neuropsychol. Soc.* 9, 967–982 (2003).
41. Osteen RT. Breast Cancer. In: *The American Cancer Society's Clinical Oncology*. Lenhard RE, Osteen RT, Gansler T (Eds). The American Cancer Society, Inc, GA, USA (2001).
42. Lassman AB, DeAngelis LM. Brain Metastases. *Neurol. Clin*. 21(1), 1–23 (2003).
43. Brufsky A, Martin K, Cleary D *et al.* A Phase II study of carboplatin and docetaxel as first line chemotherapy for metastatic breast cancer. *Proceedings of the American Sociey of Clincal Oncology* 21, 526 (2002).
44. Neuwelt EA. Mechanism of disease: the blood–brain barrier. *Neurosurgery* 54, 131–142 (2004).
45. Greig NH. Drug delivery to the brain by blood–brain barrier circumvention and drug modification. Implications of the blood–brain barrier and its manipulation. In: *Basic Sciences Aspects (Volume 1)*. Neuwelt EA. Plenuna Publishing Corp., NY, USA (1989).

- **Reviews drug delivery across the BBB.**

46. Burris HA, Hurwitz HI, Dees EC *et al.* Phase I safety, pharmacokinetics, and clinical activity study of lapatinib (GW572016), a reversible, dual inhibitor of epidermal growth factor receptor tyrosine kinases in heavily pretreated patients with metastatic carcinoma. *J. Clin. Oncol.* 23(23), 1–9 (2005).
47. Spector NL, Xia W, Burris H *et al.* Study of the biologic effects of lapatiib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J. Clin. Oncol.* 23(11), 2502–2512 (2005).
48. Neuwelt EA, Specht HD, Hill SA. Permeability of human brain tumor to ^{99m}Tc-glucoheptonate and ^{99m}Tc-albumin: implications for monoclonal antibody therapy. *J. Neurosurg.* 65, 194–198 (1986).

- **Demonstrates problem of protein delivery across the BBB.**

49. Fortin D, Desjardins A, Benko A, Niyonsega T, Boudaris M. Enhanced chemotherapy delivery by intraarterial infusion and blood–brain barrier disruption in malignant brain tumors. The Sherbrooke Experience. *Cancer* 103, 2606–2615 (2005).
50. Remsen LG, Trail PA, Hellstrom I, Hellstrom KE, Neuwelt EA. Enhanced delivery improves the efficacy of a tumor-specific doxorubicin immunoconjugate in a human brain tumor xenograft model. *Neurosurgery* 46(3), 704–709 (2000).
51. Muldoon LL, Neuwelt EA. BR96-DOX immunoconjugate targeting of chemotherapy in brain tumor models. *J. Neurooncol.* 65(1), 49–62 (2003).

•• **Reviews antibody delivery to brain tumors.**

52. Staverosky J, Muldoon L, Guo S, Evans A, Neuwelt E, Clinton G. Herstatin, an autoinhibitor of the epidermal growth factor receptor family, blocks the intracranial growth of glioblastoma. *Clin. Cancer Res.* 11, 335–340 (2005).

53. Grunwald V, Hidalgo M. Developing inhibitors of the epidermal growth factor receptor for cancer treatment. *J. Natl Cancer Instit.* 95(12), 851–867 (2003).
54. Molina MA, Saez R, Ramsey EE *et al.* HN_2-terminal truncated HER-2 protein but not full-length receptor is associated with nodal metastasis in human breast cancer. *Clin. Cancer Res.* 8(2), 347–353 (2002).
55. Williams PC, Henner WD, Neuwelt EA *et al.* Toxicity and efficacy of carboplatin and etoposide in conjunction with disruption of the blood–brain tumor barrier in the treatment of intracranial neoplasms. *Neurosurgery* 37(17), 17 (1995).
56. Neuwelt EA, Brummett RE, Bubalo JS *et al.* First evidence of otoprotection against carboplatin-induced hearing loss with a two-compartment system in patients with central nervous system malignancy using sodium thiosulfate. *J. Pharm. Exp. Ther.* 286, 77 (1998).
57. Doolittle ND, Muldoon LL, Neuwelt EA *et al.* Delayed sodium thiosulfate as an otoprotectant against carboplatin-induced hearing loss in patients with malignant brain tumors. *Clin. Cancer Res.* 7, 493 (2001).
58. Evans A, James J, Cornford E *et al.* Brain metastases from breast cancer: identification of a high-risk group. *Clin. Oncol.* 16, 345–349 (2004).
59. Miller K, Weathers T, Hanley L *et al.* Occult central nervous system involvement in patients with metastatic breast cancer: prevalence, predictive factors and impact on overall survival. *Ann. Oncol.* 14, 1072–1077 (2003).
60. Lai R, Dang C, Malkin M, Abrey L. The risk of central nervous system metastases after trastuzumab therapy in patients with breast carcinoma. *Cancer* 101, 810–816 (2004).
61. Kim L, Huang S, Lu W, Lev DC, Price J. Vascular endothelial growth factor expression promotes the growth of breast cancer brain metastases in nude mice. *Clin. Exp. Metastasis* 21, 107–118 (2004).
62. Yoneda T, Williams P, Hiraga T, Niewolna M, Nishimura R. A bone seeking clone exhibits different biological properties from the MDA-MB-231 parental human breast cancer cells and a brain-seeking clone *in vivo* and *in vitro*. *J. Bone Min. Res.* 16, 1486–1495 (2001).
63. Lockman P, Koziara J, Roder K *et al. In vivo* and *in vitro* assessment of baseline blood–brain barrier parameters in the presence of novel nanoparticles. *Pharm. Res.* 20, 705–713 (2003).
64. Doolittle N. Abrey L, Bleyer W *et al.* New frontiers in translational research in neuro-oncology and the blood–brain barrier: Report of the tenth annual blood–brain barrier disruption consortium meeting. *Clin. Cancer Res.* 11, 421–428 (2005).
65. Neuwelt EA. Mechanism of disease: the blood-brain barrier. *Neurosurgery* 54, 131–142 (2004).

•• **Reviews blood–brain barrier issues.**

66. Peereboom DM. Chemotherapy in brain metastases. *Neurosurgery* 57(4), 54–65 (2005).
67. Lassman AB, Abrey LE, Begemann M *et al.* Systemic high dose methotrexate (HD MTX) for cnetral nervous system (CNS) metastases. Presented at: *The Annual Meeting of the American Society of Clinical Oncology.* Abstract 2083 (2005).
68. Burstein HJ, Lieberman G, Slamon DJ, Winer EP, Klein P. Isolated central nervous system metastases in patients with HER2 overexpressing advanced breast cancer treated with first-line trastuzumab-based therapy. *Ann. Oncol.* 16, 1772–1777 (2005).

Affiliations

Rose Marie Tyson, ANP, MSN
Oregon Health & Science University,
Department of Neurology, Portland,
OR, 97239, USA
Tel.: +1 503 494 5626
Fax: +1 503 494 5627
tysonr@OHSU.edu

Dale F Kraemer, PhD
Oregon State University,
Department of Pharmacy Practice, Portland,
OR, 97239, USA
Tel.: +1 503 494 2258
Fax: +1 503 494 8797
kraemerd@ohsu.edu

Matthew A Hunt, MD
Oregon Health & Science University,
Department of Neurosurgery, Portland,
OR, 97239, USA
Tel.: +1 503 494 8211
Fax: +1 503 494 5627
huntmat@ohsu.edu

Leslie L Muldoon, PhD
Oregon Health & Science University,
Portland, OR, 97239, USA
Tel.: +1 503 494 7179
Fax: +1 503 494 5627
muldoonl@ohsu.edu

Peter Orbay, MD
Országos Idegsebészeti Tudományos Intézet,
1145 Budapest, Amerikai út 57, Hungary
Tel.: +36 1467 9300
Fax: +36 1251 8869
orbapet@oiti.hu

Leeza Maron, PhD
Oregon Health & Science University,
Department of Psychiatry, Portland,
OR, 97239, USA
Tel.: +1 503 494 8144
Fax: +1 503 494 6170
maronl@ohsu.edu

Kristoph Jahnke, MD
Oregon Health & Science University,
Department of Neurology,
Portland, OR, 97239, USA
Tel.: +1 503 494 5626
Fax: +1 503 494 5627
jahnkek@ohsu.edu

Edward A Neuwelt, MD
Oregon Health & Science University,
Department of Neurology, Portland,
OR, 97239, USA
Tel.: +1 503 494 5626
Fax: +1 503 494 5627
neuwelte@ohsu.edu