Published in final edited form as:
Eur J Cancer. 2010 May ; 46(7): 1204–1210. doi:10.1016/j.ejca.2010.02.033.

Translational research in brain metastasis is identifying molecular pathways that may lead to the development of new therapeutic strategies

Brunilde Gril, **Lynda Evans**, **Diane Palmieri**, and **Patricia S. Steeg**
Women's Cancers Section, Laboratory of Molecular Pharmacology, National Cancer Institute, National Institute of Health, Bethesda, MD 20892, USA

Abstract

Central nervous system (CNS) or brain metastasis is an emerging area of interest in organ-specific metastasis research. Lung and breast cancers are the most common types of primary tumors to develop brain metastases. This disease complication contributes significantly to the morbidity and mortality of both of these common cancers; as such, brain metastasis is designated an unmet medical need by the US Food and Drug Administration. Recently, an increase in incidence of CNS disease has been noted in the literature for breast cancer, while it has been an ongoing major complication from lung cancer. Progress in treating brain metastases has been hampered by a lack of model systems, a lack of human tissue samples, and the exclusion of brain metastatic patients from many clinical trials. While each of those is significant, the major impediment to effectively treating brain metastatic disease is the blood–brain barrier (BBB). This barrier excludes most chemotherapeutics from the brain and creates a sanctuary site for metastatic tumors. Recent findings on the biology of this disease and translational leads identified by molecular studies are discussed in this article.

Clinical Overview

Brain metastasis is the most common form of adult CNS tumors, outnumbering primary brain tumors by 10:1 (1). While multiple primary tumor sites metastasize to the brain, 40–50% of brain lesions originate from lung cancer and 20–30% from breast cancer (2). The natural history of the disease is very different for these two primary tumor types. Lung cancer patients develop brain metastases early – within the first two years – after primary tumor diagnosis while breast cancer patients tend to recur in the CNS after the development of systemic metastatic disease and multiple rounds of chemotherapy. Regardless, brain metastasis is a major cause of morbidity and mortality. Tumors in the CNS strongly affect patient quality of life, impairing sensory and motor neural functions and inducing headaches (24–48%), nausea, vomiting, and seizures. The mainstay of treatment is radiation therapy, which can add additional morbidity, particularly neurocognitive complications from whole brain radiotherapy.

The incidence of brain metastatic disease appears to be rising as a result of multiple factors. First, there is an increase in the aging population. Second, improvements in systemic

The authors declare no conflict of interest.

chemotherapy, particularly in breast cancer, have increased the number of metastatic patients either responding to treatment or with stable disease who are at risk of brain progression. Third, the increased awareness of the warning signs and risk factors has led to frequent screening for disease in the brain. However, more research is needed to identify patients at high risk of developing brain metastasis.

Lung Cancer

Small cell (SCLC) and non-small cell (NSCLC) are the major histological types of adenocarcinoma of the lung. It has been estimated that 50% of both SCLC and NSCLC patients will develop metastatic disease in the brain. For SCLC, 10% of patients already have CNS involvement at the time of primary tumor diagnosis (3). Treatment options are limited and standard of care is generally whole brain radiation therapy (WBRT) with corticosteroids to alleviate edema. Overall survival ranges from three to six months after diagnosis of CNS disease. Favorable prognostic factors that affect survival include Karnofsky's performance status, patient age (>65), control of primary tumor, and absence of extracranial metastatic disease (4).

Recently, the use of prophylactic cranial irradiation (PCI) has been investigated in clinical trials for both SCLC and NSCLC. PCI entails a series of low-dose radiation exposures to the whole brain to eliminate micrometastases. The short- and long-term neurocognitive consequences of PCI are still debated, particularly what portion of decline is from disease itself versus the therapy. While PCI has been believed to be beneficial in SCLC for some time, this benefit was thought to be limited to patients who had a complete response to chemotherapy for their primary disease. Slotman et al. conducted a randomized trial of patients with extensive SCLC disease and found that patients who received PCI were at a lower risk of developing symptomatic brain metastasis (hazard ratio 0.27, $p<0.001$) (5). Significant, yet modest, increases in disease-free (12 weeks compared with 14.7 weeks with PCI) and overall survival (5.4 months compared with 6.7 months with PCI) were also noted. In NSCLC, randomized trials have shown that PCI can prevent brain metastasis, but a survival benefit has not been demonstrated (6). The risk of significant neurocognitive decline following PCI has been studied and debated. A discussion of that literature is beyond the scope of this review; however, the need to identify lung cancer patients at risk of developing brain metastasis to minimize the number of patients receiving PCI is important.

Few studies have identified risk factors for the development of brain metastasis after diagnosis of lung cancer. This is in contrast to breast cancer where several risk factors have been identified that place breast cancer patients at high risk of developing of brain metastasis. Grinberg-Rashi et al. reported twelve candidate genes whose overexpression was associated with brain or general metastasis in 142 NSCLC primary tumors to identify a predictive pattern of high risk (7). Multivariate Cox regression analysis showed that the expression values of three genes (CDH2, KIFC1, and FALZ) in primary tumors had prognostic value. The role of CDH2 (N-Cadherin) is discussed further below.

Breast Cancer

Historically, brain metastases develop in ~15–20% of patients with systemic metastatic disease (8). At autopsy, asymptomatic metastatic lesions are found in the brains of more than 30% of breast cancer patients (9). Additionally, breast cancer is the solid tumor most commonly giving rise to leptomeningeal metastases, lesions in the tissue that line the brain and spinal cord (10). Several studies have reported risk factors for the development of brain metastases (Table 1). These studies generally examined characteristics of primary tumors from patients who did and did not develop brain metastases. Despite differences in the patient and tumor characteristics between studies, several trends emerged. Breast cancer

patients who were (a) young, (b) had ER-negative primary tumors, but overexpression of HER2 and/or epidermal growth factor receptor (EGFR)-positive primary tumors, and (c) had lymph node-positive disease or distant metastases were at high risk of brain metastatic relapse. Breast cancer is a disease with a number of subtypes and patients with metastatic triple-negative breast cancer (ER-, PR-, HER2 unamplified) tend to develop brain metastasis at a high rate (11). For the HER2 amplified subtype, the frequency of brain metastasis has been reported to be as high as 35% (12). As noted above, one reason for the increased incidence of brain metastatic disease is the improvement in patient survival due to effective control of systemic disease. This is particularly the case for breast cancer patients with HER2 amplified tumors treated with trastuzumab, a large monoclonal antibody to HER2 that does not cross the blood-brain barrier (BBB). Many of these patients had stable disease or were responding to treatment systemically at the time of brain relapse (13–18). Patients whose breast cancer has metastasized to the brain have an estimated one-year survival rate of 20%. Other than cranial radiotherapy and in limited cases, surgery, no effective treatment options exist.

Molecular Pathways Mediating Brain Metastasis

Gene Expression Analyses

In order to identify pathways specific to the development of brain metastasis and novel molecular targets/translational leads, several groups have undertaken gene expression studies in animal models and human tissue cohorts. Gene expression analyses of surgically resected brain metastases or of model systems consisting of parental and brain metastasizing variant cell lines have been reported for both lung and breast cancers. (19,20). For most of the model systems, a metastatic tumor cell line is injected either into the left cardiac ventricle or the carotid artery, so that the brain is a direct capillary bed. Cells are then isolated from the brain and re-injected for several rounds to establish a tissue-specific subline. It is necessary to compare and contrast the gene expression of patients and model systems to attain an accurate molecular portrait of brain metastases.

In a cohort of primary tumors from lung cancer patients who developed brain metastases, Grinberg-Rashi et al. found CDH2 (N-cadherin) to be significantly increased (p=0.009) (7). The cadherins are a family of Ca2+ dependent cell–cell adhesion molecules. N-cadherin is involved in multiple processes including inducing invasion and migration and promoting survival of cancer cells, regulating adhesion and neurite outgrowth (reviewed in (21)). Clinically, N-cadherin overexpression has been shown to be associated with decreased survival in poorly differentiated small cell lung carcinomas (excluding squamous cell carcinomas and adenocarcinomas) (22). An N-cadherin antagonist, a polypeptide known as ADH-1 or Exherin, is currently in clinical trials. Two Phase 1 clinical trials have been reported showing the compound to be generally well tolerated and to induce partial responses and stable disease in the treatment of solid tumors (23),(24).

Activation of the WNT/TCF pathway has also been identified as playing a role in lung cancer spread to the brain and bone. Nguyen et al. developed a T-cell factor 4 (TCF4) signature prognostic for lung metastasis to multiple organs using a bioinformatic analysis of a cohort of 107 human lung adenocarcinoma samples. Functional validation of these findings identified three genes from the TCF4 signature that were highly correlated with metastatic development – LEF1, HOXB9, and BMP4. Treatment of a brain-seeking lung cancer cell line H2030-BrM3 with Wnt3a significantly increased LEF1 and HOXB9. Confirming that LEF1 and HOXB9 are involved in metastasis, overexpression of the two genes led to an increase in bone and brain metastases whereas knockdown of each gene decreased metastatic incidence (25). Supporting the hypothesis that TCF4 may play an important role in lung cancer development, Xu et al. found increased TCF4 overexpression

in patients with stage III–IV lung cancer compared with stages I–II (26). Furthermore, expression of Wnt3a in a four-gene signature predicted increased mortality rates in lung cancer patients (27). Taken together, these studies suggest that a treatment targeting TCF4/Wnt3a might show efficacy in targeting lung cancer metastases, including those tumors that develop in the brain. These experiments bring up the questions, which remains unanswered, of whether brain metastases are molecularly linked to other systemic metastases – especially in lung cancer, where both develop simultaneously and early in the course of the disease – and to what degree do site-specific metastatic pathways actually exist?

In breast cancer, the Massague laboratory identified several genes that may mediate the spread of metastatic tumor cells to the brain. Bos et al. derived brain-seeking cells from the triple-negative human MDA-MB-231cell line and from the tumor of an ER- patient (CN34) (28). They found 243 genes differentially expressed between the brain metastasis and parental cell lines. Of those, the expression of 17 genes was correlated with brain relapse in patient samples. These 17 genes showed no association with bone, liver, or lymph node metastasis, although six genes correlated with a previously derived lung metastasis signature, including COX-2, MMP-1, ANGPTL4, LTBP1, fascin-1, and RARRES3 (29). Both the brain and lung sets also expressed an EGFR ligand (HB-EGF in the brain metastases and EREG in the lung metastases) indicating that the EGFR pathway may play an important role in breast cancer metastasis to the brain. To investigate the role of COX-2 and the EGFR pathway, Bos et al. used siRNA to inhibit COX-2 or treated with cetuximab to inhibit EGFR and, in both instances, observed a decrease in brain metastases ($p \leq 0.0195$ and $p \leq 0.002$ respectively) (28).

To identify genes that were responsible for brain specificity, Bos et al. also determined the gene expression patterns of organ-tropic MDA-MB-231 bone-, lung-, and brain-seeking cells and CN34 brain-seeking cells. They identified 26 upregulated genes in the brain-seeking cells including ST6GALNAC5, and a-2,6-sialyltransferase whose expression is typically found in the brain. Analysis of the presence of sialylic groups in resected metastatic breast tumors found that 50% had the carbohydrate group present in brain metastases compared with 18% in lung metastases. When animals were inoculated with ST6GALNAC5 knockdown cells in addition to treatment with cetuximab, an additive effect was observed compared with each method alone ($p=0.019$), indicating the importance of these pathways in the development of brain metastases (28).

In a gene expression analysis of resected human primary tumors and unlinked brain metastases (matched for TNM stage, hormone receptor status, and patient age), Palmieri et al. found hexokinase 2 (HK2) to be upregulated 1.5-fold in tumor cells in the brain ($p=0.13$). High HK2 expression predicated poor patient survival following a craniotomy ($p=0.028$) (30). HK2 is a member of the Hexokinase family that catalyzes the phosphorylation of glucose, thereby shunting glucose into a number of metabolic pathways. HK2 is often overexpressed in cancer cells and in gastric and hepatocellular carcinomas, the enzyme is associated with higher grade tumors, increased invasion, and poor patient survival (31–33). As a glucose-converting enzyme, HK2 is a regulator of cellular metabolism. Chen et al. found that the brain-seeking breast cancer cells upregulate a number of enzymes involved in cellular metabolism, which indicated that the cancer cells in the brain derive most of their energy from glucose (34). While this proteomic study did not specifically identify HK2, the upregulation of HK2 mRNA is consistent with these findings in that it may be the first step in many of the metabolic pathways dysregulated in tumor cells growing in the brain.

Integrins

Integrins are a class of heterodimeric proteins that, when activated, promote cell to extracellular matrix adhesion. $\alpha v\beta 3$ is a vitronectin-binding integrin known to be

dysregulated in a variety of cancers including breast cancer, glioma, ovarian cancer, and melanoma (35). Expression of αvβ3 was associated with increased metastatic potential in human cancer cells implanted into the mammary fat pad of mice, presumably through its ability to allow the cancer cells to interact with platelets in the blood stream, thereby arresting the cancer cells in the blood flow (35). Interestingly, the expression of a constitutively active form of αvβ3 promoted the growth of breast cancer cells in the brain, reduced the amount of hypoxia in the metastatic lesions by approximately 12-fold, and promoted angiogenesis by approximately 2-fold in normoxic regions of the tumor compared with the wild-type integrin (36).

Integrins have also been implicated in helping promote brain metastasis in lung cancer. Yoshimasu et al. developed a brain-seeking cell line from EBC-1 lung cancer cells and found that the EBC-1/brain cells were more adherent to fibronectin, type I collagen, and laminin than the parental EBC-1 cell line. Analysis of integrin expression revealed that the α3 subunit was increased in the brain-seeking line compared with the parental and EBC-1 bone-seeking line. Furthermore, inhibition of α3 with a blocking antibody resulted in a decrease in brain metastases by 83%. As α3 is only known to heterodimerize with β1, the authors surmise that α3β1 is important for EBC-1 metastasis to the brain (37).

HER2

Brain metastases are relatively common in metastatic breast cancer patients whose tumors have amplified the HER2 tyrosine kinase receptor gene. The specific scenario of HER2-positive (HER2+) breast cancer patients experiencing recurrence in the brain, while trastuzumab controlled their systemic disease, was described above, and suggests that a major contributing factor to this high incidence might be the inability of trastuzumab to cross the BBB. However, it also remains possible that HER2 overexpression alters the natural history of breast cancer to promote brain metastasis. HER2 was transfected into the brain-seeking cell variant of the MDA-MB-231 (231-BR-HER2) to study its effect on brain colonization in an experimental metastasis mouse model (38). Palmieri et al. showed that HER2 overexpression does not affect tumor cell arrival or intravasation into the brain, but it increases brain colonization. Specifically, there was a 2.5- to 3-fold increase in the formation of large metastases.

Lapatinib, an orally available small-molecule competitive tyrosine kinase inhibitor that binds reversibly to the cytoplasmic ATP-binding site in the kinase domains of EGFR and HER2, is a potential new therapy for HER2+ patients. The FDA-approved lapatinib in 2006 in combination with capecitabine for patients with HER2+ tumors who had progressed after treatment with regimens including an anthracycline, a taxane, and trastuzumab. In a preclinical study, lapatinib, when administered three days after intracardiac injection of HER2 and EGFR expressing 231-BR brain-seeking cells, inhibited the formation of large brain metastases by 54% and significantly reduced the phosphorylation level of HER2 in the brain metastases ($p<0.001$), suggesting clinical activity in the prevention setting (39).

This finding is supported by a randomized Phase III clinical trial of metastatic breast cancer. Lapatinib added to capecitabine improved the response rate and statistically significantly ($p = 0.002$) prolonged the time to disease progression compared with capecitabine alone in patients with metastatic HER2+ breast cancer whose disease had progressed following trastuzumab-based therapy (40). An update from the same trial showed a small but significant preventive effect of lapatinib in the cohort treated with lapatinib plus capecitabine. Four patients (2%) developed brain metastasis as a first site of progression versus 13 (6%) in the group of patients receiving capecitabine only ($p = 0.045$) (41). Confirmation of these trends will occur in ongoing adjuvant trials (Trans-ALLTO and TEACH). In contrast, only modest single-agent lapatinib activity has been observed in

patients with recurrent brain metastases from HER2+ breast cancer following trastuzumab and cranial radiotherapy, with 2.6–6% partial responses and stable disease for a period of 16 weeks in 13–14.7% of patients. A 20% volumetric reduction in brain metastasis was observed in 21% of patients treated with single agent lapatinib (42,43). These data argue that lapatinib will be more useful in preventing rather than treating brain metastasis of breast cancer.

Angiogenesis

Angiogenesis is considered a key component of tumor growth. As such, anti-angiogenic therapies that prevent the development of new tumor vasculature, thereby depriving the tumor of oxygen and nutrients, are being actively pursued. Certain anti-angiogenic agents could also transiently "normalize" the abnormal structure and function of tumor vasculature to make it more efficient for oxygen and drug delivery. However, the role of angiogenesis in metastatic brain disease has not been established, even as bevacizumab (a monoclonal antibody to VEGF) and sunitinib (a multi-kinase inhibitor including VEGFR) are in clinical trials for brain metastasis of breast and lung cancer.

As the brain is a highly vascularized organ, neo-angiogenesis may not be necessary for maintaining the growth of metastatic tumors (44). Experimental brain metastasis studies using either breast cancer or melanoma showed that tumor cells were growing along the pre-existing vessels, co-opting blood vessels rather than inducing neo-angiogenesis (45–48). Kusters et al., using a melanoma cell line injected into the internal carotid artery, showed that a brain metastasis could grow up to 3 mm without inducing the angiogenic switch described by Folkman (49), but instead modulated pre-existing blood vessels (45). Carbonell et al. used several breast and melanoma cell lines to show that the growth of micrometastasis in the brain was dependent on the co-option mechanism without induction of sprouting angiogenesis (48). Interestingly, the study showed that vessel density was even lower in areas of metastatic growth compared with normal brain. This finding has been confirmed independently in a MDA-MB-231-BR-HER2 overexpressing brain metastasis model (personal communication, Paul Lockman, Texas Tech University Health Sciences Center). Furthermore, Carbonell et al., reported that the co-option process is an active adhesive mechanism between the tumor cells and the exterior of the blood vessels. They showed that β1 integrin expressed by the tumor cell lines is the key component of co-option through its specific interaction with the vascular basement membrane (VBM). Indeed, the β1integrin-VBM interaction induces growth signaling pathway activation allowing metastatic growth in the brain (48).

While several reports excluded the requirement for neo-angiogenesis in brain metastatic tumor formation, Kim et al. reported an increase in blood vessel density as well as vascular remodeling (50). They used variants of human MDA-MB-231 breast cancer cells isolated from the brain after repeated cycles of carotid artery injection. The brain metastasis-selected variants released significantly more VEGF-A into the culture supernatant and showed increased potential for brain colonization and higher microvessel density in vivo compared with parental MDA-MB-231 cells. Subsequently, they evaluated the efficacy of a VEGF receptor kinase inhibitor (PTK787) in mice injected with the brain metastasis-selected variants and showed reduced brain metastasis burden and decreased microvascular density. However, there was no significant increase in survival compared with vehicle treated controls. This may not be surprising given the recent findings of both Paez-Ribes et al. and Ebos et al. (51,52). Combined, these authors used five model systems and four different anti-angiogenic compounds to show that, while anti-angiogenic therapies decreased primary tumor growth they actually increased local invasion and distant metastasis. This worrisome adverse effect of anti-angiogenic therapy demonstrates the need for additional preclinical studies to elucidate a clear mechanism of action, particularly in brain metastasis where side

effects (i.e., hemorrhage) are an important issue. That said, Socinski et al. recently completed a Phase II trial in patients with NSCLC and brain metastasis to assess the safety of bevacizumab. Bevacizumab in combination with either carboplatin, pemetrexed or erlotinib was associated with a low incidence of CNS hemorrhage (53).

Areas for Future Translation

An important aspect of translational research on brain metastasis that must be considered when identifying new molecular targets is the BBB. Consider the following: a retrospective study by Chen et al. showed that NSCLC patients who had a pathological complete response were at high risk of developing brain metastasis (54). Omuro et al. followed NSCLC patients treated with gefitinib and the five-year incidence of brain metastasis was 60%, and 33% of responding patients developed brain metastases as their first site of relapse (55). In breast cancer, HER2+ patients responding to trastuzumab have a high frequency of brain metastasis (12). These data support what has long been proposed: that many agents cannot cross the BBB, so that tumor cells that do transverse the barrier are protected from these drugs. The BBB, or more precisely the vasculature of the brain, is composed of endothelial cells that are sealed together by tight junctions. These cells lack fenestra and are further surrounded by basement membrane, pericytes, and the feet of astrocytes. This composition severely limits passive diffusion of molecules into the brain. If a drug can make its way across the barrier, then it may be immediately shuttled back out of the brain by a number efflux pumps. The need for effective drugs that can cross the BBB and achieve therapeutic levels in the brain is obvious. Chemical characteristics of compounds with a higher potency to cross the BBB include being small (<500 mw), nonpolar, and not substrates for efflux pumps, although numerous other considerations are important. It remains a high priority that BBB permeable lead compounds be selected as drug development progresses, so that brain efficacy can be maximized.

Another translational goal for brain metastasis is to improve the efficacy of radiation therapy. Radiation therapy comes in two forms: stereotactic radiosurgery (“Gamma knife”), which delivers a high dose to the bed of a metastasis and whole brain radiation therapy (WBRT). The ability to improve on the results of WBRT would be of great benefit and as such radiation sensitizing agents are an area of intense interest. Randomized control trials have tested lonidamine, metronidazole, misonidazole, motexafin gadolinium, bromodeoxyuridine, and efaproxiral as sensitizing agents and failed to show benefit (56). Identifying agents that synergize with stereotactic radiation therapy is also a goal for the treatment of established brain metastases. Recently, the HDAC inhibitor vorinostat was shown to synergize with radiation and extend lifespan in the 231-BR breast cancer brain metastasis model system ($p=0.038$) (57). This preclinical work has led to a Phase I clinical trial investigating this combination in patients with brain metastasis (NCT00838929, www.clinicaltrials.gov).

The molecular pathways described above identify some of the important findings and considerations for translational research for brain metastases. The research suggests the hypothesis that it might be more realistic to prevent the metastatic colonization of the brain than to reduce the size of an already established brain metastasis. To this end, it would be beneficial if preclinical drug studies clearly tested an agent when given just after tumor cell inoculation as well as after micrometastases or large metastases had formed. This would identify a drug as a prevention or a treatment agent. This type of experiment was performed with vorinostat in the 231-BR model system. When drug treatment was delayed until after the formation of micrometastases, a progressive loss in efficacy was observed compared with an experimental group that received vorinostat immediately following tumor cell inoculation, indicating only a preventive effect for vorinostat (58). In contrast, most brain

metastasis clinical trials are conducted on patients with established metastases, and the drug is asked to significantly decrease the size or volume of a large tumor.

What kind of trial should be conducted when the preclinical data are exclusively preventative? We favor a design enrolling cancer patients with established brain metastasis, who are therefore at high risk of the development of additional brain metastases. Patients could be on chemotherapy, but should not have received whole brain radiotherapy. The primary endpoint would not be shrinkage of the initial metastasis, but time to progression, i.e., the development of a new brain metastasis (personal communication, Minesh Mehta, University of Wisconsin). These time-to-progression studies could provide sufficient data to support a full metastatic trial, which would involve greater numbers of patients, and more funding and time.

Close coordination between molecular biologists, medicinal chemists, BBB pharmacologists, radiation oncologists, and medical oncologists will be needed to address the vital issues of treating brain metastatic disease. New clinical trial designs as noted above and novel preclinical approaches may be needed. However, the potential for clinical benefit is great for this population of at risk and affected patients.

Acknowledgments

This work was supported by the intramural program of the National Cancer Institute (NCI).

References

1. Weil R, Palmieri D, Bronder J, Stark A, Steeg P. Breast cancer metastasis to the central nervous system. Am. J. Pathol 2005;167:913–920. [PubMed: 16192626]
2. Patel R, Mehta M. Targeted therapy for brain metastases: improving the therapeutic ratio. Clin. Cancer Res 2007;13(6):1675–1683. [PubMed: 17363520]
3. Castrucci WA, Knisely JP. An update on the treatment of CNS metastases in small cell lung cancer. Cancer J 2008;14(3):138–146. [PubMed: 18536552]
4. Schwer AL, Gaspar LE. Update in the treatment of brain metastases from lung cancer. Clin Lung Cancer 2006;8(3):180–186. [PubMed: 17239293]
5. Slotman B, Faivre-Finn C, Kramer G, Rankin E, Snee M, Hatton M, et al. Prophylactic cranial irradiation in extensive small-cell lung cancer. N Engl J Med 2007;357(7):664–672. [PubMed: 17699816]
6. Lester JF, MacBeth FR, Coles B. Prophylactic cranial irradiation for preventing brain metastases in patients undergoing radical treatment for non-small-cell lung cancer: a Cochrane Review. Int J Radiat Oncol Biol Phys 2005;63(3):690–694. [PubMed: 15913909]
7. Grinberg-Rashi H, Ofek E, Perelman M, Skarda J, Yaron P, Hajduch M, et al. The expression of three genes in primary non-small cell lung cancer is associated with metastatic spread to the brain. Clin Cancer Res 2009;15(5):1755–1761. [PubMed: 19190132]
8. Barnholtz-Sloan JS, Sloan AE, Davis FG, Vigneau FD, Lai P, Sawaya RE. Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. J Clin Oncol 2004;22(14):2865–2872. [PubMed: 15254054]
9. Stemmler HJ, Heinemann V. Central nervous system metastases in HER-2-overexpressing metastatic breast cancer: a treatment challenge. Oncologist 2008;13(7):739–750. [PubMed: 18614587]
10. Kesari S, Batchelor T. Leptomeningeal metastases. Neurol Clin 2003;21:25–66. [PubMed: 12690644]
11. Lin NU, Claus E, Sohl J, Razzak AR, Arnaout A, Winer EP. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. Cancer 2008;113(10):2638–2645. [PubMed: 18833576]

12. Lin NU, Winer EP. Brain metastases: the HER2 paradigm. Clin Cancer Res 2007;13(6):1648–1655. [PubMed: 17363517]
13. Pestalozzi BC, Zahrieh D, Price KN, Holmberg SB, Lindtner J, Collins J, et al. Identifying breast cancer patients at risk for Central Nervous System (CNS) metastases in trials of the International Breast Cancer Study Group (IBCSG). Ann Oncol 2006;17(6):935–944. [PubMed: 16603601]
14. Bendell J, Domchek S, Burstein H, Harris L, Younger J, Kuter I, et al. Central Nervous System Metastases in Women who Receive Trastuzumab-Based Therapy for Metastatic Breast Carcinoma. Cancer 2003;97:2972–2977. [PubMed: 12784331]
15. Gabos Z, Sinha R, Hanson J, Chauhan N, Hugh J, Mackey JR, et al. Prognostic significance of human epidermal growth factor receptor positivity for the development of brain metastasis after newly diagnosed breast cancer. J Clin Oncol 2006;24(36):5658–5663. [PubMed: 17102066]
16. Shmueli E, Wigler N, Inbar M. Central nervous system progression among patients with metastatic breast cancer responding to trastuzumab treatment. Eur J Cancer 2004;40(3):379–382. [PubMed: 14746856]
17. Clayton A, Danson S, Jolly S, Ryder W, Burt P, Stewart A, et al. Incidence of cerebral metastases in patients treated with trastuzumab for metastatic breast cancer. Br J Cancer 2004;91:639–643. [PubMed: 15266327]
18. Lower E, Blau R, Bismayer J, Brennan L, Broun R, Dannenman W, et al. Increased brain metastasis detected in metastatic breast cancer patients receiving herceptin. Breast Cancer Res Treat 2001;69:271. Abstract 351.
19. Kikuchi T, Daigo Y, Ishikawa N, Katagiri T, Tsunoda T, Yoshida S, et al. Expression profiles of metastatic brain tumor from lung adenocarcinomas on cDNA microarray. Int J Oncol 2006;28(4):799–805. [PubMed: 16525627]
20. Saito N, Hatori T, Aoki K, Hayashi M, Hirata Y, Sato K, et al. Dynamics of global gene expression changes during brain metastasis formation. Neuropathology 2009;29(4):389–397. [PubMed: 19019174]
21. Derycke LD, Bracke ME. N-cadherin in the spotlight of cell-cell adhesion, differentiation, embryogenesis, invasion and signalling. Int J Dev Biol 2004;48(5–6):463–476. [PubMed: 15349821]
22. Nakashima T, Huang C, Liu D, Kameyama K, Masuya D, Kobayashi S, et al. Neural-cadherin expression associated with angiogenesis in non-small-cell lung cancer patients. Br J Cancer 2003;88(11):1727–1733. [PubMed: 12771988]
23. Perotti A, Sessa C, Mancuso A, Noberasco C, Cresta S, Locatelli A, et al. Clinical and pharmacological phase I evaluation of Exherin (ADH-1), a selective anti-N-cadherin peptide in patients with N-cadherin-expressing solid tumours. Ann Oncol 2009;20(4):741–745. [PubMed: 19190075]
24. Beasley GM, McMahon N, Sanders G, Augustine CK, Selim MA, Peterson B, et al. A phase 1 study of systemic ADH-1 in combination with melphalan via isolated limb infusion in patients with locally advanced in-transit malignant melanoma. Cancer 2009;115(20):4766–4774. [PubMed: 19637344]
25. Nguyen DX, Chiang AC, Zhang XH, Kim JY, Kris MG, Ladanyi M, et al. WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. Cell 2009;138(1):51–62. [PubMed: 19576624]
26. Xu HT, Wei Q, Liu Y, Yang LH, Dai SD, Han Y, et al. Overexpression of axin downregulates TCF-4 and inhibits the development of lung cancer. Ann Surg Oncol 2007;14(11):3251–3259. [PubMed: 17768662]
27. Raz DJ, Ray MR, Kim JY, He B, Taron M, Skrzypski M, et al. A multigene assay is prognostic of survival in patients with early-stage lung adenocarcinoma. Clin Cancer Res 2008;14(17):5565–5570. [PubMed: 18765549]
28. Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, et al. Genes that mediate breast cancer metastasis to the brain. Nature 2009;459(7249):1005–1009. [PubMed: 19421193]
29. Minn AJ, Gupta GP, Padua D, Bos P, Nguyen DX, Nuyten D, et al. Lung metastasis genes couple breast tumor size and metastatic spread. Proc Natl Acad Sci U S A 2007;104(16):6740–6745. [PubMed: 17420468]

30. Palmieri D, Fitzgerald D, Shreeve SM, Hua E, Bronder JL, Weil RJ, et al. Analyses of resected human brain metastases of breast cancer reveal the association between up-regulation of hexokinase 2 and poor prognosis. Mol Cancer Res 2009;7(9):1438–1445. [PubMed: 19723875]

31. Mathupala SP, Ko YH, Pedersen PL. Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. Oncogene 2006;25(34): 4777–4786. [PubMed: 16892090]

32. Peng SY, Lai PL, Pan HW, Hsiao LP, Hsu HC. Aberrant expression of the glycolytic enzymes aldolase B and type II hexokinase in hepatocellular carcinoma are predictive markers for advanced stage, early recurrence and poor prognosis. Oncol Rep 2008;19(4):1045–1053. [PubMed: 18357395]

33. Rho M, Kim J, Jee CD, Lee YM, Lee HE, Kim MA, et al. Expression of type 2 hexokinase and mitochondria-related genes in gastric carcinoma tissues and cell lines. Anticancer Res 2007;27(1A):251–258. [PubMed: 17352240]

34. Chen EI, Hewel J, Krueger JS, Tiraby C, Weber MR, Kralli A, et al. Adaptation of energy metabolism in breast cancer brain metastases. Cancer Res 2007;67(4):1472–1486. [PubMed: 17308085]

35. Felding-Habermann B, O'Toole T, Smith J, Fransvea E, Ruggeri Z, Ginsberg M, et al. Integrin activation controls metastasis in human breast cancer. Proc. Natl. Acad. Sci USA 2001;98:1853–1858. [PubMed: 11172040]

36. Lorger M, Krueger JS, O'Neal M, Staflin K, Felding-Habermann B. Activation of tumor cell integrin alphavbetα3 controls angiogenesis and metastatic growth in the brain. Proc Natl Acad Sci U S A 2009;106(26):10666–10671. [PubMed: 19541645]

37. Yoshimasu T, Sakurai T, Oura S, Hirai I, Tanino H, Kokawa Y, et al. Increased expression of integrin alphα3beta1 in highly brain metastatic subclone of a human non-small cell lung cancer cell line. Cancer Sci 2004;95(2):142–148. [PubMed: 14965364]

38. Palmieri D, Bronder JL, Herring JM, Yoneda T, Weil RJ, Stark AM, et al. Her-2 overexpression increases the metastatic outgrowth of breast cancer cells in the brain. Cancer Res 2007;67(9): 4190–4198. [PubMed: 17483330]

39. Gril B, Palmieri D, Bronder JL, Herring JM, Vega-Valle E, Feigenbaum L, et al. Effect of lapatinib on the outgrowth of metastatic breast cancer cells to the brain. J Natl Cancer Inst 2008;100(15): 1092–1103. [PubMed: 18664652]

40. Geyer C, Forster J, Lindquist D, Chan S, Romieu G, Peinkowski T, et al. Lapatinib plus capecitabine for HER-2-positive advanced breast cancer. N Engl. J. Med 2006;355:2733–2743. [PubMed: 17192538]

41. Cameron D, Casey M, Press M, Lindquist D, Pienkowski T, Romieu CG, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analyses. Breast Cancer Res Treat 2008;112(3):533–543. [PubMed: 18188694]

42. Lin N, Carey L, Liu M, Younger J, Come S, Bullitt E, et al. Phase II trial of lapatinib for brain metastases in patients with HER2+ breast cancer. J. Clin. Oncol 2006;24(18S):503.

43. Lin NU, Dieras V, Paul D, Lossignol D, Christodoulou C, Stemmler HJ, et al. Multicenter phase II study of lapatinib in patients with brain metastases from HER2-positive breast cancer. Clin Cancer Res 2009;15(4):1452–1459. [PubMed: 19228746]

44. Fidler IJ, Yano S, Zhang RD, Fujimaki T, Bucana CD. The seed and soil hypothesis: vascularisation and brain metastases. Lancet Oncol 2002;3(1):53–57. [PubMed: 11905606]

45. Kusters B, Leenders WP, Wesseling P, Smits D, Verrijp K, Ruiter DJ, et al. Vascular endothelial growth factor-A(165) induces progression of melanoma brain metastases without induction of sprouting angiogenesis. Cancer Res 2002;62(2):341–345. [PubMed: 11809675]

46. Leenders W, Kusters B, Verrijp K, Maass C, Wesseling P, Heerschap A, et al. Antiangiogenic Therapy of Cerebral Melanoma Metastases Results in Sustained Tumor Progression via Vessel Co-Option. Clin Cancer Res 2004;10:6222–6230. [PubMed: 15448011]

47. Fitzgerald D, Palmieri D, Hua E, Hargrave E, Herring J, Qian Y, et al. Reactive glia are recruited by highly proliferative brain metastases of breast cancer and promote tumor cell colonization. Clin. Exp. Metast 2008;25:799–810.

48. Carbonell WS, Ansorge O, Sibson N, Muschel R. The vascular basement membrane as "soil" in brain metastasis. PLoS One 2009;4(6):e5857. [PubMed: 19516901]
49. Folkman J. What is the evidence that tumors are angiogenesis-dependent? J Natl Cancer Inst 1990;82:4–6. [PubMed: 1688381]
50. 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 2004;21:107–118. [PubMed: 15168728]
51. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 2009;15(3):232–239. [PubMed: 19249681]
52. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 2009;15(3):220–231. [PubMed: 19249680]
53. Socinski MA, Langer CJ, Huang JE, Kolb MM, Compton P, Wang L, et al. Safety of bevacizumab in patients with non-small-cell lung cancer and brain metastases. J Clin Oncol 2009;27(31):5255–5261. [PubMed: 19738122]
54. Chen AM, Jahan TM, Jablons DM, Garcia J, Larson DA. Risk of cerebral metastases and neurological death after pathological complete response to neoadjuvant therapy for locally advanced nonsmall-cell lung cancer: clinical implications for the subsequent management of the brain. Cancer 2007;109(8):1668–1675. [PubMed: 17342770]
55. Omuro AM, Kris MG, Miller VA, Franceschi E, Shah N, Milton DT, et al. High incidence of disease recurrence in the brain and leptomeninges in patients with nonsmall cell lung carcinoma after response to gefitinib. Cancer 2005;103(11):2344–2348. [PubMed: 15844174]
56. Eichler AF, Loeffler JS. Multidisciplinary management of brain metastases. Oncologist 2007;12(7):884–898. [PubMed: 17673619]
57. Baschnagel A, Russo A, Burgan WE, Carter D, Beam K, Palmieri D, et al. Vorinostat enhances the radiosensitivity of a breast cancer brain metastatic cell line grown in vitro and as intracranial xenografts. Mol Cancer Ther 2009;8(6):1589–1595. [PubMed: 19509253]
58. Palmieri D, Lockman PR, Thomas FC, Hua E, Herring J, Hargrave E, et al. Vorinostat inhibits brain metastatic colonization in a model of triple-negative breast cancer and induces DNA double-strand breaks. Clin Cancer Res 2009;15(19):6148–6157. [PubMed: 19789319]
59. Clark G, GW Sledge J, Osborne C, McGuire W. Survival from first recurrence: relative importance of prognostic factors in 1,015 breast cancer patients. J. Clin. Oncol 1987;5:55–61. [PubMed: 3806159]
60. Slimane K, Andre F, Delaloge S, Dunant A, Perez A, Grenier J, et al. Risk factors for brain relapse in patients with metastatic breast cancer. Ann Oncol 2004;15(11):1640–1644. [PubMed: 15520065]
61. Evans A, James J, Cornford E, Chan S, Burrell H, Pinder S, et al. Brain metastases from breast cancer: identification of a high-risk group. Clin. Oncol 2004;16:345–349.
62. Miller K, Weathers T, Hanley L, Timmerman R, Dickler M, Shen J, et al. Occult central nervous system involvement in patients with metastatic breast cancer: prevalence, predictive factors and impact on overall survival. Ann. Oncol 2003;14:1072–1077. [PubMed: 12853349]
63. Carey LA, Ewend MG, Metzger R, Sawyer L, Dees EC, Sartor CI, et al. Central nervous system metastases in women after multimodality therapy for high risk breast cancer. Breast Cancer Res Treat 2004;88(3):273–280. [PubMed: 15609130]
64. Hicks D, Short S, Prescott N, Tarr S, Coleman K, Yoder B, et al. Breast cancers with brain metastases are more likely to be estrogen receptor negative, express the basal cytokeratin CK5/6 and over-express Her-2 or EGFR. Am. J. Surg. Pathol 2006;30:1097–1104. [PubMed: 16931954]
65. Sezgin C, Gokmen E, Esassolak M, Ozdemir N, Goker E. Risk factors for central nervous system metastasis in patients with metastatic breast cancer. Med Oncol 2007;24(2):155–161. [PubMed: 17848738]

Table 1

Risk factors for the development of brain metastasis from breast cancer

Risk Factors	Reference
Node-positive (< 0.01) Tumor grade, size > 2 cm., ER-negative, HER2-positive (each < 0.01)	Pestalozzi et al. (13)
ER-negative (0.0025)	Clark et al. (59)
Lung metastases (0.0003) ER-negative (0.002)	Slimane et al. (60)
Young age (0.0002) ER-negative (0.0003)	Evans et al. (61)
HER2+ tumor (0.02) Number of sites of metastatic disease (0.03)	Miller et al. (62)
Young age (median 40) (0.007) Lymphovascular invasion in primary tumor (< 0.0001) Relapse in bone or liver (< 0.006)	Carey et al. (63)
Young age (0.001) ER-negative (<0.001) High grade (0.002) CK5/6+ (<0.001)	Hicks et al. (64)
Lung metastasis (0.02) High p53 expression (0.05)	Sezgin et al. (65)