Toward Precision Medicine in Brain Metastases

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Abstract

Brain metastases (BMs) reflect an area of high clinical need, as up to 40% of patients with metastatic cancer will develop this morbid and highly fatal complication. Historically, treatment strategies have relied on local approaches including radiosurgery, whole-brain radiotherapy, and neurosurgical resection. Recently, targeted and immune-modulating therapies have shown promising responses and have been introduced in the clinical management of patients with BMs. Recent improvements in genomic technologies have enriched our understanding of BMs and have demonstrated that BMs present with significant genetic divergence from the originating primary tumor, such that potentially targetable genetic alterations are detected only in the BMs. However, this genetic divergence also results in genetic alterations associated with resistance to targeted therapies. A deeper insight on the genetic alterations of BMs and the interaction with the brain microenvironment will likely reveal new treatment targets, moving toward more precision therapies for patients with BMs.

Keywords
- brain metastases
- branched evolution
- targeted therapies
- clinical trials

Brain metastases (BMs) are a highly lethal neurologic complication occurring in up to 40% of patients with metastatic cancer. Upon the symptomatic occurrence of BMs, only limited treatment options are available, most commonly local therapies such as radiosurgery, resection, or whole-brain radiotherapy (WBRT).[1,2] Although several targeted and immune-modulating therapies have recently changed the clinical course of several solid extracranial malignancies, patients with BMs are typically excluded from phase III trials.[3–5] Indeed, systemic therapies might face several challenges in BMs as the biology of the brain metastatic cascade, and in consequence the molecular characteristics, differs from extracranial metastases, and the components of the blood–brain/tumor barrier might at least partly limit the efficacy of systemic therapies.[6,7] Consequently, intracranial recurrences are frequently observed, and approximately one-third to half of the patients die due to isolated intracranial progression, despite the controlled extracranial disease.[8] Therefore, the following review concentrates on the important biological characteristics of BMs as well as the application of targeted therapies and the development of BM-specific trials.

Brain Metastases: Current Standard of Care

The standard of care for BMs depends on several factors including the number and size of BMs, the presence or absence of neurologic symptoms, the histology of the

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primary tumor, the patient's age, and the performance status of the patient.[1,2] Symptomatic or oligometastatic BMs are typically treated with local therapies such as resection or stereotactic radiosurgery (SRS). Neurosurgical resection should especially be considered in patients with large (>3 cm) symptomatic single BM or BM without a known primary cancer diagnosis.[1] SRS is the method of choice in patients with three to five BMs, although several studies have suggested the feasibility of SRS for more than five BMs, and prospective studies are underway to answer this question.[9] Although adjuvant WBRT after SRS or surgery in patients with oligometastatic brain disease has been considered the standard of care, data are emerging that WBRT can be withheld due to a lack in overall survival benefit conferred by WBRT and the neurotoxicity associated with WBRT.[10,11] However, in patients with multiple disseminated BMs, where local therapy is not feasible, WBRT is still considered the standard of care, although this could change in the future as improved systemic therapies with blood–brain barrier penetration are developed. To reduce neurologic and neurocognitive side effects, recent studies have reported promising results with hippocampal sparing WBRT.[12]

In patients with asymptomatic (to oligosymptomatic) BMs, a primary systemic therapy that has central nervous system (CNS) penetration (►**Table 1**) can be considered.[1] Furthermore, patients with active extracranial disease should receive a systemic therapy after the local brain therapy, and agents with known intracranial efficacy should be used.[1]

Genetic Heterogeneity in Brain Metastases

Historically, selection of targeted therapy for patients with BMs has relied on the analysis of the initial primary tumor resection. However, genomic sequencing of primary tumors and extracranial metastases revealed a marked locoregional genetic heterogeneity and further genetic differences from primary tumor to metastases, suggesting that metastases undergo genetic evolution.[14,15] Indeed, analysis of 104 primary tumors and matched intracranial as well extracranial metastases revealed the principle of "branched evolution" in BMs: most BM specimens present with some common mutations with the original primary tumor or extracranial metastases, whereas other mutations are specific to BMs (►**Fig. 1**).[16] Although BMs are markedly divergent from the primary tumor, the majority are still clearly clonally related, as only 4/86 (4.6%) specimens were shown to be clonally unrelated to the analyzed primary lesion. In contrast, anatomically and temporally distinct BMs from the same patient still demonstrated a more homogenous genetic relation to each other when compared with the originating primary tumor (►**Fig. 1**), arguing that specific genetic signatures are required for the brain metastatic process. Indeed, several studies have suggested that specific upregulation of certain pathways such as phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), epidermal growth factor receptor (EGFR), or human EGFR 2 (HER2) are of pivotal importance for the specialized process of brain metastatic spread.[17,18] Furthermore, alterations in the CDK pathway are frequent in BMs.[16]

This genetic divergence has important clinical implications in BMs. Genetic comparison of primary tumor and matched intra- and extracranial metastases revealed accumulation of potentially targetable mutations in the BM specimen not present in the primary tumor tissue. In fact, more than 50% of BMs harbor clinically actionable alterations not detected in the primary tumor.[16] Importantly, the matched extracranial metastases were also genetically divergent from the BMs, arguing that a therapeutic option can be missed if only the genetic analysis of the primary tumor or the extracranial metastasis is available.[16] The differential genetic profile of BMs and primary tumors likely, at least in part, results in the clinical phenomenon of mixed responses to targeted therapies, with responding or stable extracranial disease and progressing intracranial disease.[19] Indeed, several predictive genetic aberrations such as overexpression of HER2 in breast cancer have been shown to be different between the primary tumor and matched BMs.[20] A conversion in both directions was observed, with initially overexpressing tumors converting to negative ones and vice versa. Overexpression of estrogen receptor is frequently lost in BMs from breast cancer, especially if the patients have been treated with endocrine therapy.[20] Similarly, EGFR mutation status in non-small cell lung cancer was shown to change in up to 27% of cases.[21] Actionable PI3K mutations were also enriched in BMs compared with extracranial sites from melanoma.[11] In contrast, other predictive biomarkers such as the B-Raf murine sarcoma viral oncogene homolog B (BRAF) V600E point mutation in melanoma or anaplastic lymphoma kinase (ALK) translocations in nonsmall cell lung cancer were shown to be relatively stable between lesions from different sites.[22–24]

Given the remarkable responses recently observed with newer targeted therapies in BMs (►**Table 1**), it is critical to understand the genetic heterogeneity of intracranial metastases as we consider the application of targeted therapies to BMs. When clinically available, the presence or absence of targetable alterations should be assessed in BM tissue. As BM tissue is not always available for analysis, a deeper insight on how liquid biopsies, circulating tumor cells, or cell-free DNA can provide information on potential targetable driver mutations is urgently needed.[25,26]

Therapeutic Targets of the Brain-Specific Microenvironment

According to Paget's "seed-and-soil" theory, the characteristics of the (brain) metastasis-initiating tumor cells (seed) are not the only determinants of the brain metastatic process; the brain parenchyma (soil) also plays a role.[27] The specific characteristics of the brain's immune response is of recent interest, as immune-modulating therapies such as checkpoint inhibitors have shown remarkable responses in primary tumors that have a high incidence of BMs, such as non-small cell lung cancer and melanoma.[3,4] The brain's immune response is tightly regulated to prevent overwhelming immune responses in this organ, with little restorative capacities, therefore theoretically limiting the efficacy of

Table 1 Selected BM-specific trials or trials with BM-specific subgroups on targeted therapies

Study population	Targeted therapy	Predictive biomarker	Intracranial disease control rate (SD + PR + CR)	Extracranial disease control rate (SD + PR + CR)	OS	Reference
Nonsmall cell lung cancer Untreated, asymptomatic BMs ($n = 23$)	Erlotinib or gefitinib	EGFR mutation[a]	73.9%	82.6%	18.8 mo	Kim et al[71]
Nonsmall cell lung cancer ($n = 136$; $n = 50$ with measurable CNS disease; 30% without previous CNS treatment)	Alectinib	ALK translocation	90% (measurable BMs) 85.3% (measurable and/or nonmeasurable BMs)	N.A.	Median CNS disease control rate 10.8 mo (measurable BMs) 11.1 mo (measurable and/or nonmeasurable BMs)	Gadgeel et al[72]
Nonsmall cell lung cancer Asymptomatic or neurologically stable BMs ($n = 100$)	Ceritinib	ALK translocation	80% (assessable in 99 patients)	82.2% (assessable in 20 patients)	Median PFS: 5.4 mo	Crinò et al[73]
Nonsmall cell lung cancer Untreated, asymptomatic BMs ($n = 18$)	Pembrolizumab	None	57% (assessable in 14 patients)	44%	6.8 mo	Goldberg et al[33]
Breast cancer BMs ($n = 45$)	Lapatinib (plus capecitabine)	HER2 overexpression	84% (assessable in 44 patients)	91% (assessable in 34 patients)	Median PFS: 5.5 mo	Bachelot et al[13]
Melanoma Cohort A: newly diagnosed, asymptomatic BMs ($n = 89$) Cohort B: previously treated, asymptomatic BMs ($n = 83$) Total: $n = 172$	Dabrafenib	BRAF V600E or BRAF V600K point mutation	BRAF V600E mutant: Cohort A: 81.1% Cohort B: 89.2% BRAF V600K mutant: Cohort A: 6.7% Cohort B: 22.2%	BRAF V600E mutant: Cohort A: 79.7% Cohort B: 8.1% BRAF V600K mutant: Cohort A: 46.7% Cohort B: 50%	BRAF V600E mutant: Cohort A: 33.1 wk Cohort B: 31.4 wk BRAF V600K mutant: Cohort A: 16.3 wk Cohort B: 21.9 wk	Long et al[74]
Melanoma Cohort 1: newly diagnosed, asymptomatic BMs ($n = 90$) Cohort 2: previously treated, asymptomatic BMs ($n = 56$) Total: $n = 146$	Vemurafenib	BRAF V600E point mutation	Cohort 1: 18% Cohort 2: 20%	Cohort 1: 33% Cohort 2: 23%	Cohort 1: 8.9 mo Cohort 2: 9.6 mo	McArthur et al[75]
Melanoma Cohort 1: newly diagnosed, asymptomatic BMs ($n = 76$) Cohort 2: previously treated, asymptomatic BMs ($n = 16$) Cohort 3: asymptomatic BMs with or without previous treatment ($n = 16$) Cohort 4: symptomatic BMs with or without previous treatment ($n = 17$)	Dabrafenib plus trametinib	BRAF V600E, V600K, V600R or V600D point mutation	Cohort 1: 78% Cohort 2: 88% Cohort 3: 75% Cohort 4: 82%	Cohort 1: 79% Cohort 2: 69% Cohort 3: 94% Cohort 4: 65%	Cohort 1: 10.8 mo Cohort 2: 24.3 mo Cohort 3: 10.1 mo Cohort 4: 11.5 mo	Davies et al[76]

(*Continued*)

Table 1 (*Continued*)

Study population	Targeted therapy	Predictive biomarker	Intracranial disease control rate (SD + PR + CR)	Extracranial disease control rate (SD + PR + CR)	OS	Reference
Melanoma Cohort A: asymptomatic BMs ($n = 51$) Cohort B: symptomatic BMs ($n = 21$)	Ipilimumab	None	Cohort A: 25% Cohort B: 10%	Cohort A: 33% Cohort B: 10%	Cohort A: 7 mo Cohort B: 3.7 mo	Margolin et al[34]
Melanoma Untreated, asymptomatic BMs ($n = 18$)	Pembrolizumab	None	42%	50%	Median OS not reached after median follow-up of 11.6 mo	Goldberg et al[33]

Abbreviations: ALK, anaplastic lymphoma kinase; BM, brain metastasis; BRAF, B-Raf murine sarcoma viral oncogene homolog B; CNS, central nervous system; CR, complete response; EGFR, epidermal growth factor receptor; HER2, human EGFR 2; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease.
[a]EGFR mutation was not tested in the reported study.

immune-enhancing therapies in BMs.[28,29] However, important predictive factors such as infiltration with tumor-infiltrating lymphocytes (TILs) and expression of programmed cell death ligand 1 (PD-L1) were also observed in BMs, although analysis of the matched primary tumor revealed that BMs are more frequently immunologically "cold," as defined by the absence of TILs and PD-L1 expression.[28,30–32] Nevertheless, intracranial responses to immune checkpoint based therapy have been observed in patients with asymptomatic melanoma or non-small cell lung cancer BMs (►**Table 1**).[33,34]

Besides inflammatory cells, other host cells in the brain, such as astrocytes, influence brain metastatic behavior, as the specific interaction of astrocyte and BM-initiating tumor cells determines metastatic potential.[35] Similar to the connection between glioma cells forming a cancer network through gap junctions, BM cancer cells form gap junction connections to astrocytes through connexin 43.[35,36] In the resulting astrocyte-cancer cell network, the BM cells transfer second messenger cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) to astrocytes and thereby activate the production of inflammatory cytokines. Importantly, these BM-promoting connections can be disturbed by gap junction inhibitors, which have shown activity in the preclinical model.[35] Furthermore, astrocytes promote reversible phosphatase and tensin homolog (PTEN) loss in BM cancer cells through messenger ribonucleic acid (RNA) released from astrocyte-derived exosomes and thereby promote cancer growth and invasion.[37,38] Consistent with this observation, human BM specimens frequently exhibit loss of PTEN,[16] and its loss is associated with shorter survival.[37] The observed PTEN loss is exclusive to BMs and is apparently not as prevalent in the metastatic colonization of other organs.[38] Targeting these specific interactions of astrocytes and BM-initiating cancer cells might facilitate promising targets for BM treatment as well as prevention in the future.

Applying Precision Medicine in Patients with Brain Metastases: The Blood–Brain Barrier

The introduction of targeted therapies and immune checkpoint inhibitors has dramatically changed the natural course of several cancers with a high propensity of BMs, such as melanoma, non-small cell lung cancer, or breast cancer, providing impressive, durable responses. Unfortunately, patients with BMs are frequently excluded from clinical trials, resulting in only limited knowledge on the efficacy and CNS penetration of targeted and immune-modulating monotherapies in patients with BMs.

Indeed, circumventing the blood–brain barrier is an obstacle for the use of targeted therapies in patients with BMs. Although most BMs present with contrast enhancement and therefore, by definition, blood–brain barrier disruption, some parts of the BMs might still be protected by the blood–brain barrier. Compelling data by Steeg et al demonstrate that BMs have a blood–tumor barrier, as the

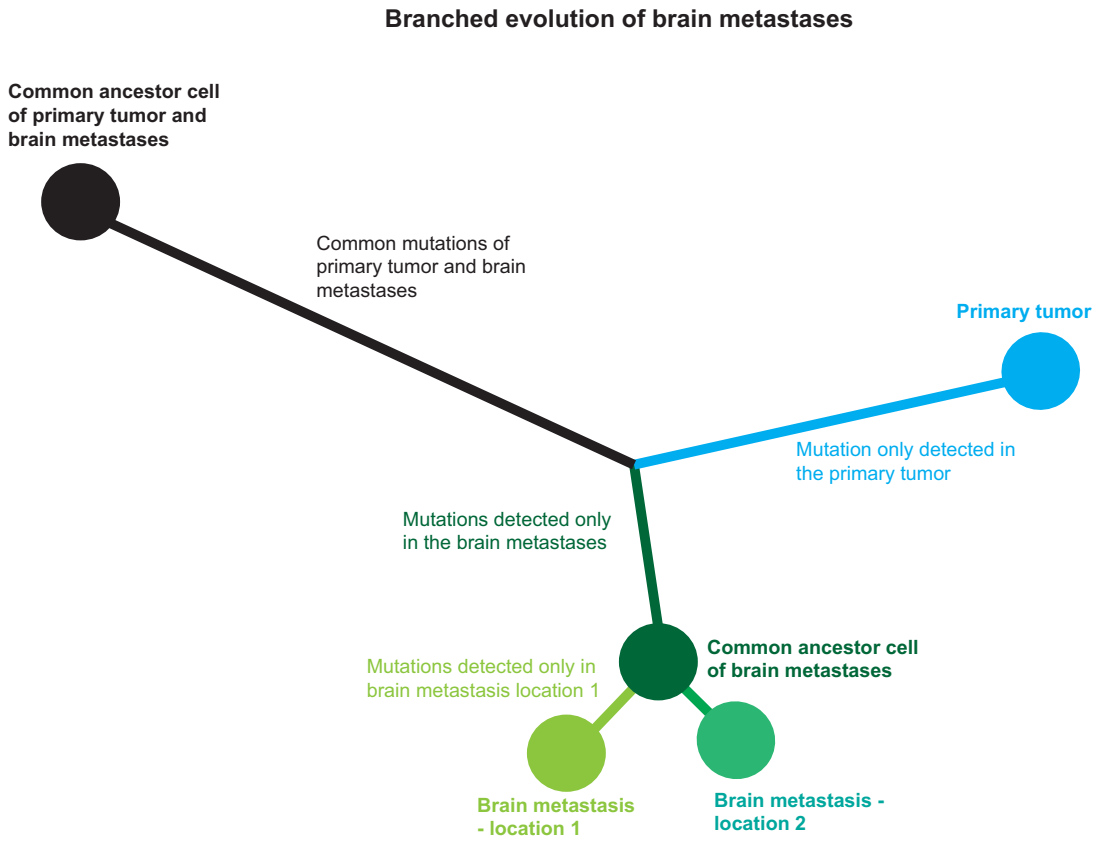

Fig. 1 Illustration of the genetic, branched evolution of primary tumors and their matched brain metastases (BMs). A subset of mutations are shared by the primary tumor and the brain metastasis, many of which are known drivers of cancer. Furthermore, BMs harbor specific "private" mutations that are likely to drive brain metastatic behavior. Two different BMs are genetically more homogenous in comparison to the primary tumor.

composition and properties of the vascular system in BMs differ from the blood–brain barrier.[6,39] Passive diffusion of small molecules with a molecular weight under <400 to 500 Da should be possible even in parts with an intact blood–brain barrier, arguing that tyrosine kinase inhibitors more so than monoclonal antibodies should achieve therapeutically meaningful concentrations in BMs. However, evaluation of BM resection specimens of patients receiving the HER2 tyrosine kinase inhibitor lapatinib showed significantly varying concentrations.[40]

Furthermore, the drug efflux pump P-glycoprotein also limits the efficacy of systemic therapies for BMs. Several targeted therapies, such as the MEK inhibitors cobimetinib and trametinib, the EGFR tyrosine kinase inhibitor erlotinib, the vascular endothelial growth factor (VEGF) receptor inhibitor axitinib, the BRAF inhibitor vemurafenib, the antiestrogen tamoxifen, and the ALK inhibitor ceritinib, are substrates for the P-glycoprotein drug efflux pump, resulting in reduced BM diffusion for these agents despite blood–brain barrier permeability.[41–49] Molecular modification of therapies resulting in a lower affinity for efflux pumps such as P-glycoprotein can result in a higher efficacy, as shown for appropriately designed PI3K inhibitors in a preclinical model.[6]

Whether the blood–brain barrier also limits the clinical efficacy of immune checkpoint inhibitors is an area of investigation.[28] In theory, although immune checkpoint inhibitors are monoclonal antibodies too large to cross the intact blood–brain barrier, activated T cells can cross the intact blood–brain barrier and induce a tumor-specific immune response. Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibodies such as ipilimumab trigger the tumor-specific immune response in the local lymph nodes, making effective penetration of ipilimumab into the local tumor microenvironment not as critical to drug action. Similarly, PD1 inhibitors such as nivolumab or pembrolizumab have therapeutic potential in BMs by interfering with the crosstalk between tumor cells and T cells in the local tumor microenvironment.[50] Indeed, intracranial clinical responses for both CTLA4 and PD1 inhibitors have been observed in patients with BMs, supporting the idea that the diffusion restriction of antibodies might not be clinically meaningful in this setting.[33,34]

Prevention of Brain Metastases: The More Precise Approach?

Targeting brain-specific pathways in patients without intracranial disease could, in theory, prevent brain metastatic spread before it becomes clinically evident.[51] A small number of clinical trials have aimed to investigate the preventive potential of targeted therapies. For example, the CERBEL trial investigated the BM preventive potential of the HER2

tyrosine kinase inhibitor lapatinib versus the HER2 antibody trastuzumab.[52] In theory, the small molecule lapatinib is able to cross an intact blood–brain barrier more efficiently than trastuzumab. The large monoclonal antibody might therefore create a sanctuary site for BM development as the extracranial disease is controlled.[53] However, no statistically significant difference in BM occurrence was observed, although the data have to be interpreted with caution as the study was closed early.[52]

Preclinical data suggest that targeted therapies administered in the setting of micrometastases may be more effective than in macrometastatic disease. PI3K inhibitor treatment in a preclinical model showed only slowing of tumor growth in established macrometastases, whereas single perivascular cells were successfully eliminated, arguing that targeted therapies might be more effective in early brain metastatic disease and even more in a prevention setting.[6,51] Application of the VEGF inhibitor bevacizumab prevented the outgrowth of macrometastases when applied early in a preclinical model, whereas almost no impact was observed if applied in the case of already established macrometastases.[7] Importantly, the underlying histology might influence the targetable characteristics of the brain metastatic cascade as neoangiogenesis is a specific hallmark of non-small cell lung cancer, whereas breast cancer or melanoma more frequently presents with a cooptive growth pattern alongside preexisting vessels.[7,54] Consistent with this hypothesis, the addition of bevacizumab significantly reduced BMs as the first site of recurrence in non-small cell lung cancer, but not in breast cancer patients in phase III trials of bevacizumab plus standard chemotherapy.[55] This observation might be translated to other targetable hallmarks of the brain metastatic cascade to prevent the occurrence of symptomatic BMs in a population at a high risk of BMs, such as stage III/IV non-small cell lung cancer, metastatic melanoma, or metastatic triple-negative or HER2-positive breast cancer. Here, a deeper understanding of the pivotal factors determining the success of the brain metastatic cascade is needed to guide further clinical development.

Considerations for BM-Specific Trial Design

Recently, BM-specific trials have shown that in patients with asymptomatic BMs, targeted and immune-modulating therapies have comparable intra- and extracranial efficacy and should be considered in the treatment algorithm (►**Table 1**).[1]

BM-specific trials have additional challenges, as several characteristics influence the survival of patients with BMs.[58] First, patient selection needs to be consistent to allow comparisons across clinical trials. Most prognostic models of patients with BMs include clinical variables such as age, number of BMs, extent of extracranial disease, performance score, and primary tumor histology.[59] Either stratifying these variables or detailed reporting of these variables when reporting trial outcomes will enable physicians to infer generalizability of trial outcomes to their patients. However, the Karnofsky performance score might also underlie subjective bias, and inclusion of further radiological or laboratory factors (e.g., presence of targetable mutations or known oncogenic drivers) adds meaningful information, leading to a more precise prognostic evaluation.[60–62] Ideally, the primary histology should be considered in the conduct of clinical trials, given the wide range of prognostic differences across different histologies.[58,59]

As outlined earlier, the genetic heterogeneity in BMs is a challenge when applying precision medicine in patients with BMs. The presence of predictive biomarkers can change between extra- and intracranial sites. BM tissue is not always clinically available to reevaluate the status.[24] In addition, it will be critical to evaluate whether targeting the alterations found exclusively in the BMs will lead to improved survival. Clinical trials are underway to answer this question (NCT02896335).

The application of radiolabeled drugs might provide another opportunity to analyze the presence of predictive biomarkers. For example, radiolabeled EGFR tyrosine kinase inhibitors or radiolabeled trastuzumab may not only show the presence of targetable mutations but also demonstrate clinical meaningful penetration of the drug into the BM lesion despite possible restrictions of the blood–brain barrier.[63,64] The application of radiolabeled drugs needs to be further explored in clinical trials.

Formulation of BM-specific end points might be challenging as they can be diluted by the activity of the extracranial disease. Therefore, although BM-specific trials are warranted, analysis of BM-specific questions as secondary end points in phase III trials might provide meaningful information.[58,65] Indeed, several clinical trials in non-small cell lung cancer allowed inclusion of stable or asymptomatic BM patients, providing insight on the clinical activity of targeted therapies in intracranial disease.[66]

The "Response Assessment in Neuro-Oncology" (RANO) group recently released consensus recommendations to guide response assessment in patients with BMs. Here, use of magnetic resonance imaging (MRI) is preferred over computed tomography (CT) scans to evaluate intracranial responses. Uni- or bidimensional measurements are recommended, as insufficient evidence exists to use volumetric measurements as a standard approach. Furthermore, need for steroid use and clinical signs/symptoms should be included in the response and progression assessment. Application of these comparable response assessment guidelines across trials is crucial to allow cross-trial comparisons[58]

The question of sufficient brain penetration of new targeted therapies is important to consider as outlined earlier. To address this point, "window-of-opportunity" studies can provide precise information on the intratumoral accumulation of a new drug. Application of a drug several days before resection, followed by comparable analysis between plasma and BMs, might reveal diffusion restrictions and help plan future trial designs.[65]

Conclusion

Precision medicine is rapidly playing an important role in patients with BMs, as several targeted and immune-modulating therapies have shown remarkable clinical efficacy.[5,67–69] Although BM-specific clinical trials are challenging, they are urgently needed.[65] The RANO guidelines provide important recommendations on patient selection and clinically meaningful outcome parameters for BM-specific trials.[58,70] Genetic heterogeneity between extracranial sites and BMs has to be considered in clinical treatment evaluations, and retesting of BM tissue should be considered if clinically feasible.[24] New insights into the biology of the BM-initiating tumor cells, including oncogenic drivers as well as the interaction of BM tumor cells with brain parenchymal cells, might reveal new treatment targets in the future.

Conflict of Interest
None.

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