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Chapter

Non-Small Cell Lung Cancer Brain Metastasis: The Link between Molecular Mechanisms and Novel Therapeutic Approaches

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Abstract

The prognosis of patients suffering from non-small cell lung carcinomas (NSCLC) worsens significantly when brain metastasis occurs. Seeding to the brain usually happens relatively early in the course of disease and therefore, new therapies anticipating this complication would result in considerable improvement in outcomes. In this review, we address recent molecular data of NSCLC with a focus on the risk of the formation of brain metastasis. Included is new data on the involvement of miRNAs and lncRNAs in the rise of the cerebral seeding of NSCLC. We summarize novel therapeutic approaches developed in the light of these recent molecular discoveries.

Keywords: brain metastasis, lung cancer, blood-brain barrier, microRNAs, targeted therapy, immunotherapy

1. Introduction

Lung carcinoma is among the deadliest cancers and its treatment is an important challenge for oncologists. Approximately 16–20% of patients with lung cancer develop brain metastasis, regarded as the most life-threatening complication of the disease. Population-based incidence proportions for brain metastasis are highest for lung cancer (20% against 9.6% for all common cancers) [1]. The frequency of the diagnosis of lung cancer brain metastasis (LCBM) has increased and the reasons for this are not entirely clear. Certainly, advances in radiology have resulted in increased sensitivity for tracing brain metastatic sites. In addition, metastatic tumor cells behind the blood-brain barrier (BBB) are less vulnerable to chemotherapeutic agents ("pharmacologic sanctuary"). Further, there are effects of the increasing age of the population [2]. The incidence and severity of the cerebral symptoms vary from minimal to severely debilitating. Less than 4% of patients with metastatic NSCLC live longer than five years after the diagnosis [3–5]. Obviously, protecting patients from developing brain metastases would significantly alleviate the disease burden and improve outcomes. Knowledge of the subsequent steps tumor cells need to take before growing as metastases in the brain is essential. In this review, we will summarize

current knowledge on the genes and pathways operative in the development of brain metastasis of NSCL and summarize the application of targeted drugs.

2. Brain metastasis development and the blood-brain-barrier

A significant part of the disease burden and death of cancer is caused by the seeding of tumor cells to the brain [6]. The stages of the development of brain metastasis include the detachment of cancer cells from the primary tumors and their penetration of the BBB followed by extravasation, colonization, and macro-metastatic growth [7]. The detachment of tumor cells from the primary tumor mass depends on cell adhesion molecules (CAMs) including immunoglobulins (IgCAMs), selectins, integrins, and cadherins [8–10]. The tumor cells require the loss of functional E-cadherin (CDH1) in order to increase their motility, and close relation between reduced E-cadherin expression and poor outcome due to tumor spread in NSCLC exists [11]. CDH1 regulates EGFR activity through receptor tyrosine kinases (RTKs) and provides functions in intracellular signaling. Subsequent events include the epithelial-mesenchymal transition (EMT), a crucial phenomenon in the dissemination and motility of cancer cells [12, 13]. The process of EMT relays on proteases such as secreted matrix metalloproteinases (MMPs) that degrade the extracellular matrix (ECM) components including proteoglycans, collagen, fibronectin, and laminin, and modify the structural and mechanical features of the ECM [14]. MMPs also break down cell-ECM - and cell-cell connections by cleaving CDH1 and CD44. MMP-1, MMP-2, and MMP-9 are particularly associated with metastases of lung cancer [15]. Once detached and motile, the tumor cells enter the circulation to become circulating tumor cells (CTCs). Some CTCs resist the forces of the blood flow and by using surface receptors adhere to the endothelial cells. Subsequently, the cells will migrate through the endothelial layer by the expression of selectins, integrins, and chemokines. This process is accompanied by the creation of a permissive immune microenvironment through the activation of integrins and the release of cytokines such as vascular endothelial growth factor (VEGF) [16]. VEGF is vital in the process of neovascularization and takes part in the creation of high endothelial venules, to increase lymphocyte extravasation and infiltration in the perivascular niches (PVN) at the metastatic sites [17]. The altered microenvironment promotes further migration of CTCs to the brain parenchyma by secreting site-specific chemokines such as CXCR4 and its ligand, CXCL12 [17, 18]. There is high expression of CXCR4/CXCL12 in brain metastases of NSCLC and, together with integrins, CXCR4 enhances further tumor cell invasion. The metastatic cells in the PVNs activate tumor-associated macrophages (TAMs) and microglia. TAMs play a role in the survival of CTCs and induce extravasation and colonization by expressing survival factors such as epidermal growth factor (EGF) [19]. While the TILs try to combat the tumor cells, the microglia switches from the M1 (anti-tumor) phenotype to the M2 (anti-inflammatory) phenotype by factors secreted from tumor cells [20] and display tumor-supporting activity. The M2 microglia counteracts TILs activity via the induction of immunosuppressive factors including programmed cell death protein 1(PD1) /programmed death-ligand 1 (PD-L1) [21]. Also, activated astrocytes promote the proliferation and brain invasion of the tumor cells [22]. Obviously, cell types and pathways that initially are activated to counter-act the metastatic process become collaborators in progressive colonization of the brain later on. So far, therapeutic interventions aimed at the elimination of the tumor cells growing in the brain.

Future therapeutic strategies may target any of the preceding events, including CTC trafficking and penetration of the BBB.

3. Genetic alterations in NSCLC associated with brain metastasis

Targeted therapies that successfully combat tumor cells outside the brain may fail to be effective behind the BBB. There are several reasons for this, one of which are differences in genetic alterations between the primary tumors and their metastases [23]. Patients suffering from NSCLC have been classified according to the genetic changes in the primary tumor, which include epidermal growth factor receptor (EGFR), Kirsten rat sarcoma (KRAS), and anaplastic lymphoma kinase (ALK). NSCLC brain metastasis-specific mutations can be detected in the cerebrospinal fluid (CSF) and can also be used to evaluate the presence of disease and response to therapy [24].

3.1 EGFR mutations

EGFR is a receptor for extracellular growth factors such as epithelial growth factor (EGF) and tumor growth factor-α (TGFα). Binding of these factors causes a structural change and activation of the receptor complex, resulting in the activation of signaling pathways that promote cell proliferation, motility, and survival. Dysregulation of the receptor is associated with various human cancers. The prevalence of EGFR mutations is dependent on a variety of factors, including ethnicity, gender, smoking, tumor heterogeneity, and tumor progression. EGFR is often overexpressed in NSCLC and the two most frequent EGFR mutations encountered involve exon 19 (deletions) and 21 (L858R mutations) [25, 26]. [27]. There is data supporting that CNS metastases of NSCLC are promoted by EGFR-activated mesenchymal–epithelial transition (MET) through mitogen-activated protein kinases (MAPK) signaling. EGFR activates signal transducer and activator of transcription 3 (STAT3) via the expression of interleukin-6 (IL-6) which would increase the risk of BM [28]. NSCLC patients with EGFR mutations at the time of diagnosis or in the early stages of the disease seem to have two times higher risk of brain metastasis [29–31]. In a series of 30 primary tumor/metastasis series, there was discordance between EGFR status as measured by IHC of one-third of sample pairs and a little less by FISH [32]. In 14 out of 54 paired samples of lung adenocarcinomas, EGFR alterations of EGFR were restricted to the brain metastases [33]. In a recent paper by Haim et al., the EGFR mutational status of brain metastasis could be predicted with an accuracy of almost 90% by using clinical, radiological, and molecular data for deep learning strategies [34]. Obviously, the presence of CNS metastases leads to poorer outcomes (viz., 11.6 months vs 18.7 months) as shown in a study on 101 EGFR positive metastatic NSCLC previously treated with either combination chemotherapy or oral TKI [35]. The progression of the cerebral lesions is also relatively high during treatment in these patients and there is a connection between the EGFR mutations and EMT-related tumor invasion [36, 37].

3.2 KRAS mutations

The K-Ras protein is encoded by the KRAS gene and is part of the RAS/MAPK pathway, where it transfers signals to proliferate and divide from extracellular into the nucleus. A single substitution of a nucleotide may serve as an activator of the signaling pathway turning tissue hyperplasia into invasive cancers. Although it is believed

that KRAS and EGFR mutations are mutually exclusive [38, 39], yet cases of simultaneous occurrence were found [40–42]. Nearly 15–30% of NSCLCs have activating mutations in the KRAS gene that are associated with adenocarcinoma initiation and clinical aggressiveness [38, 43, 44]. There is a clear connection between KRAS mutations and smoking history [45, 46]. In a study of 482 lung adenocarcinomas (LADC), it was found that KRAS mutations also occur in patients who had never smoked, but the mutations differ from those in the tumors of smokers. For instance, transition mutations (G > A) prevail in those who never smoked while transversion mutations (G > T or G > C) are typical for NSCLCs in smokers [47]. The relation between KRAS mutations in NSCLC and propensity for brain metastasis is still unknown and need to be further studied [36, 42]. Approximately 25% of brain metastatic tumors with KRAS mutations were observed in smokers [44]. Other mutations, including ROS proto-oncogene 1, liver kinase B1 (LKB1), and hepatocyte growth factor receptor (HGFR), were associated with the development of lung carcinoma [48–50], but their relations with brain metastasis of lung cancer is also unknown. LKB1 is inactivated in nearly 30% of all NSCLCs [46] and its effects are synergistic with those of KRAS mutation on the progression of lung cancer and the development of metastases in general [51, 52]. In a study of 154 patients with NSCLC, Zhao et al. concluded that KRAS mutations in combination with low LKB1 copy numbers (CNs) are related to a 20-fold increase in brain metastasis [53]. So far, therapeutic KRAS targeting has been unsuccessful.

3.3 ALK translocations

ALK gene mutations, copy number changes, or fusion with other genes have oncogenic effects. Similar to EGFR mutations, translocations of ALK are predictive of response to Tyrosine Kinase Inhibitors (TKIs) [54]. ALK testing is mostly recommended for non-squamous cell lung cancers lacking EGFR mutations. The fusion between ALK and EML4 (echinoderm microtubule-associated protein-like 4) produces molecular variants with diverse biological functions and affects various signaling pathways [55, 56]. The incidence of cerebral metastases in NSCLC with ALK mutations is high and ALK translocations of primary tumors and their brain metastases are often similar. Interestingly, the progress of brain metastases of tumors with ALK mutations slows down significantly when treated with targeted therapy: over 45% of patients with BM had overall survival rates of three years [57]. Because nearly 45% of patients with ALK-positive NSCLC have developed BM at death [58], cerebral seeding is an important clinical challenge for developing strategies for personalized care in NSCLC [59].

3.4 MET and RET mutations

The large variety of mutations in EMT: (mesenchymal epithelial transition factor) affects a range of cancers, including NSCLC. The MET gene codes for a tyrosine-kinase receptor that plays role in developmental processes and wound healing. Hepatic growth factor/scatter factor (HGF/SF) and their splice isoforms NK1 and 2 are the only known ligands of the MET receptor. In cancer, abnormal MET activation triggers proliferation, angiogenesis, and metastasis. The MET pathways interfere with the key oncogenic pathways RAS, P13K, STAT3, and beta catenin. In general, mutations consist of duplications of mutant alleles, intronic splice site alterations, and mutations affecting the receptor downstream targets. In NSCLC BM, the majority of

MET mutations found at metastatic sites affect the extracellular SEMA: Semaphorin superfamily domain of the receptor [60–62]. Remarkably, mutations in MET occur more frequently in CNS metastasis from NSCLC than in their primary tumors. RET chromosomal rearrangements have been detected in 1–2% of all patients with NSCLC, particularly in patients with adenocarcinoma. The rearrangements are mutually exclusive with EGFR, ALK, or RAS mutations [63]. Importantly, NSCLC with RET rearrangement is associated with an increased risk of BMs [64].

4. The role of MicroRNAs in NSCLC brain metastasis

MicroRNAs (miRNAs) are conserved short endogenous RNA molecules (21–25 nt) that play critical roles in gene expression patterning by interfering with target mRNAs [65]. MiRNAs regulate cellular functions including cell growth, cell differentiation, and cell death. About half of the miRNAs participate in tumorigenesis [66]. The expression of miRNAs may lead to the rise of tumors by activating the pathways implicated in carcinogenesis. The function of miRNAs in the development of tumor metastasis to brain has recently attracted attention and various studies have addressed the role of miRNAs in the progression of brain metastases of lung cancers in particular (**Table 1**) [83, 84]. The effects of miRNAs vary widely, depending on the expressional cascades they influence.

In **Table 1**, a summary of currently known miRNA associations with NSCLC and their brain metastases is presented. MiRNA-184 and miRNA-197 are highly expressed in EGFR-mutant NSCLCs of patients with cerebral metastases and may serve as biomarkers for the risk of cerebral seeding [67]. Expression of miRNA-9 and miRNA-1471 has also been found in lung cancer with brain metastasis. Up-regulation of miRNA-145 inhibits the proliferation of human tumor cells in lung adenocarcinomas via targeting of c-Myc and EGFR [79]. [85, 86]. MiRNA-146a is overexpressed in NSCLC and is associated with down-regulation of heterogeneous nuclear ribonucleoprotein (hnRNP) C1/C2 and up-regulation of β-catenin, resulting not only in tumor cell invasion and migration but also in the metastatic potential to brain [87, 88]. Also, MiRNA-95-3p is upregulated in lung adenocarcinoma but overexpression of this MiRNA seems to suppress the formation of brain metastasis via down-regulation of cyclin D1 [75]. MiRNA-378 is overexpressed in NSCLC and their brain metastases and increases tumor growth and metastasis via the upregulation of MMP-7, VEGF, and MMP-9 [74]. Also, MiRNA-328 is overexpressed in NSCLC and allegedly promotes the formation of brain metastases via PRKCA and urokinase-type plasminogen activator (uPA) [71]. PRKCA mediates the expression, resulting in the migration of the cancer cells [89]. Lastly, increased miRNA-21 levels suppress cell death and promote the proliferation and invasion of NSCLC and lung adenocarcinoma cells [68, 90].

Some miRNAs are downregulated in the context of cerebral seeding of lung cancer. MiRNA-768-3p is downregulated in lung cancer cells co-cultured with astrocytes, leading to increased KRAS expression, tumor outgrowth, and propagation of brain metastasis [91]. MiRNA-375 is another miRNA that reportedly is down-regulated in primary NSCLC and reduced levels of miRNA-375 are associated with NSCLC brain metastasis [72]. In tumors in which miRNA-375 was downregulated MMP9 and VEGF were found overexpressed [72]. Reduced miRNA-145 levels also seem to promote brain metastasis in lung adenocarcinoma, while overexpression reduces tumor dissemination [69].

miRNA	Tissue	Target	Tumor suppressor/ Oncogene	Effect	References
miR-184, miR-197	EGFR-mutant lung tumors				[67]
miR-21	*In vivo*	SPRY2, TIMP3, CDKN1A, SERPINB5 and PTEN	Oncogene	Initiating cell proliferation promoting brain metastasis-	[68]
miR-145-5p	Brain and lung tumors	TPD52	Suppressor	Inhibited cell invasion and migration	[69]
miR-142-3p	TCGA data	TRPA1	Suppressor	Suppressing NSCLC progression	[70]
miR-328	Brain and lung tumors	PRKCA	Oncogene	Increasing cell migration	[71]
miR-375	Brain and lung tumors	VEGF and MMP-9	Suppressor		[72]
miR-590	Lung tumors	ADAM9	Suppressor	Suppressing tumorigenesis and invasion	[73]
miR-378	Brain and lung tumors	MMP-2, MMP-9 and VEGF	Oncogene	Promoting migration, invasion, and angiogenesis	[74]
miR-95-3p	*In vivo*	Cyclin D1	Suppressor	Inhibiting invasion and proliferation	[75]
miR-330-3p	Lung tumors	GRIA3	Oncogene	Promoting growth, tumor invasion, and migration.	[76, 77]
miR-490-3p	Brain tissues	PCBP1	Oncogene	Promoting proliferation, invasion, and migration	[78]
miR-145	Brain and lung tumors		Suppressor	Inhibiting cell proliferation	[79]
miR-423-5p	Lung tumors	MTSS1	Oncogene	Promoting cell invasion and migration.	[80]

miR-15a, miR-210, miR-214	Lung tumor			Predicting brain metastasis in patients with lung adenocarcinoma	[81]
miR-4317	Lung tumors	FGF9 and CCND2	Suppressor	Inhibiting proliferation, migration, colony formation, and invasion	[82]

TPD52: tumor protein D52; TRPA1: transient receptor potential ankyrin 1; GRIA3: glutamate receptor, ionotropic, AMPA 3; PRKCA: protein kinase C-α; MMP: matrix metalloprotease; ADAM9: a disintegrin and metalloproteinase 9; PCBP1: poly r(C)-binding protein 1; MTSS1: metastasis suppressor protein 1; FGF9: fibroblast growth factor 9; CCND2: cyclin D2; and TCGA: The Cancer Genome Atlas.

Table 1.
MicroRNAs associated with brain metastasis from NSCLC.

Taken together, miRNAs appear to have great potential for cancer diagnosis, prognosis, and treatment at the molecular level, but the use of miRNAs for the clinical treatment of brain metastases requires further investigation. Many studies focused on the identification of alterered expression patterens of miRNAs after outgrowth in the brain microenvironment, but validation of data in larger groups of tumor samples is needed [31].

5. Role of lncRNAs in NSCLC brain metastasis

Long non-coding RNAs (lncRNAs) are non-coding transcripts comprising > 200 nucleotides that have substantial functions in various physiological and pathological pathways. Similar to miRNAs, lncRNAs also regulate a variety of molecular targets by various mechanisms. Recently, the effective role of lncRNAs in tumorigenesis was shown [92]. lncRNAs are important regulators of lung cancer progression. Some lncRNAs serve different functions in various types of cells [93]. MALAT1 (Metastasis-associated lung adenocarcinoma transcript 1) is a large non-coding RNA gene that is highly conserved in mammals and regulates gene expression via splicing-independent mechanisms in NSCLC metastasis [94]. MALAT1 is located on chromosome 11q13 and increased MALAT1 levels were recently discovered in patients with NSCLC who had developed cerebral metastasis, while not in patients without brain locations [95, 96]. In addition, functional studies revealed that overexpression of MALAT1 leads to overex-pression of vimentin in highly invasive metastatic lung cancer cell lines while silencing MALAT1 affects EMT programming and suppresses metastasis of the lung cancer cells [96]. Moreover, RNAi-mediated repression of MALAT1-RNA has a negative impact on the migration and outgrowth of human NSCLC cell lines. Overexpression of MALAT1 in NIH/3T3 fibroblasts significantly enhanced migration [97] and stimulated cell motility via the regulation of related genes [98]. The oncogene c-MYC influences cerebral metastasis of NSCLC by inducing the overexpressing of Non-coding RNA BCYRN1 (brain cytoplasmic RNA 1) in NSCLC cells [99, 100]. c-MYC-activated BCYRN1 induces NSCLC metastasis by the expression of MMP9 and MMP13, members of the matrixin subfamily that behave as ECM-degrading enzymes [101–103]. HOTAIR (HOX transcript antisense RNA) is a lncRNA that is highly expressed in NSCLCs with brain seeding [104]. *In vitro* studies have revealed that HOTAIR expression enhances

tumor cell migration and outgrowth [105]. At this point, the relationship between MALAT1 and HOTAIR in NSCLC brain metastasis is still unknown [104].

6. Novel therapeutic approaches

For many years, the rise of brain metastases of lung cancer has been considered the final stage of the disease. Patients were treated with standard therapeutic options such as palliative care or whole brain radiotherapy (WBRT). However, since the discovery of new systemic and targeted therapies, additional effective treatments for lung cancer were introduced with the aim to enhance local control and survival [106].

6.1 Targeted systemic therapy

The BBB is an obstacle to enter the brain for many agents and has limited the application of drugs used for systemic therapy [107]. The application of drugs targeting EGFR and ALK has heightened the interest in utilizing systemic agents to treat brain metastases [108–111]. In **Table 2** clinical trials of targeted therapy for NSCLC brain metastases are listed.

6.2 EGFR tyrosine kinase inhibitors

Patients with tumors harboring EGFR mutations are prone to develop brain metastases [112, 113]. Although the efficacy of EGFR-TKIs for NSCLCs with EGFR mutations has been proven, its effectiveness is not clear in patients with brain metastases since they were excluded from controlled clinical trials. In a prospective study, 41 patients with unselected NSCLC brain metastasis were treated with Gefitinib resulting in 10% intracranial partial responses (PR) with an average response period of 13.5 months [114]. However, most information on the efficacy of TKIs in patients with brain metastases was obtained from retrospective studies [110]. Firstly, it appeared that recorded concentrations of Afatinib, Erlotinib, and Gefitinib in cerebrospinal fluid (CSF) clearly exceeded those needed to inhibit the growth of cells with EGFR mutations *in vitro*. In patients with lung adenocarcinoma, about 70% intracranial tumor response was obtained with Gefitinib or Erlotinib as first-line treatment [115]. Other retrospective clinical studies revealed that patients with brain metastases from EGFR-mutant NSCLC have more favorable responses to WBRT or TKI therapy than patients with brain metastases from EGFR–wild-type NSCLC [116]. The progression periods were 11.7 months for patients with EGFR-mutant NSCLCs treated with Erlotinib and 5.8 months for patients with EGFR-wild-type NSCLCs, respectively [117]. The potent EGFR-TKI, AZD3759 showed significant penetration of the BBB in pre-clinical models for the treatment of EGFR-mutant NSCLC with brain metastasis [118]. Moreover, the third-generation EGFR inhibitors osimertinib and rociletinib targeting the T790M-EGFR resistance mutation in NSCLC appeared effective in treating patients with NSCLCs with these mutations [119, 120]. Unfortunately, a phase 3 trial conducted by RTOG using WBRT plus SRS with Temozolomide and Erlotinib in unselected patients with a maximum of three brain metastases was closed prematurely because of low accrual [121]. No significant benefit of adding Gefitinib to WBRT in phase 2 trials in patients with unselected NSCLC with brain metastasis was recorded [122]. At present, there is not sufficient data to draw conclusions on TKI

Targeted agent	Target	Pretreatment with radiotherapy	Progression-free survival (month)	Phase	Status	NCT identifier
Alectinib, bevacizumab	ALK, VEGF	No	NA	I/II	Recruiting	NCT02521051
AT13387, Crizotinib	c-MET, ALK, ROS1, Hsp90	No	NA	I/II	Completed	NCT01712217
MK-3475	PD-L1	No	NA	II	Completed	NCT02085070
Sunitinib	VEGF, KIT, PDGF, FLT-3	Yes	2.1	II	Completed	NCT00372775
GRN1005	-	Yes	NA	II	Completed	NCT01497665
Dasatinib	BCR-ABL	Yes	NA	II	Completed	NCT00787267
Cetuximab	EGFR	Yes	NA	II	Completed	NCT00103207
Certinib	ALK	No	NA	II	Active, not recruiting	NCT02513667
Erlotinib	EGFR	Yes	1.6	III	Completed	NCT01887795
Afatinib	HER2, EGFR, HER4	No	NA	III	Completed	NCT02044380
Osimertinib	EGFR	No	NA	IIIb/IV	Completed	NCT03790397

c-MET: tyrosine-protein kinase Met, hepatocyte growth factor receptor; ROS1: Proto-oncogene tyrosine-protein kinase ROS; Hsp90: heat shock protein 90; KIT: Proto-oncogene c-KIT; PDGF: Platelet-derived growth factor; FLT-3: fms like tyrosine kinase 3, CD135; HER2: human epidermal growth factor receptor 2; and HER4: human epidermal growth factor receptor 4.

Table 2.
Clinical trials of targeted therapy for the treatment.

therapy plus CNS-directed radiation therapy for patients with NSCLCs with EGFR mutations.

6.3 ALK tyrosine kinase inhibitors

ALK rearrangements are found in about 4–8% of NSCLCs, representing a distinct subgroup [110]. ALK-TKIs are active against CNS metastases and novel drugs are effective in treating brain metastases, even in patients with multiple intracranial tumors [123]. Crizotinib (Xalkoric) was the first ALK-TKI for metastatic ALK-positive NSCLCs. In phase 3, randomized clinical trial with a single-arm of crizotinib for patients with NSCLC with cerebral metastases, better intracranial response was obtained for patients who were also treated with RT [124] and median survivals of almost 50 months were recorded for patients with ALK-positive tumors [58]. Second-generation ALK-TKIs including brigatinib, ceritinib (Zykadia), and alectinib (Alecensa) have shown a better BBB penetration and activity against BM in crizotinib-resistant tumors [125]. Ceritinib appeared to be a powerful drug for patients with metastatic ALK-positive NSCLCs in whom treatment with crizotinib was not effective anymore. Ceritinib also showed activity against crizotinib-resistant tumors in the mouse models [126]. Patients with ALK-positive tumors with CNS lesions were treated with alectinib against crizotinib in the ALEX trial. The results showed that patients treated with alectinib had a longer progression-free survival (PFS) rate than patients treated with crizotinib [123]. In addition, nearly half of patients with ALK-positive NSCLCs with cerebral metastasis improved significantly upon treatment with alectinib. Taken together, these results indicate that Alectinib can be used as an effective treatment option for patients with NSCLC-positive ALK with cerebral metastasis [123].

6.4 MET inhibitors

In recent years, several MET inhibitors have been approved and have entered clinical trials. There are limited data available on the role and efficacy of monoclonal antibodies that inhibit MET in brain metastasis [127]. The effectiveness of Sym015, which consists of two monoclonal antibodies targeted to non-overlapping epitopes of MET, was high in inhibiting MET-amplified tumors as compared to emibetuzumab, a humanized monoclonal antibody developed for patients with NSCLC [128]. Among the new small inhibitors, cabozantinib, an inhibitor of MET, RET, and VEGFR2, appeared effective in radiation-resistant MET-mutated BM in renal cell carcinoma [129]. In addition, cabozantinib yields rapid responses in crizotinib-resistant NSCLC harboring a MET exon 14 alteration [130]. Simultaneous activation of the MET receptor and the ALK fusion gene in NSCLC yielded effective responses to crizotinib in patients with brain metastases [131]. The oral administered selective MET inhibitor capmatinib came with controllable toxicity profiles in treatment-naive patients with MET-exon14 positive NSCLC. Preliminary studies in mice that were injected with human BM cells from NSCLC showed that capmatinib is able to cross the BBB and is active in the brain. In *in vivo* models, the combination of capmatinib and afatinib was found to suppress tumor growth [132]. Recently it was demonstrated that bozitinib, another novel orally administered PLB-1001 compound, better penetrated the BBB as compared to other MET inhibitors in MET-mutated glioblastoma [133]. These preliminary results raise hopes for the effectiveness of PLB-1001 in the treatment of secondary brain lesions from various primary sites.

6.5 RET inhibitors

Cabozantinib and vandetanib are oral multi-kinase, non-selective RET inhibitors that have a modest advantage but significant toxicity. Cabozantinib is effective in RET-rearranged NSCLC and has limited activity against RET, while vandetanib more effectively targets RET. No specific activity against CNS seedings of NSCLC has been reported for these drugs [134, 135]. Selpercatinib and pralsetinib are small highly selective RET inhibitors approved by the FDA for the treatment of NSCLC with RET fusion [136, 137]. Selpercatinib (LOXO-292) is an oral tyrosine-kinase inhibitor specifically targeting the RET kinase domain. Its activity profile and clinical safety were evaluated in phase I/II clinical trial LIBRETTO-001. The study included patients with advanced RET-positive NSCLC who had progressed disease after platinum-based chemotherapy in patients who were treatment naïve. In the phase II trial, 105 patients were pretreated with platinum-based chemotherapy. The ORR was 64% (95% confidence interval (CI): 54% to 73%) with a median duration of response of 17.5 months. A major advantage was observed among the 39 treatment naïve patients, with an ORR of 85% (95% CI: 70% to 94%). Selpercatinib was also designed to have an effect on the CNS. Eleven patients with BM participated and Intracranial responses were observed in 10/11 patients with response rates of 91% (95% CI: 6.7% to NE) [138]. The FDA granted to accelerate the approval of selpercatinib for treating patients with metastatic RET-positive NSCLC, regardless of specific treatment strategy. The RET kinase domain inhibitor Pralsetinib (BLU-667) is currently applied in a multicenter phase I/II ARROW trial. Based on the results of this trial, the FDA approved the efficacy of pralsetinib in patients with RET alteration-positive NSCLC with/without prior therapy. Patients with asymptomatic BM were allowed to be included in this trial. In total, 79 patients participated, and the majority were pretreated primarily with chemotherapy (76%) and immunotherapy (41%). CNS metastasis at the baseline observed in 39% of patients. Efficacy was based on 57 patients, all of whom had at least one follow-up evaluation [139, 140].

6.6 Immune therapy

Although the immune system plays a role in all stages of the development of cerebral metastasis, so far therapeutic interference was limited to the immune response around the tumor cells present in the brain. The inflammatory microenvironment of brain metastases mainly consists of infiltration by tumor-infiltrating lymphocytes (TIL) expressing immunosuppressive factors like programmed death-1 (PD-1) ligand (PD-L1). Immunotherapeutic agents include anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), anti- PD-1, and PD-L1 monoclonal antibodies (mAbs). There are limited data available on the efficacy and safety of immunotherapy for patients with NSCLC brain metastasis. Approximately, 15% of patients participated in studies and all had stable BM or had been treated for BM, while patients with symptomatic BM were excluded from trials [16]. The available data were derived from single-arm phase I/II trials [141–143], pre-arranged analyses of phase III trials [143–145], and expanded access programs [146, 147]. In the phase I multi-cohort CheckMate 012 study of the tolerability and safety of nivolumab in patients with NSCLC with BM only twelve patients were included. Their median PFS and OS were 1.6 months and 8.0 months, respectively, and no more than two intracranial responses were observed [143].

In a phase 2 trial, the PD-1 blockade by pembrolizumab was studied in patients with advanced NSCLC with untreated brain metastases. Forty-two patients were treated with Pembrolizumab. The cohort with PD-L1 ≥1% 1, 29.7% of patients had a BM response,

while the patients with PD-L1 <1% did not show a response. The median OS and PFS of patients in cohort 1 was 9.9 months and 1.9 months, respectively, confirming that pembrolizumab activity in CNS metastasis is limited to NSCLC with higher PD-L1 expressions. Moreover, the PD-L1 expression was associated with long-term OS [142]. In two nivolumab EAP studies conducted in Italy and France, 409 and 130 patients respectively, were included with advanced NSCLC and asymptomatic and stable BM. Part of the patients received corticosteroids and the other part underwent concomitant brain radiotherapy. The OR was 17% in the Italian study and 12% in the French study; the OS was 8.6 and 6.6 months, respectively [146, 147]. In another pooled analysis of larger trials on pembrolizumab monotherapy (KEYNOTE 001, 010, 024, and 042) and pembrolizumab combined with chemotherapy (KEYNOTE 021, 189 and 407), the OS of patients who received pembrolizumab alone or with chemotherapy was better as compared to patients who received chemotherapy alone [144, 145].

7. Conclusions

Brain metastasis of NSCLC is most life-threatening for patients and the treatment is a major challenge. Traditional therapies do not eradicate cerebral cancer cells and recurrent disease is common. A significant obstacle in treating patients with brain metastases is the BBB, which prevents chemotherapeutic agents from entering the brain. Due to this obstacle and the failure of conventional therapies, novel therapeutic approaches are being explored. Despite recent advances in lung cancer treatment, a better understanding of the molecular mechanisms and pathways implicated in lung cancer is essential to identify appropriate targets to prevent brain metastasis. It is undeniable that many factors in the tumor microenvironment contribute to the outgrowth of tumor cells, not only at the primary site but also at the sites of seeding in distant organs. The formation of brain metastases is largely the result of tumor-microenvironment interactions. The brain micro-environment not only contributes to colonization by tumor cells but also affects the results of therapeutic interventions. Obviously, detailing the entire spectrum of genomic alterations and molecular mechanisms involved in lung cancer brain metastasis is important to develop effective treatments. Specifically scrutinizing the mechanisms by which cancer cells cross the BBB is important for establishing preventive brain metastases strategies.

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