Chapter 6
Possibilities of Targeted Therapies for Brain Metastasis

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Abstract In the era of therapies successfully targeting distinct molecular pathways in cancer, the incidence and relevance of brain metastases are rising. Generally, the old therapeutic nihilism with respect to brain metastasis has given way to a more pragmatic approach, aiming to optimally combine (radio)surgery, whole brain radiotherapy, and sometimes systemic chemotherapy. However, local approaches inevitably fail to address the multifocal nature of the disease, whole brain radiotherapy shows relevant neurotoxicity, and systemic chemotherapy faces the obstacle of the blood-brain/tumor-barrier. Therefore, judicious addition of targeted agents to the therapeutic armamentarium for brain metastases holds the promise to make a real difference for patients suffering from this devastating disease. Unfortunately, because of their unfavorable prognosis, patients with brain metastases have traditionally been excluded from studies with targeted therapies. This is changing now for several reasons, making it likely that we will obtain relevant clinical data in the next few years. The following chapter gives an overview of new therapies targeting molecular pathways both in the tumor stroma and in cancer cells, covering its theoretical and reported activity against brain metastases. A special emphasis will be placed on prophylaxis, i.e. prevention of macrometastasis formation.

1 Introduction

Brain metastasis (BM) therapy faces the challenge to efficiently target the cancer cell, or its supportive relationship with the brain parenchyma, without damaging the delicate organ it is colonizing. Therapeutic agents targeting distinct molecular

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pathways of cancer cells hold the promise to do just that [1]. Furthermore, the fact that the majority of BM patients suffer from multiple metastases that occur unpredictably at different sites during the course of the disease makes a systemic therapy treating macro- and also micrometastases most adequate. Optimally, a systemic therapy might even prevent brain colonization altogether, or at least arrest single cells or micrometastases in a dormant state. Even though we are far away from having such a weapon with proven clinical efficacy at hand, there is some reason to be cautiously optimistic.

First, one of the greatest challenges of systemic brain tumor therapy can be overcome: the blood-brain/blood-tumor barrier. For example, antiangiogenic agents targeting the VEGF pathway have to reach only the endothelial cell, but do not have to cross the entire blood-tumor barrier, consisting of additional layers of thickened basement membrane, irregular pericyte coverage, and occasionally astrocyte foot processes in the brain [2–4]. In contrast, this is mandatory for all chemotherapeutics or targeted drugs that have to reach the cancer cell to exert their action. Furthermore, drug penetration to the cancer cell is also hindered by the aberrant and highly heterogeneous blood flow in brain tumor vessels lacking the normal hierarchical structure of normal brain vasculature. Finally, increased interstitial fluid pressure hinders extravasation into the tumor [5]. All in all, it is a futile challenge for most drugs available today to overcome these barriers between the blood stream and the brain tumor cell, at least in meaningful concentrations. Like antiangiogenic agents, immunomodulators targeting cells responsible for anti-tumor immunity (e.g., Ipilimumab) do not need to reach the cancer cell to exert their action.

Second, targeted small molecule and even antibody inhibitors can be designed to efficiently cross the blood-brain barrier (BBB), or linked to an agent that is actively transported over it [6]. Until recently, pharmaceutical companies did not show great interest in developing such agents. However, the rising incidence of brain diseases like Alzheimer's or Parkinson's changed this, and today pharmaceutical companies start drug development programs to select and/or design agents with maximum BBB penetration capabilities, including antitumor agents.

Third, it is proven that macrometastatic outgrowth in the brain can be effectively prevented – by prophylactic whole brain irradiation which targets the whole organ. Applied during a short time frame (2–3 weeks) early in the beginning of the disease, prophylactic whole brain irradiation decreases the incidence of (macro)metastasis formation by more than 50%, an effect that continues over the next 24 months [7]. This matches well with common preclinical and clinical experience that prevention of a disease is much easier than treating it when it is fully developed. Since targeted agents can be applied over long periods of time, are active in the whole body, and do not show the neurotoxicity of whole brain radiotherapy (WBRT), they seem to be perfect future candidates for brain metastasis prevention.

In the last 10 years, targeted cancer therapy [8] has grown explosively and is now established for many tumor entities. However, like established cytotoxic therapies, its role in influencing the occurrence of metastases has rarely been systemically addressed [9, 10]. Furthermore, virtually no targets for molecular therapies have been identified in small cell lung cancer (SCLC) yet, which makes the tumor entity

with the highest incidence of BM formation still largely terra incognita. In general, clinical trials are not (yet) designed to prospectively investigate the rate of metastasis formation. Taken that the vast majority of cancer patients die of metastasis and not the primary tumor, this appears to be one of the most important issues for future cancer research. This chapter provides an overview of those targeted therapies that seem most suited for use in BM therapy, both of established macrometastases and of early metastatic events. Furthermore, the clinical data available today is provided, with the limitation that a controlled, prospective, randomized clinical trial testing the effect of targeted therapies on BM has not been completed yet. However, there are several clinical studies on the way that aim to explore the efficacy of targeted agents in BM therapy. At this time, patient data from small case series, retrospective analyses, or even anecdotal reports may teach us what pathways and agents might be the best candidates for future trials. In the following paragraphs, those pharmacologically targetable molecular pathways will be presented that are most promising with respect to BM therapy, because of existing clinical data or for conceptual reasons.

2 Antiangiogenic Therapy

Most antiangiogenic agents target the VEGF pathway. It is important to keep in mind that – in general – tyrosin kinase inhibitors show only moderate selectivity for one receptor (or even class of receptors) [11], which extends their activity to PDGFRs and others. During vessel formation, PDGF-BB is required for the recruitment and differentiation of pericytes, and preclinical data suggest that concomitant inhibition of VEGF and PDGF signaling can improve anti-tumor activity compared with VEGF alone [12]. It needs to be clarified if normalization of brain tumor vasculature during VEGF pathway inhibition [2] is preferable for every patient, or if agents that disturb the vasculature by disrupting pericyte support (such as PDGF receptor inhibitors) may sometimes have greater benefit. However, severe reduction or lack of pericyte coverage may also facilitate metastasis by disrupting the integrity of the vasculature [13]. In contrast, bevacizumab, the antiangiogenic agent most widely used today, inhibits only VEGF-A. Accordingly, there is rising evidence that different antiangiogenic agents can exert very different actions in vivo, which makes it problematic to generalize one finding with one inhibitor to all antiangiogenic agents. Furthermore, other pathways like the angiopoietin system are now coming into the focus of drug development. From a conceptual point of view, antiangiogenic agents that mainly prevent ligand binding/receptor activation at the endotheralial cell do not have to cross the BBB/blood-tumor-barrier, which could prove to be their most important advantage for brain tumor therapy. Therefore, the argument that large antibodies like bevacizumab do not cross the intact BBB and may therefore not be useful in brain tumors does not apply.

In primary brain tumors, antiangiogenic therapy with the humanized monoclonal anti-VEGF-A antibody bevacizumab has shown clinical activity [14], received an

accelerated FDA approval due to its excellent response rates and very good progression free survival data at 6 months, and is now widely used for patients suffering from malignant gliomas. Two large double-blind, placebo-controlled phase III studies are currently investigating bevacizumab as first-line therapy for glioblastoma in addition to radio/chemotherapy (www.clinicaltrials.gov). Both have completed accrual, recruited more than 700 patients each, and first results are expected for 2013. This high level of clinical study activity is limited to primary brain tumors though. With respect to BM, there is mainly preclinical evidence from multiple animal models that antiangiogenic agents can be effective: elevated VEGF expression has been linked to the development of BM in a murine model [15]. Kim et al. showed that treatment with the VEGF-receptor tyrosine kinase inhibitor PTK787/Z 222584 reduced angiogenesis and restricted the growth of brain metastases in a murine breast cancer model [16]. In another mouse model, inhibition of VEGF signaling using bevacizumab was able to efficiently inhibit angiogenesis and metastasis formation of lung cancer, but not melanoma cells [17]. In established brain metastatic disease, high-dose bevacizumab therapy could induce vascular normalization, and blood vessel and tumor cell regression [von Baumgarten L, Kienast Y, Winkler F; unpublished data], similar to what we have found in glioblastoma [18]. However, from what is known today, the growth pattern of different tumor (sub)types in the brain is highly different, with lung carcinoma being the most angiogenesis-dependent, and melanoma being the most angiogenesis-independent (due to the ability to grow co-optive along pre-existing brain microvessels). Breast cancer seems to be located in the middle of this continuum, but considerable variability within tumor entities is likely. It is plausible that this has great impact on the efficacy of antiangiogenic therapies [17]. Conclusively, antiangiogenic therapy has not shown efficacy in melanoma patients yet. All in all, these preclinical results argue for a serious clinical evaluation of antiangiogenic agents in BM therapy and prophylaxis. As with other tumor sites, a clinical parameter (laboratory, imaging, or histological) that predicts response to antiangiogenic therapy would be extremely helpful – but is lacking. Until then, the preclinical results strengthen the point that brain metastases from different tumor entities should be investigated separately in clinical studies.

There is limited data about the clinical activity of antiangiogenic agents in BM patients yet. This is mainly due to exclusion of patients with BM from clinical trials with antiangiogenic agents since a single patient with brain metastatic hepatocellular carcinoma (a disease with high incidence of intracranial bleedings [19]) developed a cerebral hemorrhage 2 weeks after a single dose of bevacizumab in a phase I trial [20]. Since then, large meta-analyses, retrospective case studies and a prospective phase II trial have shown that bevacizumab therapy does not increase the incidence of clinically relevant intracranial bleedings in patients with central nervous system (CNS) metastases [21–23]. This seems to be also true for tyrosine kinase inhibitors [24]. Consequently, the contraindication for BM has been removed from the bevacizumab label in Europe and most likely will be removed also in the US in due time. Several phase I and II trials evaluating bevacizumab alone or in combination with cytotoxic compounds in BM patients have been initiated and are ongoing (Table 6.1). Other drugs with antiangiogenic properties that are investigated

Table 6.1 Overview of targeted agents that are currently being investigated in ongoing clinical studies (http://clinicaltrials.gov)

Type of treatment	Investigational agent	Tumor type per trial	Trial phases
Anti-angiogenic agents	Bevacizumab	All, NSCLC, breast cancer, melanoma	I, II
	Cilengitide	Lung cancer	I
	Sorafenib	All, kidney cancer	I, II
	Sunitinib	All, NSCLC, kidney cancer, melanoma, breast cancer	I, II
	Thalidomide	All, melanoma	I, II, III
BRAF inhibitors	GSK2118436	Melanoma	II
	Vemurafenib	Melanoma	II
EGFR inhibitors	Afatinib	All	II
	Erlotinib	NSCLC	I, II, III
	Gefitinib	NSCLC, lungadenoc arcinoma	II
	Lapatinib	Breastcancer, lungcancer	I, II
	Trastuzumab	Breastcancer	II
	Nimotuzumab	NSCLC	II
Gamma-secretase/ notch inhibitor	RO4929097	Breastcancer	I/II
HDAC inhibitors	Panobinostat	All	I
	Vorinostat	All, NSCLC	I
Immunomodulatory agents	Ipilimumab	Melanoma	II
	Interferon alfa-2a	Breastcancer	II
mTor inhibitors	Everolimus	Breastcancer, NSCLC	I, II
PARP inhibitors	ABT-888	All	I
	Iniparib	Breastcancer	II
Protein kinase C beta inhibitor	Enzastaurin	SCLC	II
Radiation sensitizers	Cytochlorandtetrahy drouridine	All	I
	Efaproxiral	All, breastcancer	III

From [1]

in clinical trials enrolling BM patients include sunitinib, sorafenib, and cilengitide. Older trials evaluated non-specific antiangiogenic agents such as thalidomide, in combination with WBRT, without demonstration of improved efficacy, but high numbers of dropouts due to severe side effects [25]. Hopefully, the newer studies with more specific antiangiogenic agents will provide us with data on the efficacy of antiangiogenic drugs in established brain metastases.

The experience with antiangiogenic agents in primary brain tumors highlights several issues that may require special attention also in brain metastases [26]. VEGF-targeting drugs like bevacizumab have a BBB-stabilizing effect which leads to a reduction of brain edema and radiological contrast media uptake. This effect, which has also been shown in brain metastases [27], may lead to

overestimation of tumor shrinkage and requires stringent application of adequate response criteria and clinical trial endpoints [28]. Interestingly, treatment of mice with glioblastoma with cediranib prolonged survival despite persistent brain tumor growth in mice by reducing brain edema [29]. It remains to be clarified whether a potential tumor growth-inhibitory or an anti-edematous effect is responsible for the clinical benefit in humans.

Prophylactic administration of VEGF antagonists seems also feasible and is an attractive approach that can be tested in patients at high risk for developing BM [17, 30]. In a novel preclinical animal model we used in vivo multiphoton microscopy for real-time imaging, and tested the prophylactic effect of VEGF-A blockade on the outgrowth of individual metastasizing lung cancer cells in the mouse brain [17] (Fig. 6.1). One experimental group received the anti-VEGF-A antibody bevacizumab just after tumor cell inoculation into the internal carotid artery. Bevacizumab completely prevented early angiogenic events in micrometastases, and thereby induced prolonged dormancy of micrometastatic tumors (maximum ten cells). We did not observe any effects on any other essential steps of the metastatic cascade (initial arrest at vascular branch points; early extravasation; perivascular position with close physical contact to a brain microvessel). Bevacizumab had no effect on the metastatic colonization of melanoma cells in the brain, which showed a non-angiogenic growth pattern under normal conditions. Further preclinical studies are required to determine how discontinued versus prolonged inhibition of VEGF, and combination with other treatment modalities, influences the establishment and growth of micrometastatic disease. An interesting retrospective analysis from the clinic has shown that patients with renal cell carcinoma who received sorafenib had lower incidence of brain metastases than those patients who did not receive sorafenib (3% vs. 12%). This effect stayed statistically significant over 2 years [31]. Even though both groups consisted of considerably low numbers of patients, the prophylactic properties of antiangiogenic agents is an area of important future clinical research.

It is also important to mention several caveats regarding antiangiogenic therapy for brain tumors. In 2009, anti-VEGF monotherapy became controversial with respect to tumor metastasis: accelerated tumor invasiveness and metastasis was observed in mice after pharmacological blockade of the VEGF pathway [13, 32]. However, this did not translate into impaired animal survival (partly to the contrary), and – as stated above – is not in accordance with current signals from the clinic. Furthermore, in glioblastoma, bevacizumab treatment has been suggested to increase the rate of intracerebral distant and diffuse tumor progression by increasing the tendency of glioma cells to invade the brain parenchyma along pre-existing vasculature [33, 34]. However, this view has been challenged lately in better controlled clinical studies which failed to demonstrate a different pattern of relapse in bevacizumab-treated glioblastomas [35]. Increased vascular co-option has been shown for bevacizumab-challenged brain metastatic lung cancer [17] and melanoma cells [36] in the experimental setting. Like in glioblastoma, we have to closely monitor potential pro-invasive effects of antiangiogenic therapies in controlled clinical trials of BM. Finally, the vasculature of brain metastases differs significantly

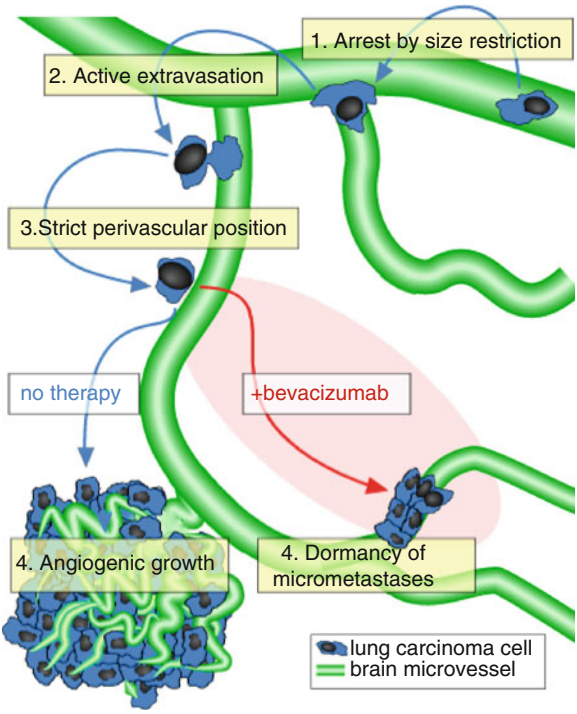

Fig. 6.1 Prophylaxis of brain metastasis formation, as demonstrated in a novel preclinical animal model [17]. Continuous antiangiogenic therapy with the anti-VEGF-A antibody bevacizumab has the potential to interrupt the metastatic cascade by forcing micrometastases into a state of chronic dormancy. This is due to interruption of an early angiogenic switch that is crucial for successful macrometastasis growth of angiogenesis-dependent cancer cells

from that of the primary tumor [37]. This strengthens the point that results from clinical trials investigating the systemic effects of antiangiogenic therapy cannot be transferred to the CNS setting one-to-one.

3 HER2 in Breast Cancer

HER2 amplification or overexpression is found in around 20% of primary breast tumors and is associated with poor prognosis and with the development of BM [38–41]. The incidence of BM in patients with HER2 amplified breast cancer is 25–40%. The reasons for the increased incidence of BM are unclear and are likely multifactorial: First, there is ample data that HER 2 overexpression increases the outgrowth of metastatic tumor cells in the brain by a direct biological effect [42–44]. The exact mechanism of how HER2 modulates BM formation is not known yet; it might involve HER2-induced activation of the angiogenic VEGF pathway [45–47]. Compared to HER2 amplified primary breast tumors, HER2 mRNA levels were increased fivefold in breast cancer BM [48], which supports an important role of HER2 for breast cancer metastasis growth in the brain microenvironment. In support of this, MDA-MB-231 human breast carcinoma cells transfected with HER2 produced threefold larger brain metastases than control transfected cells [43].

3.1 Trastuzumab

Another cause of the increased incidence of BM in HER2 overexpressing breast cancer could be that trastuzumab, a recombinant humanized monoclonal antibody against HER2 that is significantly improving the survival of women with HER2 amplified systemic breast cancer, is not active against breast cancer cells in the brain. Trastuzumab does not penetrate the BBB, which makes the brain a "sanctuary site" for metastatic cells [10]. Poor cerebrospinal fluid (CSF) penetration of trastuzumab was found even after WBRT and in the presence of leptomeningial carcinomatosis [49]. In line with this, several studies showed that more than two thirds of trastuzumab-treated patients present with BM at a time of systemic disease control [38, 50]. The systemic disease control with trastuzumab seems to endure even after diagnosis of BM [51]. The CNS delivery problems of systemic trastuzumab therapy have lead to attempts to bypass the BBB: trastuzumab has been injected directly into the CSF of patients that suffer from leptomeningeal carcinomatosis, with casuistic evidence of impressive and prolonged clinical activity [52, 53]. Since the HER2 status is largely (87%) consistent between matched primary tumors and cerebral metastases [44], it appears promising to investigate smaller HER2 inhibitors that have the chance to cross the BBB in sufficient concentrations for HER2-positive breast cancer patients with BM.

3.2 Lapatinib

Lapatinib is an orally available inhibitor that binds reversibly to the cytoplasmatic ATP-binding site of the HER2 and EGFR tyrosine kinases and is primarily used for treatment of trastuzumab-resistant advanced breast cancer. Its brain penetration might be compromised by drug efflux transporter activity in the BBB [54]. In fact, a recent preclinical study has found highly heterogenous lapatinib concentrations in brain metastases that depended on local BBB permeability; generally, only 10–20% of the drug concentration in peripheral metastases was reached [55]. Accordingly, two phase II trials investigating lapatinib in breast cancer patients with BM have been completed and have shown no certain [56] or only modest [57] single agent activity. In a recent study, lapatinib plus capecitabine achieved a good objective response rate of 38%, but no signs of response were found for lapatinib plus topotecan, again questioning the role of lapatinib [58]. Trials investigating lapatinib in combination with other antineoplastic agents are ongoing. The Radiation Therapy Oncology Group (RTOG) is in the process of initiating a clinical trial for women with HER2-positive breast cancer and BM; the two treatment arms will test WBRT with or without lapatinib, in the context of a randomized phase II trial. These trials should provide a better idea whether lapatinib has relevant CNS activity or not.

It is noteworthy that there might be a decreased incidence of CNS relapses in patients treated with lapatinib in Phase III trials [59, 60], even though this was not

the primary study objective , and the low patient numbers resulted in borderline significance. In a mouse model, Gril et al. tested the efficacy of early onset lapatinib treatment in breast cancer cells with HER2 overexpression, and showed an inhibition of the formation of large brain metastases by approximately 50% [61]. Taken together, lapatinib might not have a great therapeutic effect if large metastases have formed, but might very well be of preventive ("prophylactic") benefit with respect to brain metastasis formation. Remarkably, a large ongoing phase III study can illuminate the prophylactic potential of lapatinib in brain metastasis formation. Patients with recurrent systemic HER2 positive breast carcinoma are randomized to receive lapatinib plus capecitabine vs. trastuzumab plus capecitabine, and the primary outcome measure is the incidence of CNS metastases as the site of first relapse (www.clinicaltrials.gov; NCT00820222). This is one of the very few phase III clinical trial addressing the role of targeted therapies in BM, in this case the prevention of it.

3.3 Other HER2 Targeting Agents

Afatinib is an orally available next generation tyrosine kinase inhibitor that irreversibly inhibits HER2 and EGFR tyrosine kinases. In higher doses of 40 mg/day, clinical responses of brain metastases have been observed [62]. A phase II randomized multicenter trial is now enrolling patients with HER2 positive breast carcinoma with recurrent or progressive brain metastases after trastuzumab or lapatinib treatment into three treatment arms: afatinib 40 mg/day; afatinib plus vinorelbine; investigator's choice of treatment (www.clinicaltrials.gov; NCT01441596).

4 EGFR in Non-small Cell Lung Cancer

Ten percent (US) to 25% (Asia) of non-small cell lung cancer (NSCLC) cases (mainly adenocarcinomas) carry EGFR activating mutations; these numbers might be higher in BM [63, 64]. A recent study has shown that EGFR mutations are found in 44% of BM from NSCLC, and are associated with a doubled median survival of patients. This was due to better intracranial and also extracranial disease control; 78% received EGFR inhibitor therapy after diagnosis of BM [63]. The oral EGFR tyrosine kinase inhibitors gefitinib and erlotinib are approved and routinely used for the treatment of NSCLC: gefitinib for NSCLC with mutations of EGFR and erlotinib for locally advanced or metastatic NSCLC that has failed at least one prior chemotherapy regimen. A number of case reports, small retrospective and prospective case series and non-randomized phase II trials indicate that EGFR inhibitors may be active in NSCLC BM (Table 6.2), particularly in cases with activating EGFR mutations [65–76]. Erlotinib seems to produce higher CSF concentrations than

Table 6.2 Overview of clinical study results showing that EGFR tyrosine kinase inhibitors may have clinical activity in NSCLC brain metastases, although adequately powered and designed studies are missing[a]

Drug (dose)	n patients	Disease	Study type	RR	PFS[b] (months)	OS (months)	Reference
Gefitinib (250 mg)	76	NSCLC	Prospective	33.3%	5	9.9	73
Gefitinib (250 mg)	41	NSCLC	Prospective	27%	3	5	72
Gefitinib (250 mg)	40	NSCLC-adenocarcinoma	Phase II	32%	9	15	70
Gefitinib (250 mg) or erlotinib (150 mg)	23	NSCLC-adenocarcinoma	Prospective	73.9%	7.1	18.8	69
Gefitinib (250 mg)	15	NSCLC	Retrospective	60%	8.7	8.3	68
Gefitinib (250 mg) + WBRT	21	NSCLC	Phase II	81%	10	13	71
Erlotinib (150 mg)	17	NSCLC with EGFR mutations	Retrospective	82.4%	11.7	12.9	66
Gefitinib (250 mg)	14	NSCLC	Retrospective	86%	7.7	9.1	67

[a]Only studies fulfilling the following criteria were included in this compilation: results reported for >10 patients with brain metastases of NSCLC, drug monotherapy with erlotinib or gefitinib (From [1])

[b]Abbreviations (alphabetical order): *EGFR* epidermal growth factor receptor, *mg* milligram, *NSCLC* non-small cell lung cancer, *OS* overall survival, *PFS* progression-free survival, *RR* response rate

gefitinib and therefore may be preferable [77]. Unfortunately, definite results from randomized and adequately powered trials are not available [78]. Case reports suggest that dose escalation strategies should be considered, especially for patient who develop BM under standard dose EGFR inhibitor therapy [64]. This has also been shown for leptomeningeal carcinomatosis [79]. Interestingly, a recent retrospective study demonstrated a potential prophylactic role of EGFR tyrosine kinase inhibitors in patients with advanced NSCLC and somatic EGFR mutations. The cumulative risk of CNS progression at 1 and 2 years was 5% and 21% in patients receiving erlotinib or gefitinib vs. 24% and 31% in the chemotherapy group, indicating a potential prophylactic role of EGFR inhibitors [80].

5 BRAF in Melanoma and Beyond

Activating mutations of the serine threonine kinase v-RAF murine sarcoma viral oncogene homolog B1 (BRAF) are found in a wide range of human cancers and are frequently found in melanoma (60% of cases). More than 95% of BRAF mutations are of the V600E type, which leads to the substitution of valine by glutamic acid in the activating segment of the kinase domain of BRAF. This aberration leads to constitutive kinase activity of BRAF, thereby enhancing the proliferative and metastatic tumor potential through downstream activation of the mitogen-activated protein kinase (MAPK) signal transduction pathway. BRAF mutations seem to be associated with an increased risk for BM formation in patients [81], which makes this mutation overrepresented in BM and the BRAF pathway an interesting therapeutic target. Furthermore, it matches well with preclinical experience that the small proportion of melanoma cell lines that forms parenchymal BM in animals have mutated BRAF.

Several specific inhibitors of BRAF V600E mutated protein are under preclinical and clinical development and have shown favorable clinical activity in metastatic melanoma. Vemurafenib (PLX4032) produced compelling response rates of up to 70% and improved overall and progression-free survival times in BRAF V600E mutated metastatic melanoma patients [82]. Unfortunately, patients with active brain metastases have been excluded from current vemurafenib trials. However, there are favorable preliminary efficacy data on GSK2118436, another inhibitor of mutant BRAF, in patients with brain metastatic melanoma. In a phase I/II study enrolling patients with metastatic melanoma, GSK2118436 lead to shrinkage and even some complete responses of previously untreated asymptomatic brain metastases in a subpopulation of ten patients [83]. Based on these preliminary observations, a large non-randomized phase II study exploring the effect of GSK2118436 on the radiological response rate in patients with BRAF V600 mutated melanoma brain metastases was launched and almost completed (NCT0166967). Also, a phase II trial evaluating efficacy and safety of vemurafenib in patients with brain metastatic melanoma has been initiated (NCT01378975). Such systemic approaches are very promising, as expression of the therapeutic target (BRAF V600E-mutant protein) has

been shown to be homogenous throughout the tumor tissue and to be consistent between different tumor manifestations in individual patients [84]. However, although most patients with BRAF V600E mutated melanomas initially show response to BRAF inhibitors, a significant number of patients develop secondary resistance and experience disease relapse. Treatment resistance may be explained by mechanisms like platelet derived growth factor (PDGFR)-beta upregulation or acquisition of N-RAS mutations or MET mutations [85, 86].

6 Cytotoxic T Lymphocyte Antigen 4 Immunomodulators in Melanoma

Ipilimumab, a human IgG1 monoclonal antibody to cytotoxic T-lymphocyte Antigen 4 (CTLA-4), activates T-cells by blocking the inhibitory action of CTLA-4. CTLA-4 ligation down-regulates T-cell responses and its clinical effects. Overall survival of patients with advanced malignant melanoma was prolonged in two randomized, double-blind multi-national phase 3 trials of ipilimumab as monotherapy [87] and in combination with dacarbazine chemotherapy [88]. Furthermore, anecdotal data and subgroup analyses imply that ipilimumab can show clinical activity against melanoma BM [89–90]. These studies demonstrated the activity of ipilimumab as a monotherapy with responses measured as tumor reduction (objective tumor response). Partial responses were noted in about 25% of patients not on corticosteroids and 5% of those on corticosteroids. Importantly, the current data implies an acceptable safety profile, including patients who previously received CNS radiation. This point has to be followed closely, since previous effective immunotherapies against targets in the CNS showed meningitis and encephalitis including serious brain swelling [91].

7 WNT Pathway

The WNT pathway has been strongly implicated in cancer including cancer stem cell maintenance [92], with 80% of colorectal cancers harboring WNT pathway mutations. Nguyen et al. identified activation of the canonical WNT/TCF pathway as a major factor for metastatic spread to the brain and the bones in NSCLC [93]. Remarkably, WNT signaling was also strongly associated with BM in breast carcinoma patients [94]. In a preclinical study, it was found that microglia promotes brain tissue colonization by breast cancer cells in a WNT-dependent manner [95]. Since the WNT pathway is critical for tissue regeneration and for the ability of stem cells in the bone marrow and gut to self-renew, there is concern that WNT pathway inhibitors could have serious side effects. Accordingly, gastrointestinal and wound healing defects were seen in animals, although these were reversible after drug removal [96]. Therefore, several researchers and pharmacological companies are

moving steadily forward with WNT pathway inhibitors. At this time, a handful of WNT inhibitors are already being investigated in Phase I clinical trials, although none of them in the context of BM. Taken the strong evidence for WNT pathway involvement in BM formation, this is one of the most promising future targets for clinical trials.

8 Predictive Markers

There is currently no validated predictive marker that tells a clinician which BM will respond to a specific targeted therapy. However, it is plausible to assume that the laws of general oncology can be transferred to the brain metastatic setting. Furthermore, there seems to be a high consistency (generally around 90%) for molecular alterations in the primary tumor and the BM. This makes it reasonable to take the genetic or gene expression information from the primary tumor as stratification for BM therapy, when (a) the molecular marker is validated to be predictive for the extracranial disease, and (b) the molecular marker has been demonstrated to be consistent between primary tumor and BM. At the moment, those requirements are fulfilled for HER2 status in breast carcinoma and BRAF status in melanoma. However, it is preferable to note that the tissue from the brain metastatic lesion itself is lacking. One very promising potential predictive marker in brain metastasis is BRAF V600E in brain metastatic melanoma. Correct identification of candidate patients for BRAF inhibitors requires reliable identification of BRAF V600E mutated tumors. So far, DNA-based methods have been primarily used and a real-time PCR test kit has been approved by the FDA for diagnostic purposes. However, the feasibility of DNA-based methods in the routine diagnostic setting is limited. The mutation-specific monoclonal antibody VE1, which allows immunohistochemical detection of BRAF V600E protein in formalin-fixed, paraffin-embedded tissue samples including brain metastases, has recently been generated (Fig. 6.2) [84, 97]. Immunohistochemistry using VE1 seems to be an attractive tool for the diagnostic setting and facilitates mutation screening in large tumor series, even in entities with low mutation frequencies. Finally, one ongoing area of research is the identification of predictive markers for antiangiogenic therapy. Despite intensive research in this area, no biomarker could be validated yet; candidates for brain tumors include changes in distinct MRI sequences, circulating endothelial cells, and plasma levels of cytokines, receptors, and components of the vascular basement membrane [98]. The most straightforward approach, measurement of VEGF-A and/or its receptors, did not prove successful yet. However, new retrospective analyses from large phase III trials now point towards a potential predictive role for plasma-VEGF-A in extracranial tumors; this needs to be evaluated in a prospective setting. In general, it is likely that the advent of effective targeted therapeutics will further increase the necessity of molecular analysis from BM, which might increase the future role of surgical procedures (resection, or biopsy).

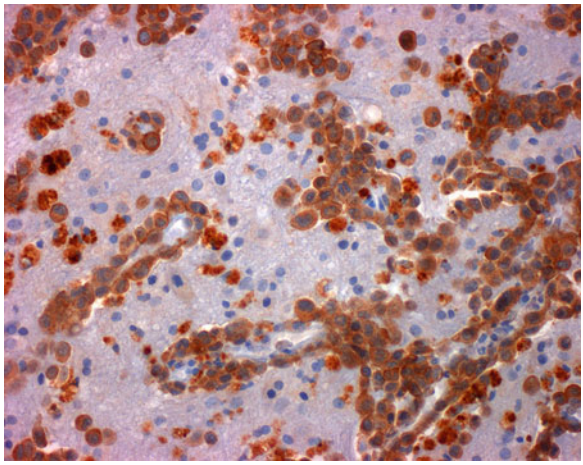

Fig. 6.2 BRAF V600E mutated protein visualized by immunohistochemistry in a melanoma brain metastasis (VE1 immuno-staining, original magnification × 200). There is homogenous expression of the aberrant protein in all tumor cells. Note the perivascular growth pattern of the tumor cells (vascular co-option). (From [1])

9 Outlook

The advent of targeted therapies will hopefully facilitate the shift from the current practice of treating BM according to a rather crude algorithm, in many cases not even considering the histological tumor type, to rational treatment based on individual tumor characteristics. Established BM may be amenable to targeted inhibition of signaling pathways, at least in a proportion of cases. Patients with BM have long been systematically excluded from clinical trials, although there is a growing recognition in the international community that there is no rationale to continue to do so [99]. Hopefully, this will result in the realization of more high-quality trials for BM. Such studies need to take into account the large diversity of cancer entities producing brain metastases and should implement molecular stratification factors whenever possible. Basic and translational investigations are needed to identify novel molecular targets and also to understand secondary resistance mechanisms that are expected to limit lasting effects of many targeted drugs. The use of Response Evaluation Criteria in Solid Tumors or RECIST criteria to measure tumor response of molecular targeted agents might underestimate their effectiveness, as prolonged tumor stabilization should also be considered as a common mode of action. Furthermore, clinical trials should routinely include neurocognitive status and quality-of-life metrics, as both parameters are important to inform decisions regarding the individualized, therapeutic strategies in patients with BM.

A particularly interesting approach is the development of prophylactic systemic therapy to decrease the incidence of BM in high-risk patients. We see advantages, in recent years, to identify these patient subgroups by molecular and/or histological subtype. There are several approaches one can think of: After prevention of intravasation in the primary tumor, the next approach would be to interfere with tumor cell migration through the BBB with drugs targeting selectins, integrins or other adhesion

molecules. Another possibility could be to inhibit growth of micrometastases by blocking ECM-degrading substances (e.g. heparanase, MMP) or early angiogenesis, as successfully exemplified with bevacizumab in experimental NSCLC [17] (Fig. 6.1). The latter seems most promising, since tumor metastasis is regarded as an early event today, which would make it likely that disseminated tumor cells or even micrometastases are already residing in the brain at the time of diagnosis of cancer. For clinical metastasis prevention studies, optimized trial designs are mandatory. Only those patients with a high risk of future BM formation should be included: when one to three brain metastases received successful local treatment, and/or when the tumor type has a known high propensity to metastasize to the brain, including SCLC, NSCLC, and breast carcinoma of the basal-like, triple-negative, and/or HER2 overexpressing subtype. Further molecular stratification approaches (e.g., WNT pathway, chemokine receptor status, BRAF) are on the horizon. The primary end point should be time to progression measured by development of new brain metastases, and secondary end points should include new BM formation thereafter, next to brain metastasis-related morbidity and mortality. Finally, drugs that are normally developed and tested in vivo with respect to their growth inhibitory effect on established tumors are not necessarily effective in terms of metastasis prevention, or might even promote metastasis formation. Therefore, a careful preclinical evaluation of candidate agents is needed before moving to clinical trials of metastasis prevention. In this regard, prophylactic WBRT could at best be displaced by systemic treatment options that are less neurotoxic and have additional effects on systemic metastasis prevention. It is reasonable to assume that this might be a targeted therapy, maybe in a low-dose regimen.

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