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# Precision medicine biomarkers in brain metastases: applications, discordances, and obstacles

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**Abstract**

Brain metastases (BM) present a common cause of mortality and morbidity in several metastatic cancer entities. New therapeutic developments during the last decades, including targeted and immune-related therapies, have shown considerable extra- and intracranial response rates in specific subgroups of BM patients. However, differences in the molecular alteration in the BM tumor tissue compared to extracranial tumors leads to heterogeneous therapeutic responses. Therefore, an accurate molecular analyzation of BM tissue, if possible, has become an essential part in therapeutic decision making in BM patients. The concordance of predictive molecular biomarkers between multiple sites including extracranial and intracranial tumor tissue have been analyzed for some but not all biomarkers routinely applied in modern precision medicine approaches. In the present review, we summarize the current evidence of predictive biomarkers for personalized therapy approaches in the treatment of parenchymal BM.

**Keywords**

biomarkers in brain metastases | immunotherapy in brain metastases | targeted therapy in brain metastases

For a long time, the brain has often been considered a "sanctuary site" for tumor cells and less accessible for a successful systemic therapy due to the blood–brain barrier. However, in preclinical trials, the blood–brain barrier shows an increased vascular permeability with an irregular blood flow and become leaky in further course of brain metastatic disease.[1,2] Indeed, studies of radiolabeled trastuzumab or erlotinib specially binding to the HER2 receptor in HER2 overexpressing breast cancer or the EGF-receptor in NSCLC, showed that radiolabeled antibodies specifically accumulate in brain metastases (BM), resulting in sufficient concentration for clinical efficacy.[3,4]

Immune checkpoint inhibitors and targeted therapies have revolutionized the treatment in several entities which commonly predispose to BM including metastatic non-small-cell lung cancer (NSCLC), melanoma, and breast cancer over the last decade. Furthermore, these emerging treatment approaches present remarkable intracranial efficacies—especially in neurological asymptomatic patients—and established a new field of treatment strategies in brain metastatic patients.[5] However, the efficacies of tyrosine kinase inhibitors (TKI) and immune checkpoint inhibitors can be associated with the presence of specific, predictive molecular biomarkers.[6] Therefore, the identification of baseline biomarkers predicting the likelihood of response is of high clinical importance for precision medicine approaches in BM treatment.

Genomic sequencing of primary tumors and metastases postulated a marked locoregional genetic heterogeneity and differences from primary tumor to metastases suggesting that cancer undergo a genetic evolution between the sites. Indeed, an analysis of 104 primary tumors and matched BM revealed that specific targeted biomarkers and mutations detected in extracranial tumors were altered or not present in BM supporting the principle of "branched evolution" in

brain metastatic diseases.[7] Furthermore, more than 50% of BM can harbor potentially targetable alterations that are not present in the primary tumor or extracranial sites indicating that these mutations may not be detected from single sampling of extracranial tumor manifestations.[8] These divergent genetic profiles of BMs and extracranial sites may be the underlying reason for mixed responses to targeted therapies, with responding or stable extracranial but progressing intracranial disease. In fact, a National Cancer Institute-sponsored cooperative group trial is now underway where patients are treated with targeted therapy based on the genetic alterations in the BM (NCT03994796).

In the following review, we summarize the current relevant predictive biomarkers for precision medicine approaches in BM treatment from the most frequently BM causing primary entities: lung cancer, breast cancer, and melanoma (Table 1). Unfortunately, so far, there are no information on predictive biomarker expression and their consequences on therapeutic decisions in BM from less frequently BM causing entities.

## Biomarkers in BM From Lung Cancer

Biomarker testing is recommended for all patients diagnosed with NSCLC and already implemented in diagnostic and therapeutic routine workups.[9] Approximately 10%–15% of Caucasian and up to 60% of Asian patients present with molecular alterations at time of diagnosis with NSCLC enabling the approach of targeted therapies.[10] Importantly, patients with targetable mutations are associated with a higher incidence of BM, but also with a prolonged survival prognosis, particularly after the introduction to targeted therapy.[11]

Small-cell lung cancer (SCLC) patients have a higher propensity to develop BM as up to 20% of patients present with BM at diagnosis of the primary tumor and up to 50% experience BM subsequently during the later course of disease.[12] Despite extensive research no meaningful targetable driver mutations have been investigated in SCLC during the last decades. Recently, studies with checkpoint inhibitors have shown promising response rates in a subset of SCLC patients. However, reliable predictive biomarkers to better define the population benefiting from immunotherapy are still on-going.[13]

### Epidermal Growth Factor Receptor (EGFR) Gene Mutations in NSCLC

Approximately 10%–20% of Caucasian and 40% of Asian NSCLC patients present with an EGFR mutation, with a higher prevalence in non- or light smokers, adenocarcinomas and women.[14,15] Therefore, routine testing of EGFR mutation is recommended in metastatic NSCLC with nonsquamous histology.[16]

EGFR mutations in exon 18–21 may be divergent between extracranial- and intracranial specimens with a reported discordance rate up to 27% in BM patients.[17–19] Variabilities in EGFR status were also described in up to 36% of extracranial sites advising a re-evaluation of the EGFR status in resected metastatic tissue.[20] Indeed, 50% of the patients with EGFR mutations in primary lung tumors had lost the mutations in metastases.[17] Therefore, receptor testing in progressive NSCLC should be mandatory for accurate treatment decisions.

However, in BM, patients are often confronted with difficulties in accessing suitable material for analysis. In case of insufficient amounts of tumor tissue available for comprehensive molecular analyses, a so called liquid biopsy—a biomarker testing from circulating tumor cells, cell-free DNA, or membranous extracellular vesicles in the blood—may be useful for noninvasive biomarker screening. Especially in BM patients, this approach may be of particular interest as biopsy or resection of BM are associated with relatively high procedural risks. With regards to NSCLC, EGFR mutation detection by liquid biopsy is already accepted for making treatment decisions among NSCLC patients.[21]

In addition, a recent study postulated the cerebrospinal fluid (CSF) as a new form of liquid biopsy as the detection rate of the L858R mutation in exon 21 and the exon 19 deletions of the EGFR was higher in the CSF than in the peripheral blood of NSCLC BM patients.[22] Indeed, most of the patients who had EGFR mutations in CSF presented remarkable response rates with EGFR-tyrosine kinase inhibitor treatment.[23] Therefore, the detection of EGFR mutations in the CSF may be a possibility to analyze the EGFR status in case of CNS progression if biopsy of a parenchymal lesion is not feasible.

EGFR mutations predict response to therapy with TKIs. Specifically, the third-generation EGFR-TKI osimertinib presented remarkable intracranial response rates of over 80% in patients with EGFR-mutated metastatic lung cancer and asymptomatic BM.[24] In contrast to first- and second-generation EGFR TKIs, osimertinib is even active in the presence of an EGFR T790 mutation, a frequent point mutation of the EGFR, which is responsible for more than half of treatment resistances to first- and second-generation EGFR TKIs.[25]

### ALK Rearrangements in NSCLC

The EML4-ALK fusion protein is expressed in 2%–9% of lung adenocarcinomas with a higher prevalence in non- or light smokers and younger patients.[26] Therefore, ALK testing is recommended in patients with nonsquamous histology and never/former light smokers if EGFR mutations were not detected.[16]

The variability of ALK alterations in NSCLC differs between the ALK amplifications which can be associated with a discordance rate of 12.5% between extra- and intracranial specimens, and the ALK translocation which was shown to be relatively stable between the sites.[27]

ALK rearrangements predict response to ALK directed TKI. The next-generation ALK inhibitors including alectinib, lorlatinib, and brigatinib were associated with considerable intracranial response rates over 65%.[28–30] Importantly, ALK-TKIs are further effective in BM patients with ROS1 rearrangement.

### ROS1 Gene Rearrangements in NSCLC

ROS1 gene rearrangements are present in approximately 1%–2% of nonsquamous NSCLC patients, again with a higher frequency in female patients and never smokers, and serve as a predictive biomarker for response to ALK

**Table 1.** Selected Targetable Predictive Biomarkers and Their Therapeutic Approaches in Brain Metastases From Solid Cancers (adapted from ref. [5])

| Entity | Biomarker | Discordance Levels | Targeted Therapies | Intracranial Response Rate | Extracranial Response Rate | Progression-free Survival** | Ref |
|---|---|---|---|---|---|---|---|
| NSCLC | EGFR mutation | 0–32% | Afatinib | 81.8% | 82.1% | NG | 24,63 |
| | | | Osimertinib | 66–91.0% | 77% | NR | |
| NSCLC | ALK rearrangements | 0–12.5% | Alectinib | 78.6–85.7% | NG | 9.6 | 28–30 |
| | | | Lorlatinib | 63–66.7% | NG | NG | |
| | | | Brigatinib | 78.0% | NG | NG | |
| NSCLC | ROS1 translocation | NG | Alectinib | 78.6–85.7% | NG | 9.6 | 28–30 |
| | | | Lorlatinib | 63–66.7% | NG | NG | |
| | | | Brigatinib | 78.0% | NG | NG | |
| NSCLC | BRAF mutation | NG | Dabrafenib/Trametinib | NG | 63.2% | 5.5 | 64 |
| NSCLC | PD-L1 expression | 9–89%* | Nivolumab | 9.0% | 11.0% | 3.9 | 60,65,66 |
| | | | Pembrolizumab | 33.0% | 33.0% | 3.0–7.0 | |
| | | | Atezolizumab | NG | NG | NG | |
| Breast cancer | ER+/PR+ expression | 30–50% | Tamoxifen | NG | NG | | 67 |
| Breast cancer | HER2 overexpression | 0–14% | Tucatinib | 40.6% | NG | NG | 68–72 |
| | | | Neratinib | 33–49% | 14–43% | | |
| | | | Lapatinib | 31–57% | NG | 5.5 | |
| | | | Trastuzumab/Pertuzumab | NG | NG | 15.3 | |
| | | | Trastuzumab emtansine | 70.0% | 80.0% | 5.0 | |
| | | | Trastuzumab deruxtecan | 58.0% | NG | 18.1 | |
| Breast cancer | PD-L1 | 9–89%* | Atezoliuzumab | NG | NG | NG | 73 |
| Melanoma | BRAF mutation | 0% | Dabrafenib/Trametinib, | 44–58% | 44–75.0% | NG | 53,74 |
| | | | Vemurafinib/Cobimetinib | 18.0% | 33.0% | 4.0 | |
| Melanoma | PD-L1 expression | 9–89%* | Ipilimumab | 16.0% | 14.0% | 1.5 | 54,55,75 |
| | | | Pembrolizumab | 22.0% | 22.0% | 4.0–10.0 | |
| | | | Ipilimumab + Nivolumab | 55.0% | 50.0% | NR | |

ALK, Anaplastic lymphoma kinase; BRAF, v-Raf murine sarcoma viral oncogene homolog B; EGR-R, Epidermal growth factor receptor; HER-2, Human epidermal growth factor receptor 2; NG, Not given; NR, Not reached; NSCLC, Non-small-cell lung cancer; PD-1, Programmed cell death protein 1; PD-L1, Programmed cell death protein ligand 1; Ref, References.
*discordance rate from primary to distant metastasis, no data about BM in particular available
**intracranial progression-free survival

TKIs.[31] Routine screening for ROS1 rearrangements has not been generally recommend so far. However, considering the remarkable intracranial response rates seen in ROS1-altered lung adenocarcinomas treated with TKIs, regularly testing for ROS1 in clinical setting should be evaluated.[32]

## BRAF Mutation in NSCLC

Mutations of BRAF proto-oncogenes are most commonly associated with a valine substitution for glutamate at position 600 (V600E) within the BRAF kinase. The mutation is detected in 2%–4% of NSCLC patients, therefore, routine testing for this biomarker in BM from NSCLC are currently not generally recommended.[16] However, data from recent phase II clinical trials have postulated the clinical efficacy of dabrafenib and trametinib, a dual inhibition of BRAF and the downstream mitogen-activated protein kinase (MEK), as a therapeutic approach in metastatic BRAF V600E-mutated NSCLC. In BM, so far, only case reports have reported an intracranial activity of the combination.[5] However, considering the success of dual inhibition in BRAF V600E-mutated melanoma BM, further clinical trials focusing on the intracranial efficacy of dabrafenib and trametinib in BRAF-mutated NSCLC BM are warranted.

## PD-1/PD-L1 Expression in NSCLC

Within the last decade, the introduction of immune checkpoint inhibitors of the molecules PD-1 or PD-L1 have revolutionized the treatment of metastatic NSCLC. Either as monotherapy or in combination with chemotherapy,

immune checkpoint inhibitors presented considerable intracranial responses in brain metastatic NSCLC.[5] In particular, NSCLC patients with high PD-L1 levels (≥50% for first-line therapy and ≥1% for second-line treatment) were postulated to benefit most from these new systemic treatment approaches.[16]

However, the prevalence of PD-L1 expression in NSCLC investigated by immunohistochemistry was postulated to be heterogenous between the tumors ranging from 50% to 70%.[33] In the event of matched tumor and BM samples, PD-L1 expression on tumor cells was strongly correlated between paired primary lung and BM tissues (PD-L1 cut off >50% on tumor cells: primary NSCLC 37.5%; BM 33.3%). However, density of CD8+ TILs was concordant in only 54.16% of the paired primary lung tumors and BMs.[34] In terms of clinical efficacy, a large, multicenter retrospective analysis of NSCLC patients with new and/or growing BM without any previous local therapy reported similar intracranial (27.3%) and extracranial (22.7%) response rates with immune checkpoint inhibitor based treatment.[35]

Furthermore, the predictive role of PD-L1 was postulated as suboptimal as different studies have shown discordant therapeutic responses as well as survival prognosis regarding the expression of PD-L1. One explanation for these divergent results may be the use of non-standardized techniques for tissue sampling and antibodies used in immunohistochemistry analysis. Another possibility is the dynamic and heterogenous expression of PD-L1 levels during clinical course of disease.[36] Therefore, the potential of PD-L1 expression to predict the likelihood of response to immune checkpoint inhibitor-based therapy remains to be defined.

### PD-1/PD-L1 Expression in SCLC

In a meta-analysis, the expression of PD-L1 has been reported to be proportionally low in patients with SCLC with most studies demonstrating less than 50% PD-L1 expression in the majority of SCLC patients. Furthermore, significant heterogeneity between SCLC specimens from different sites were reported ranging from 0% to 82.8%.[37] As in NSCLC, the discrepancy may be due to varying use of antibodies and scoring systems during immunohistochemical analyses. In addition, sample size presents a problematic issue in the PD-L1 assessment of SCLC as only a small percentage of patients undergo tumor resections during course of disease, and biopsies only provide a limited amount of tumor tissue for diagnostic analysis, which is frequently not adequate for molecular testing or to accurately assess PD-L1 expression by immunohistochemistry. Interestingly, in contrast to NSCLC, expression of PD-L1 in SCLC appears to confer longer overall survival of patients.[38]

The PD-L1 inhibitor atezolizumab was the first immune checkpoint inhibitor presenting meaningful response rates as first-line treatment option for extensive-stage SCLC in combination with carboplatin and etoposide chemotherapy.[39] Although studies have shown an active immune microenvironment in SCLC BM, brain metastatic patients have not been included in registration trials so far.[40]

### Tumor Mutational Burden in NSCLC

In addition to PD-L1 expression, high tumor mutational burden (TMB) has been postulated as a hallmark of response to immunotherapy in NSCLC.[41] A phase II trial of nivolumab plus ipilimumab in NSCLC identified a tumor mutational burden of at least 10 mutations per megabase (mut/Mb) having a high effect on immune checkpoint therapy response, regardless of PD-L1 expression levels.[42] Importantly, TMB presents as a site-specific biomarker in NSCLC with spatial changes, as an increase of TMB in metastatic specimens with the highest in BM samples (13 mut/Mb) compared to other extracranial metastatic sites (10 mut/Mb) has been reported.[43] In case of BM recurrence, TMB was shown to be stable between two BM specimens at the same location but at different time points in a small cohort of NSCLC BM.[44] However, further analysis of the mutational characteristics using standardized methodology is warranted to fully guide treatment decisions in NSCLC BM.

## Biomarkers in BM From Breast Cancer

Based on molecular and histopathological profiles, several subtypes of breast cancer can be distinguished. The identification of these subtypes—usually by immunohistochemical surrogate parameters—is predictive for clinical behavior, prognosis and response to treatment strategies in the adjuvant and palliative setting.[45] In clinical routine, breast cancer is classified to four main subtypes.[46]

### Breast Cancer Subtypes

- Luminal A: expression of estrogen receptor (ER) and progesterone receptor (PR); HER2 negative;
- Luminal B: expression of ER, PR low or negative; and HER2 positive/negative.
- HER2 nonluminal: HER2 positive; lack of hormone receptor expression
- Triple-negative: HER2 negative; lack of hormone receptor expression

In general, a change of hormone receptor and HER2 status positivity has been frequently reported in distant metastases in metastatic breast cancer. In a recent multicenter analysis, a switch between breast cancer subtypes from extra- to intracranial specimens were reported in 22.8%. In particular, hormone-receptor positive breast cancer tended to a subtype switch in about 37.5% of cases.[47]

In another meta-analysis, a positive to negative conversion for ER, PR, and HER2 has been described in 22.5%, 49.4%, and 21.5%, respectively, and a negative to positive conversion in 21.5%, 15.9%, and 9.5%, respectively, of the cases. Therefore, especially in PR and HER2 a switch from positive to negative occurred statistically significantly more often than from negative to positive. Interestingly, ER discordance was statistically significant higher in BM (20.8%) and bone metastases (29.3%), while PR discordance was higher in bone (42.7%) and liver metastases

(47%) than in BM (23.3%).[48] These findings highlight the idea that breast cancer present a very heterogeneous disease arguing for a reassessment of the receptor status in the event of progressive disease for appropriate treatment planning.

### Hormone Receptor Expression in Breast Cancer

Hormone receptor expression can be observed in two thirds of breast cancer patients, however luminal breast cancer patients—in comparison to the other breast cancer subtypes—are rather infrequently associated with BM.[46]

In hormone-receptor positive breast cancer, loss of estrogen or progesterone receptor expression in BM is reported in up to 50% of patients. In addition, heterogenous expressions of the hormone receptors were also observed in extracranial sites as the receptor status can change in up to 17% of the patients, which may lead to the clinical phenomenon of mixed responses between the sites.[8] Therefore, re-biopsies should be considered in the setting of metastatic hormone receptor positive breast cancer disease.

CDK 4 and 6 inhibitors as palbociclib, ribociclib, abemaciclib in addition to endocrine therapy have shown considerable efficacies in estrogen and progesterone receptor positive metastatic breast cancer, however, BM patients were excluded from these initial registration trials resulting in limited data on the intracranial efficacy of targeted therapies in the luminal subtypes. However, the intracranial efficacy of palbociclib could be demonstrated in patients with progressive BM and CDK alterations in a recent basket trial.[49] Furthermore, the intracranial effectiveness of tamoxifen and megestrol acetate in patients with BM has been postulated in case reports and clinical case series.[5]

### Overexpression of HER2/Gene Amplification of HER2 in Breast Cancer

HER2 protein overexpression and/or HER2 gene amplification can be observed in approximately 10%–15% of breast cancer patients and is frequently associated with an earlier development of BM during the clinical course of disease. However, patients with BM from the HER2 breast cancer subtype present with a more favorable survival prognosis with in median 24 months compared to hormone receptor or triple negative breast cancer after diagnosis of BM.[50]

Importantly, only patients with a high HER2 overexpression (defined as "3+" in immunohistochemistry) or with HER2 gene amplification are supposed to benefit from anti-HER2 therapy, underlying the importance of standardized HER2 testing in breast cancer.[46] In breast cancer, HER2 status can present heterogeneously between metastatic sites resulting in varying therapeutic efficacies. Indeed, a change of the HER2 receptor status is reported in up to 14% of patients in either direction, underlying the importance of routine HER2 testing of different sites.[51]

The HER2 subtype is associated with a range of therapeutic approaches, even in the presence of BM: HER2-targeted TKI like lapatinib, neratinib, or tucatinib in combination with chemotherapy, and HER2-targeted antibodies like trastuzumab and pertuzumab in combination with chemotherapy, as well as HER2-targeted antibody drug conjugate monotherapy like trastuzumab emtansine or trastuzumab deruxtecan were associated with considerable extra- and intracranial response rates.[5,52]

### PD-1/PD-L1 Expression in Breast Cancer

The presence of PD-L1 expression in approximately 20% of triple negative breast cancer patients enables a novel treatment approach in this specific subgroup of breast cancer patients. Atezolizumab in combination with (nab-) Paclitaxel presented a survival benefit compared to chemotherapy alone in newly diagnosed, triple-negative locally advanced/ metastatic breast cancer.[5] Although BM patients were excluded from the registration trial, a current BM specific prospective trial is investigating the intracranial efficacy of atezolizumab combinations in patients with BM from triple-negative breast cancer (NCT03483012).

## Biomarkers in BM From Melanoma

Among patients with advanced melanoma, more than one-third of patients have BM at initial diagnosis of melanoma and approximately 50% develop BM during the course of their disease.[5] Considering the high propensity of metastatic spread to the brain in melanoma, therapeutic approaches with intracranial efficacies have revolutionized treatment and survival in brain metastatic melanoma during the last decade.[53–55] However, identifying patients benefiting the most from treatment strategies presents a frequent obstacle in clinical therapeutic decision making.

### BRAF Mutation in Melanoma

In approximately 50% of patients with metastatic melanoma, the targetable BRAF mutation, most frequently V600E, is detectable.

Considering the remarkable response rates of BRAF- and MEK inhibitor combinations as dabrafenib plus trametinib also in brain metastatic melanoma, testing for BRAF mutation is recommended in all patients with melanoma.[56]

Importantly, the status of BRAF mutation in melanoma was shown to be stable between extra- and intracranial disease manifestations in brain metastatic patients.[57] An analysis of paired extra- and intracranial melanoma samples reported a similar BRAF mutation consistency in lymph nodes (93%) and visceral metastases (96%), and a slight discrepancy between BM (80%) and skin metastases (75%) compared to primary melanoma. Interestingly, in half of the discrepant cases, the primary tumor was BRAF wild type, while the metastasis presented a BRAF mutation.[58] This may indicate that molecularly heterogeneous cell types coexist in primary melanoma. However, more studies are needed to understand what selective pressure induces a migration of BRAF-wild-type subclones instead of expected more aggressive BRAF-mutant subclones.

### PD-1/PD-PD-L1 Expression in Melanoma

PD-L1 expression can be observed in approximately 50% of melanoma BM and did not correlate with BRAF V600E status. Interestingly, the expression of PD-L1 was lower in primary cutaneous melanoma samples, and also slightly lower in extracranial melanoma metastases, than in BM specimens.[59]

First, the intracranial efficacies of PD-1 inhibitors in melanoma were only about half of that seen in patients with extracranial disease (22% vs. 40%), however, the observed intracranial responses were more durable than the one with BRAF inhibitors, ranging from 4 to 10 months.[54,60] In contrast to immune checkpoint inhibitor monotherapy, the combination of the CTLA-4 inhibitor ipilimumab and the PD-1 inhibitor nivolumab resulted in almost identical extra- and intracranial response rates (intracranial ORR: 55%; extracranial ORR: 47%).[55]

Even though the combination of the PD-1 inhibitor nivolumab with the CTLA-4 inhibitor ipilimumab is an established and frequent treatment approach, the utility of PD-L1 expression as predictive biomarker for the response to immunotherapy faces several problems.

While high PD-L1 expression indicates a highly inflammatory tumor that is more likely to respond to immunotherapy, the lack of PD-L1 expression, surprisingly, does not exclude a response to PD-1-directed therapy.[59]

As in NSCLC, PD-L1 expression levels show heterogeneity within tumors and present as a dynamic marker during clinical course of disease affected by treatment and local inflammation.[61] Indeed, the expression of PD-L1 was considerable associated with a dense infiltration of T-cells in BM samples of melanoma patients.[62] Furthermore, the optimal threshold level of PD-L1 expression remains uncertain.[61]

## Conclusion

Intracranial efficacies were postulated for several next-generation TKIs and immune checkpoint inhibitors during the last decades in patients with BM from different solid tumors. However, targeted- and immunotherapy were fully efficient only in a selected number of patients underscoring the need for biomarkers to support appropriate selection of individualized treatments and maximize clinical benefits for BM patients. In addition, although finding of recent studies appeal for performing molecular testing of BM, tissue availability still present a clinical obstacle.[49] So far, a small number of potential biomarkers showed clinically meaningful efficacy in BM and should be considered in treatment strategies. Newer genomically guided trials that focus genetic testing from tissue from brain metastases are ongoing (NCT02896335; NCT03994796). However, facing the clinical obstacles of those in clinical routine, more studies on predictive biomarkers are warranted to further guide sufficiently treatment decisions in brain metastatic tumor diseases.

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