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Review

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Immunotherapy in lung cancer brain metastases

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Brain metastases (BM) occur frequently in lung cancer, particularly in non-small cell lung cancer (NSCLC) patients and remain a significant cause of morbidity and mortality. Standard therapies have limited efficacy due to poor crossing of the blood-brain barrier and the distinct features between BM and the primary tumor. This review explores the immune landscape of brain metastatic disease, emerging immunotherapeutic strategies, and promising biomarkers in NSCLC patients.

Cancer is a leading cause of death globally, with almost 10 million deaths in 2022, and lung cancer is responsible for 18,7% of those deaths, with almost 2.5 million new cases[1]. Lung cancer, particularly non-small cell lung cancer (NSCLC), is one of the most common primary malignancies that disseminate to the brain[2], with a 52% incidence of brain metastases (BM) in autopsy studies[3]. BM are a significant cause of morbidity and mortality in patients[2]. The median survival after BM diagnosis is approximately 3 to 11 months, depending on the number of intracranial metastases, response to treatment, and tumor of origin[4]. Diagnosis relies on histopathological analysis for confirmation[2] and neuroimaging techniques. The standard of care treatment for BM includes surgical resection, whole-brain radiation therapy (WBRT), and stereotactic radiosurgery (SRS)[5]. Nonetheless, BM remain incurable[2], as traditional chemotherapeutic drugs have limited efficacy, possibly due to the inability to cross the blood-brain barrier (BBB), the presence of efflux pumps, and genetic differences between the primary tumor and BM[6]. Also, as patients with BM are often excluded from clinical trials, gathering data about the efficacy of innovative targeted therapies for BM becomes difficult.

The introduction of targeted therapies has revolutionized the clinical setting of lung cancer treatment. During the last two decades, new mutation-driven therapies, including *EGFR, ALK*, and *KRAS*-targeting agents, have been approved for NSCLC patients[7]. Even though only 4–7% of NSCLC patients have *ALK* rearrangements[6], BM are present at diagnosis in more than 20% of *EGFR*- and ALK-mutated lung cancer patients[8]. Among patients with these genetic alterations, the cumulative incidence of BM can increase to 52.9% in EGFR-mutant patients after 5 years, and the incidence of ALK-related BM can increase to 58.4% at 3 years after diagnosis. NSCLC patients have an incidence of 20–30% of *KRAS* mutations[9], which is associated with a 40% incidence of central nervous system (CNS) dissemination[10]. In a cohort of patients who underwent SRS analyzed from 2008 to 2020, 29% had targetable mutations, predominantly in *EGFR* and *ALK*[11]. Molecular targeted therapies were used to treat 174 patients enrolled in this study. The overall survival (OS) was 17 months in all SRS-treated patients, and treatment with chemotherapy (ChT) alone was associated with decreased survival ($p = 0.03$). In contrast, patients with targetable mutations ($p = 0.005$) and those receiving targeted treatments ($p < 0.001$) presented longer survival. Importantly, almost half of the patients experienced progression of brain metastatic disease after SRS, but targeted treatment was a predictor of better BM progression-free survival (PFS)[11]. Although the intracranial efficacy of systemic therapies may be a concern[6], there are currently more targeted agents that efficiently improve the intracranial response of NSCLC patients with BM[12]. Compared with ChT alone, the third-generation EGFR tyrosine kinase inhibitor (TKI) osimertinib has already shown good efficacy in targeting BM[13]. Recent results from

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FLAURA2[14] indicate that a combination of osimertinib with platinum-pemetrexed improves disease control, with a greater proportion of patients achieving a complete intracranial response and delaying CNS disease progression, even in leptomeningeal disease. Similarly, second- and third-generation ALK inhibitors can control and delay intracranial disease progression in NSCLC patients, such as alectinib, brigatinib, ceritinib, or lorlatinib[15,16]. These agents may be potentially used without local therapy in asymptomatic BM[17].

However, molecular targeted therapies are not an option for BM patients with non-actionable mutations, which still account for a significant number of cases[18]. Moreover, the efficacy of TKI may decrease over time due to resistance mechanisms arising in cancer cells or the tumor microenvironment (TME)[19]. Current recommendations further suggest that treatment-predictive biomarkers should be reconfirmed in BM[20], as their genetic features may differ from those of primary lung tumors and impact the therapeutic response.

In this review, we will explore the role of the TME in BM development and response to treatment, focusing on current immunotherapeutic options and innovative immune-driven treatments for patients with lung cancer and BM. We will also review preclinical studies that provide valuable insights about potential therapeutic targets for drug development. Finally, we will discuss the potential of novel immune-related biomarkers in lung cancer BM.

Immune landscape of lung cancer brain metastases

The unique microenvironment of the brain provides a challenge for cancer cells, which need to adapt and develop new interactions with CNS resident cells and immune infiltrates[21]. Differences between the BM TME and primary lung cancer tumors have been recently explored. Tumor-associated neutrophils (TANs) are a key immune population in BM[22]. Multiple single-cell approaches used in the brain TME revealed that BM TANs have extended longevity and resistance to reactive oxygen species. Elevated TANs levels are driven by the myeloid niche, via IL-8 and G-CSF, leading to the infiltration of immunosuppressive PD-L1$^+$ neutrophils. In this context, TANs contributed to increased angiogenesis pathways, altered the tumor vasculature, and led to a constant influx of cells from the periphery. TNF-α, identified as the central player of these immune alterations in the BM, may be a viable therapeutic target[22].

In the context of immune alterations in the vasculature of BM[23], brain endothelial cells (ECs) and mural cells upregulate extracellular matrix production and pathways of cell transport and adhesion, in contrast with decreases in the expression of BBB regulatory genes. Interestingly, CD276 (B7-H3), an immune checkpoint molecule that inhibits T-cell proliferation, is upregulated not only in the BM vasculature, but also in non-vascular BM cells. Inhibition of B7-H3 may be a relevant therapeutic strategy in BM, as reported for other cancers[23].

One major challenge in treatment efficacy is the ability of cancer cells to survive in metastatic sites, including the brain, by evading immune-mediated clearance. Cancer cells enter a slow-cycling state via DKK1 and upregulate stem-cell-like features[24]. This state is possible via the down-regulation of NK-cell ligands. Downregulation of these ligands and depletion of NK populations may be ChT-induced in the primary tumor, removing one of the few immune populations that keep these latent cells under control.

Tumor-infiltrating lymphocytes (TILs) are frequently present in the BM-TME, especially in individuals with magnetic resonance imaging (MRI)-detected edema. These patients often exhibit a greater presence of CD3$^+$ T-cells, particularly CD8$^+$ and CD45RO$^+$ T-cells, markers of cytotoxic and memory T-cells respectively, which are associated with better OS[25]. However, spatial transcriptomic data indicate that the majority of CD8$^+$ T-cells express exhaustion markers[26]. In fact, NSCLC BM patient samples have reduced immune activity including dendritic cell maturation, Th1 responses, and leukocyte extravasation pathways[27]. Importantly, although BM T-cell clones and T-cell receptor repertoires are mostly similar between primary tumors and metastases, BM exhibit lower T-cell density and richness, fewer CD8$^+$ T-cells, and greater infiltration of pro-tumorigenic M2-like macrophages than primary tumors[27]. This finding is in line with the genomic, transcriptomic, and immunophenotypic data of purified immune populations in lung BM harboring TP53 mutations[28]. However, in BM with a high tumor mutational burden (TMB), elevated T-cell infiltration, together with immunosuppressive myeloid populations, has been reported, suggesting that treatment with immune-checkpoint inhibitors (ICIs) could benefit these patients[28]. Similarly, in patients with leptomeningeal metastases, the cerebrospinal fluid (CSF) becomes an immunosuppressive environment following cancer cell invasion. The high abundance of epithelial malignant cells in the CSF promotes T-cell exhaustion, colonization of monocytes, and drives the M2 polarization of macrophages[29]. This process is promoted by the secretion of midkine (MDK), a growth factor secreted by malignant cells in the CSF. In osimertinib-resistant patients, lipid-associated macrophages emerge in the CSF. Activation of lipid metabolism in macrophages is key for macrophage polarization and transformation into tumor-associated macrophages (TAMs), contributing to immune suppression and tumor progression[29]. Additionally, malignant cells upregulate the expression of the immune checkpoint marker CD47, which prevents macrophage-mediated killing.

Resident CNS cell populations also contribute to BM development and play an important role in CNS immunity and therapeutic response[30]. Metastatic cancer cells activate STAT3 in astrocytes, which induces and maintains a pro-metastatic niche in the brain TME[31]. The secretome of these reactive astrocytes decreases the activation of CD8$^+$ T-cells and their anti-tumor effect. STAT3-activated astrocytes also secrete VEGF-A, lipocalin-2, TIMP-1 and MIF, which induces infiltration of immunosuppressive microglia and macrophage population[31]. Microglia and infiltrating macrophages also upregulate IL1R1/2 and TREM2[28], potential therapeutic targets[32,33] that can be used in combination with ICIs to potentiate the immune response.

In patients with brain tumors, including NSCLC BM, single-cell RNA-sequencing data of TILs and circulating T-cells revealed increased levels of activation markers in TILs, whereas circulating T-cells were more naïve[34]. In a subset of BM, CD8$^+$ TILs expressing CD39, a marker of potentially tumor-reactive T (pTRT)-cells, also expressed relatively high levels of CD103, CXCL13, PD-1, and TIM-3. These features suggest the maintenance of effector functions, but with an exhausted phenotype. This pTRT signature is enriched both in primary lung tumors and BM, associated with high levels of tumor-specific T-cell receptor clones, indicating local tumor-associated T-cell expansion. Interestingly, the presence of pTRT-cells along with TAMs was a strong predictor of ICI treatment success. The authors took advantage of multi-omics approaches to explore the immune landscape of BM patients, highlighting the importance of such techniques in improving patient's outcome.

In patients with lung adenocarcinoma, normal and tumor tissue from the lung, together with metastatic lymph nodes or BM were analyzed by single-cell RNA sequencing[35]. Treg populations arise on primary lung tumors and persist during cancer progression, as they are still found in BM, indicating ongoing immune suppression. In contrast to lung tumors and BM, normal tissues are enriched in granzyme B-secreting cytotoxic cells, decreasing cellular toxicity. Moreover, CD8$^+$ cytotoxic T-cells, which are predominant in normal tissue, are replaced by exhausted T-cells in lung tumor tissue and BM, with even greater accumulation in brain lesions. Compared with normal tissue, monocyte-derived macrophages (MDM) also have a greater immune cell proportion in BM, which correlates with increased TMB, a predictor of ICI success[35]. Increased levels of MDM infiltration may be induced by CCL2 post-irradiation[36]. The interactions between lung cancer/BM cells and MDM act on ECs via VEGF signaling, promoting angiogenesis[35]. NECTIN2-TIGIT activation in malignant cells is responsible for the inhibition of CD8$^+$ T-cells, further promoting immune suppression and tumor evasion[35].

These findings and further studies contributing to the understanding of the immune landscape of NSCLC BM are essential for defining new immune-related therapeutic targets and improving current

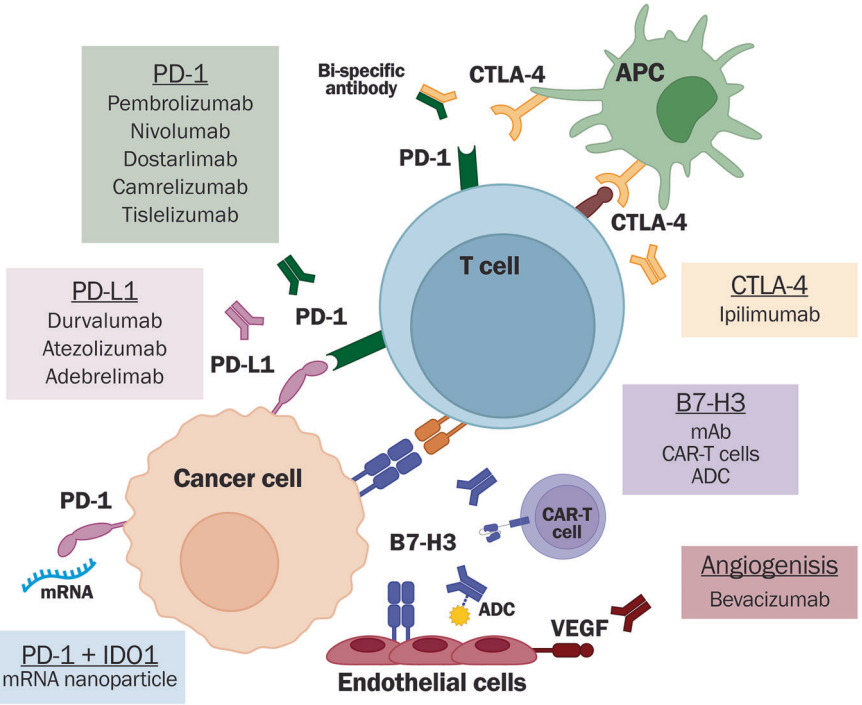

Fig. 1 | Immune targeting of NSCLC BM tumor microenvironment. Summary of current immunotherapeutic strategies described, representing both approved treatments and novel immune targets. ADC antibody-drug conjugate, APC antigen-presenting cell, CAR chimeric antigen receptor, CTLA-4 cytotoxic T-lymphocyte associated protein 4, IDO1 Indoleamine 2,3-dioxygenase 1, PD-1 programmed cell death protein 1, PD-L1 programmed death-ligand 1, VEGF Vascular Endothelial Growth Factor.

immunotherapy regimens. Figure 1 illustrates potential immune targeting in the context of the NSCLC BM microenvironment, summarizing both approved treatments and new targets.

Immunotherapies for lung cancer brain metastases

Immune checkpoint inhibitors

One of the earliest clinical trials to evaluate the efficacy of ICIs, specifically the anti-PD-1 agent pembrolizumab, in treating BM in NSCLC patients was reported in 2016. This trial demonstrated the intracranial activity of ICIs[37]. More recently, the double-blind phase II PERLA trial (NCT04581824) compared anti-PD-1 dostarlimab with pembrolizumab in patients with metastatic NSCLC. Although the study did not focus on the efficacy of these therapies on CNS lesions, the included BM patients presented an objective response rate (ORR) of 50% compared with 27% in the pembrolizumab treatment arm[38]. The KEYNOTE-189 trial (NCT02578680), which demonstrated the efficacy of pembrolizumab in combination with pemetrexed and platinum-based ChT, significantly improved the OS of NSCLC patients with (19.2 versus 7.5 months) and without BM (22.4 versus 12.1 months)[39]. These findings were recently confirmed via a pooled analysis of several clinical trials, including KEYNOTE-189, after a 5-year follow-up[40], further confirming the clinical benefit of combining anti-PD-1 therapy and ChT, including in BM patients. The single-arm phase II Atezo-Brain trial (NCT03526900) explored the use of atezolizumab, an anti-PD-L1 antibody, combined with carboplatin and pemetrexed in patients with advanced non-squamous NSCLC and BM, reporting a median PFS of 6.9 months for intracranial progression and 8.9 months for systemic progression, while the OS was 11.8 months[41]. Patients had a 12-week PFS of 62.2% and 42.7% experienced an intracranial response. The final results of this trial have not yet been reported, but the estimated 1- and 2-year OS rates were 50% and 27.5%, respectively.

A comprehensive meta-analysis evaluating immunotherapy, ChT, and radiotherapy (RT) in NSCLC BM patients revealed that combining ICIs with ChT or RT significantly improved OS and PFS compared with standard therapies alone. Results from 3160 participants in 46 trials revealed that patients receiving immunotherapy had better PFS and OS than immunotherapy-naïve patients. Of note, there were no significant differences in effectiveness among different types of ICIs targeting PD-1, PD-L1, and CTLA-4[42]. Importantly, patients who received the ICI-SRS combination had better outcomes than those who received WBRT-combined treatment. However, this meta-analysis also suggest that the administration of RT after ICI therapy may increase recurrence, which aligns with evidence that radiation-induced T-cell depletion may render immunotherapy ineffective[36]. The administration of SRS followed by ICI treatment may be more beneficial as it allows the infiltration of circulating T-cells from the periphery post-irradiation, which will then benefit from ICIs effect on immune activation.

A phase II open-label clinical trial (NCT02978404) combined nivolumab and SRS in NSCLC and renal cell carcinoma patients with BM. The one-year intracranial PFS was 45.2%, improving the reported results of SRS alone (16–27%) and decreasing recurrence from 55–78% to 19.5%[43]. Interestingly, the type of SRS treatment may induce distinct genomic signatures. Gamma-knife induces the expression of lipid transport and localization genes, whereas linear accelerator (LINAC) treatment upregulates cancer-testis antigens, which are potential immunotherapy targets[44], indicating the need for specific profiling of the BM for adequate innovative drug design and development.

PD-L1 expression is usually a good predictor of ICIs success in patients, even those with brain dissemination. Its scores are associated with better OS, regardless of brain dissemination. Compared with ChT alone, pembrolizumab improves the survival of patients with BM[45]. Ongoing studies, such as the phase II clinical trial NCT02886585, are currently evaluating pembrolizumab treatment efficacy in CNS metastases from various primary tumors[46,47]. Preliminary results indicate that patients expressing more than 1% of the PD-L1 marker may benefit from treatment with pembrolizumab. Interestingly, 3 out of 7 (43%) NSCLC patients with BM had an intracranial response. Additionally, the study reports that patients with leptomeningeal dissemination may respond to pembrolizumab, though a more precise biomarker is still required to predict therapeutic benefit[46]. Similarly, the combination of ipilimumab and nivolumab in a clinical trial revealed that survival was improved in patients with leptomeningeal carcinomatosis[48]. The data indicated that ICI-treated NSCLC patients with leptomeningeal metastases can have a clinical benefit, reporting three remarkable cases of 20-month PFS[49]. These patients received cranial radiotherapy before ICI therapy.

BM patients treated with radiation therapy and ICIs have improved survival associated with increasing levels of PD-L1 expression (PD-L1 < 1%, 1–49%, 50–89%, and ≥90%, with 11.8-, 14.4-, 29.5- and 33.1-month OS, respectively)[50]. An interim report of a clinical trial (NCT02085070) using pembrolizumab in BM patients with at least 1% PD-L1 expression yielded promising results[51]. The 2-year OS was 34% in this cohort, exceeding the historically documented survival of 14.3% in NSCLC patients with BM. The CNS PFS was 2.3 months, with no brain progression in 33% of patients at 1 year. Moreover, treatment with first-line ICI was shown to be more beneficial when PD-L1 levels in the tumor were greater than 50%[52], with no deaths associated with CNS disease.

The CheckMate 227 (NCT02477826) trial included NSCLC patients who were either treatment-naive or had recurrent disease. Patients without actionable mutations, but with PD-L1 ≥ 1% were treated with nivolumab plus ipilimumab or ChT. The ICI combination prolonged OS and five-year intracranial PFS[53]. Moreover, only 4% of patients with BM at inclusion developed new CNS lesions after treatment with the ICI combination, while 20% of the patients with the ChT regimen developed new BM. Given the encouraging results of the ICI combination treatment, a new bi-specific antibody, cadonilimab (AK104) is being tested in diverse solid tumors, including metastatic NSCLC, in the COMPASSION-01 trial (NCT03261011)[54].

Several trials for NSCLC patients with *KRAS G12C* mutations aimed to assess targeted therapy in combination with ICI, including patients with BM. The SUNRAY-01 phase 3 trial (NCT06119581) will test pembrolizumab with olomorasib, investigating patient outcomes on the basis of PD-L1 expression levels. CodeBreaK 202 (NCT05920356) evaluates sotorasib versus pembrolizumab with platinum-based ChT as first-line treatment in PD-L1-negative patients. Finally, a randomized double-blind phase 3 trial (NCT06345729) will assess pembrolizumab plus MK-1084 in patients with previously untreated metastatic NSCLC and PD-L1 ≥ 50%.

New antibody-based therapies targeting the PD-1/PD-L1 axis are currently being tested in NSCLC patients with BM. The combination of the anti-PD-1 ICI camrelizumab with brain RT and platinum-doublet ChT has shown promising results in NSCLC patients with untreated BM in the ongoing C-BRAIN trial (NCT04291092). This single-arm study reported improved intracranial response rates, with a median PFS of 16.1 months, and manageable adverse events[55]. Similarly, CTONG 2003 (NCT04768075) evaluated camrelizumab in NSCLC BM patients without EGFR or ALK alterations in a randomized placebo-controlled setting. Patients receiving platinum-doublet ChT and brain RT in combination with camrelizumab achieved a median intracranial PFS of 19.9 months, compared to 9.9 months in the placebo group[56]. The anti-PD-1 antibody tislelizumab also led to improved intracranial PFS, with a 1-year response rate of 55.8%[57].

However, patients receiving ICI alone or in combination with ChT may still progress. Since immunotherapies benefit from mismatch repair deficiency, the HUDSON study (NCT03334617) has explored the DNA damage response in advanced NSCLC patients who progressed after immunotherapy and ChT. Patients were divided based on molecular profiling into groups matched or unmatched by biomarkers. Treatment regimens included the combination of durvalumab, an anti-PD-L1 antibody, with ceralasertib (ATR kinase inhibitor) olaparib (PARP inhibitor), danvatirsen (STAT3 antisense oligonucleotide) or oleclumab (anti-CD73 antibody)[58]. Durvalumab combined with ceralasertib demonstrated the greatest efficacy, particularly in patients with *ATM*-altered tumors, improving the PFS and OS, probably due to treatment-induced immune alterations. Ceralasertib kills cancer cells under replication stress and induces IFN production. This pro-inflammatory environment benefits the anti-tumor response by promoting T-cell cytotoxic activity and decreasing exhaustion, which is further promoted by durvalumab[58]. Importantly, intermittent treatment with ceralasertib may be more beneficial since prolonged regimens result in T-cell depletion, independent of the activation state. Preliminary reports indicate the inclusion of BM patients, but there is no data available on the clinical response in this group. The IMpower150 trial (NCT02366143) explored the efficacy of ChT combined with atezolizumab and/or the anti-VEGF monoclonal antibody bevacizumab in advanced NSCLC patients[59]. This suggests that the addition of the anti-angiogenic agent may help delay BM development.

In summary, ongoing clinical trials and studies continue to demonstrate the potential of ICI in treating BM of NSCLC patients, with promising results, particularly in those with higher PD-L1 expression. Combinations of ICIs with ChT, RT, and targeted therapies may also improve patient outcome. Supplementary Table 1 lists the completed or terminated clinical trials and the ongoing studies testing immunotherapies, which include NSCLC patients with CNS metastases. Future research to improve these approaches is fundamental, exploring new therapeutic targets, testing the efficacy of novel agents, and identifying biomarkers of therapeutic response that allow patient stratification.

Preclinical studies of immunotherapies

Preclinical studies have provided valuable insights into potential therapeutic targets and strategies for treating lung cancer BM. Data on ICI indicates that anti-PD-1 treatments, reduce the frequency of intracranial progression in NSCLC patients compared with platinum-based ChT. In mouse models, after initial tumor eradication via anti-PD-1 therapy, $CD8^+$ memory T-cells retain the ability to prevent tumor growth upon re-challenge with both subcutaneous and intracranial tumors. These findings suggest that immunotherapy may have a long-lasting effect controlling intracranial tumor progression[60].

Recently, a promising target, HSP47, also known as SERPINH1, was found to be upregulated in brain lesions compared with primary tumors in both lung and breast cancer patients[61]. In mouse models, the overexpression of HSP47 leads to collagen deposition in the brain, which in turn promotes the polarization of microglia towards the M2 phenotype. This immunosuppressive state drives anti-inflammatory cytokine production and represses $CD8^+$ T-cell activity. Importantly, Col003 prevented HSP47-mediated collagen biosynthesis in these models, restoring the efficacy of anti-PD-L1 treatment, and allowing re-activation of T-cell-mediated anti-tumor responses by the resident cells in the BM TME.

Emerging immunotherapies, such as chimeric antigen receptor (CAR) T-cell therapies, have also shown promise in the context of lung cancer BM. Preclinical studies have demonstrated the effectiveness of CAR T-cells targeting EpCAM[62] or CD133[63] in suppressing tumor growth and improving survival. However, these therapies require localized delivery due to challenges in crossing the BBB. The systemic delivery of CAR T-cells may require the co-expression of other targets to ensure their infiltration into BM tissue. To address this, a novel strategy was tested using B7-H3-targeting CAR T-cells co-expressing CCL2 and CCR2b, an axis that is upregulated in BM in comparison to normal tissue and lung cancer primary tumors[64]. This modification enhanced the ability of CAR T-cells to cross the BBB in vivo, improving their infiltration into BM tissue and therapeutic efficacy. CAR T-cells-mediated killing was further validated using patient-derived organoids[64]. Patient-derived organoids that integrate autologous immune cells can inform the best strategy for patient treatment and ensure immunotherapy success[65]. These models were used to assess whether chemotherapy or radiotherapy was the best immune-priming treatment before an ICI against PD-L1 was used. As previously noted in this review, CCL2 expression in the BM increases following irradiation[36], further supporting the potential of this strategy to promote CAR T-cell infiltration after initial standard treatment of patients. Despite the strong killing ability of CAR T-cells, the efficacy of these cellular therapies in the immunosuppressive TME can be challenging[66].

Recently published data using synthetic Notch (synNotch) T-cells suggests a promising strategy to precisely target BM, enhancing tumor specificity and controlling toxicity[67,68]. Unlike conventional CAR T-cells, which may activate elsewhere, synNotch T cells are genetically engineered to be activated only upon recognition of the target antigen. Simic et al. used brevican (BCAN), a proteoglycan of the CNS extracellular matrix, as an anatomic sensor of the brain[68]. Recognition of BCAN triggers Notch cleavage, activating the loaded transcriptional programs. This led to the

activation of an anti-HER2 CAR, allowing T-cell-mediated killing of BM cancer cells. SynNotch cells can also be programmed to carry diverse payloads. Reddy et al. demonstrated that synNotch T cells could suppress the immune response by expressing cytokines, checkpoint receptors, or inhibitory ligands upon synNotch activation, or even modulate the TME to reduce neuroinflammation[67]. Adapting synNotch technology to target specific NSCLC BM antigens could increase T-cell infiltration while reducing systemic toxicity. Additional loading of these cells with immunomodulatory cytokines could further potentiate their therapeutic efficacy.

Despite ongoing efforts to develop novel strategies to target NSCLC BM, translating preclinical findings into the clinic remains challenging. More reliable experimental models, such as patient-derived organoids[69], are essential for testing new therapeutic targets and evaluate novel treatments. However, beyond better models, it is necessary to identify robust biomarkers to advance precision medicine in immunotherapy.

Integrating precision oncology approaches

Recent advancements in immunotherapeutic strategies in lung cancer BM are reshaping treatment approaches, and robust biomarkers are needed to guide therapeutic decisions. However, profiling the primary lung tumor alone may not be sufficient. A meta-analysis found a 19% disagreement between PD-L1 expression in lung tumors and BM[70], with BM presenting lower levels. BM show decreased TILs in 66% of cases, highlighting the need to monitor the immune profile during the course of the disease[70]. While PD-L1 expression remains a key biomarker in BM treatment[50,52], especially in NSCLC patients without actionable mutations, its predictive value may not always be applicable[71], underscoring the need for new biomarkers that optimize treatment outcomes. Skoulidis et al. reported that mutations in *STK11/LKB1*, a serine-threonine kinase, are key drivers of resistance to PD-1 axis inhibitors[72]. These mutations are more frequently found in *KRAS*-mutant lung cancer patients and are associated with a decrease in PD-L1 levels. *STK11/LKB1* mutations negatively impact the response to ICI, regardless of PD-L1 status, decreasing PFS and OS compared with patients with *KRAS* mutations alone.

PhenoTIL was recently reported as an innovative approach for predicting treatment outcomes[73]. This computational immune biomarker predicts treatment outcomes in patients with NSCLC by analyzing the TME via machine learning and identifying genes and pathways that categorize risk levels based on treatment. PhenoTIL was able to distinguish between low- and high-risk patients, with the potential to avoid ChT. This approach may function as a personalized biomarker for NSCLC patients but requires further validation. As previously discussed in this review, different cell components of the TME have an impact on the development of BM and response to therapy. Single-cell RNA sequencing of NSCLC BM identified tumor-reactive $CD8^+$ TILs expressing CD39, CD103, CXCL13, PD-1, and TIM-3 as potential biomarkers for ICI response, highlighting the role of transcriptomic profiling in guiding immunotherapy[34].

The BM vasculature may also provide information about ICI effectiveness. In a phase 2 study, BM patients with balanced vascular structures, comprising small and large blood vessels, responded better to pembrolizumab[74]. Peri-tumor microvasculature growth, which is detectable before visible tumor changes on MRI, was also a predictive factor for cancer progression[74]. These results further establish the need to consider vascular biomarkers together with the aforementioned B7-H3 target, which is upregulated in ECs of lung cancer BM[23]. Similarly, machine learning and radiomics of the tumoral vasculature may help predict PFS and guide personalized immunotherapy[75].

Another promising area is the prediction of immunotherapy response and disease progression via the use of circulating tumor DNA (ctDNA), a minimally invasive methodology[76]. Although liquid biopsies are not yet being used in standard clinical practice, it may be an important complementary method of evaluating disease progression. Particularly in BM patients treated with immunotherapy since inflammatory response in the tumor may mimic radiological tumor progression features[77]. The loss of genomic methylation was suggested by *Kim* et al.[78] to be a potentially good predictor of response to immunotherapy in primary tumors. Cancer cells with genomic hypermethylation avoid immune detection via the down-regulation of processes such as antigen processing and presentation. Methylation-based assays on tumoral cell-free DNA avoid contamination of methylation signals of non-tumoral cells. Moreover, this assay also predicts the response to ICI with more accuracy than the TMB and PD-L1 expression. This work did not report the intracranial treatment response[78]. However, others have reported the potential of analyzing the methylome as a biomarker of BM. The methylation profile of primary lung tumors predicts the risk of BM development, and it can be detected early in plasma ctDNA, suggesting that it may be used as a biomarker of response to treatment[79].

In NSCLC patients, increases in ctDNA mutant allele frequencies (MAF) after immunotherapy are associated with worse PFS and OS[80]. The responders did not reach the median survival in any of the parameters, indicating that the ctDNA MAF is a good non-invasive biomarker of immunotherapy response. Additionally, the TACTC-2 trial (ChiCTR2100052222) is investigating the role of ctDNA-guided treatment decisions in NSCLC patients receiving pembrolizumab and those with no actionable mutations[81]. Patients with more than 50% reduction in MAF after three weeks of treatment will maintain pembrolizumab, while others will be randomized into combination therapy with ChT or maintenance with pembrolizumab monotherapy. These results will inform about the utility of ctDNA in real-time treatment adjustments. The CR1STAL study (NCT05198154) aims to evaluate the predictive value of ctDNA in patients with advanced NSCLC to assess disease progression after 1-year PFS post-immunotherapy. Preliminary results showed that 92% of patients with disease progression had ctDNA minimal residual disease positivity[82]. Moreover, the PFS was 8.9 months in ctDNA-positive patients, whereas this parameter was not reached in the ctDNA-negative group. Another study correlated the methylation profile of ctDNA with tumor burden in NSCLC and was used to assess the response to anti-PD-1 strategies[83]. The methylation score aligns with the imaging-based disease progression and treatment response. Analysis of circulating tumor cells in NSCLC using an electrochemical sensor to measure PD-L1 levels is also a promising strategy for immunotherapy monitoring, although validation in larger patient cohorts is needed[84]. CSF can also be used to assess response to treatment since it recapitulates BM immune infiltrates[85]. Li et al. analyzed CSF cytokine profiles in patients with NSCLC BM receiving ICIs and identified immune-related biomarkers that correlated with intracranial tumor response[86]. Cytokines such as IL-6, IL-10, and TNF-α have potential as indicators of treatment efficacy in this context.

CSF analysis is particularly relevant in patients with leptomeningeal disease, where single-cell RNA and cell-free DNA profiling could provide valuable insights. An initial inflammatory response, characterized by elevated levels of IFN-γ and antigen processing signatures in the CSF, suggested potential prognostic value[87]. The development of clinical trials focused on patients with leptomeningeal metastases is essential[48,49]. These patients with leptomeningeal disease are frequently excluded from trials and have a devastating prognosis, which highlights the need to identify better biomarkers and novel treatments[88].

In conclusion, emerging biomarkers, such as immune and vascular features, computational tools, and liquid biopsy-based approaches, may significantly improve therapeutic decisions to tailor immunotherapy in lung cancer BM and impact patient outcomes.

Challenges and future directions

Despite recent advances in the treatment of lung cancer patients with BM, survival outcomes have not significantly improved. Although the 2-year survival rate for these patients increased from 9.1% to 16% between 2010–2014 and 2015–2020, the overall prognosis remains poor[89]. The treatment of NSCLC BM has significantly improved with the introduction of ICIs and immunotherapy. Nevertheless, a substantial proportion of patients still fail to respond, highlighting the need for further advancements.

As evidenced throughout this review, the TME of BM also poses significant challenges. BM can be characterized based on the immune and brain microenvironments, consisting of TILs and TAMs, and microglia and astrocytes, respectively[90]. Fibrotic lesions driven by M2 macrophages, cancer-associated fibroblasts, and astrocytes are resistant to ICI treatment. Targeting fibrosis with inhibitors of TGFβ, PDGFR, and TIMP-1 could improve treatment efficacy. It was also suggested that M2 to M1 reprogramming of macrophages via the targeting of STAT3 or TGFβ could also be effective[90]. As previously mentioned, BM tumor cells trigger a pro-metastatic response in neighboring reactive astrocytes via the STAT3 signaling pathway[31]. The inhibition of STAT3 with legasil decreased BM in experimental models and patients[31], leading to improved survival.

New immune checkpoint molecules, such as LAG-3 and B7-H3, are currently being assessed in advanced NSCLC. Preliminary results of RELATIVITY-104 (NCT04623775) presented at ESMO 2024 highlight the benefit of combining nivolumab with relatlimab, an anti-LAG3 antibody, in combination with ChT, improving PFS and ORR in comparison with nivolumab plus ChT[91]. Similarly, the RELATIVITY-1093 trial (NCT06561386) will test this approach against pembrolizumab as first-line treatment.

Upregulation of B7-H3 in BM remodels the tumor vasculature, facilitating the constant influx of cells from the periphery and promoting immune suppression, supporting the establishment and growth of brain lesions[23]. In BM mouse models, B7-H3 antibody-mediated targeting reverts vasculature leakiness and enhances the infiltration of $CD8^+$ cytotoxic T-cells[23]. In patients, the synergistic effect of anti-angiogenic therapies and ICI is under study. The BRAIN-AF01 trial (ChiCTR2300079126) will evaluate the combination of anti-PD-L1 adebrelimab and famitinib, an angiogenesis inhibitor, in NSCLC patients with BM and PD-L1 ≥ 50%[92]. Finally, the bi-specific antibody ivonescimab, which simultaneously targets PD-1 and VEGF, is under investigation in the HARMONi-2 trial (NCT05499390). The recent results suggest a PFS benefit in NSCLC patients with BM treated with ivonescimab versus pembrolizumab-treated patients[93].

Another approach involves the use of novel antibody-drug conjugates (ADCs). YL201 combines the B7H3 antibody and topoisomerase I inhibitor YL0010014. This ADC was administered to NSCLC patients with advanced disease and previously treated with anti-PD-L1 and platinum-based ChT[94]. In this trial (NCT05434234), YL201 demonstrated a good ORR.

A phase 1 study (NCT05208762) evaluated the efficacy of PD-L1V, a vedotin-based ADC, in patients with relapsed PD-L1-expressing solid tumors, including NSCLC[95]. PD-L1V demonstrated an ORR of 33% and a 66.7% disease control rate. Future studies will test it as monotherapy or in combination with pembrolizumab. The elevated AXL observed in PD-1-resistant tumors led to the design of a clinical trial (NCT04681131) using mecbotamab vedotin (BA3011), a conditionally active biologic AXL-targeting ADC. This ongoing trial is recruiting metastatic NSCLC patients to address the efficacy and safety of BA3011 alone or in combination with nivolumab in AXL-expressing patients. All the aforementioned trials allow the inclusion of BM patients with stable disease.

Antibody-independent immunotherapy approaches under investigation may open new avenues for BM treatment. Adoptive T-cell transfer was tested in a case study with three patients with *EGFR*-mutant tumors, after treatment failure with gefitinib and ChT[96]. Treatment involved the stimulation of T-cells via the use of dendritic cells transfected with tumor-associated antigens found in patients' tumors. Throughout treatment, the number of $CD69^+CD8^+$ and IFN-γ^+ cells increased, indicating enhanced T-cell activation. Brain lesions were not detected by MRI one month after treatment[96], but there was no information on patient outcomes at later stages. Finally, a lipid nanoparticle-encapsulated mRNA-based cancer vaccine targeting PD-L1 and IDO1 (mRNA-4359) stimulates antigen-specific T-cells. In early clinical trials (NCT05533697), it was well tolerated and 50% of patients achieved stable disease, promoting cytotoxic and memory T-cells and reducing suppressive immune cells[97].

Despite advancements in the treatment of BM and the identification of novel therapeutic targets, there are many challenges in translating research findings into clinical practice. Key issues include limited BBB penetration, immune-related toxicity, and the frequent exclusion of patients with BM from clinical trials[88,98]. Patients with immune-related adverse events may require treatment with corticosteroids to control peri-tumoral edema in the brain, which can reduce immunotherapy efficacy[77]. This raises concerns regarding progressive inflammatory effects in patients with intracranial tumors and may lead to interruption of immunotherapy.

Additionally, systemic therapy trials often fail to have well-defined criteria for these patients due to disease heterogeneity, and the need for accurate endpoints of intracranial response and survival benefit[98]. Standardizing the assessment and management of CNS metastasis via defined guidelines such as the Response Assessment in Neuro-Oncology (RANO) Brain Metastases working group is crucial[77,99]. The inclusion of liquid biopsies in disease monitoring also holds promise to avoid misinterpretation of radiological disease progression, assessed via enhanced contrast, an effect which may happen via immune-driven inflammation, without tumor progression[77].

All these innovative approaches hold promise, but further research is necessary to refine immunotherapeutic strategies and explore the full potential of targeting both the immune system and the unique characteristics of the brain metastatic environment.

Conclusions

Rapid advancements in immunotherapeutic strategies have significantly impacted the treatment landscape for NSCLC patients with BM. Despite improvements in survival and response rates, BM remains a critical clinical challenge. Clinical trials have demonstrated the effectiveness of ICI alone or in combination with chemotherapy and radiotherapy, particularly in patients with targetable actionable mutations such as EGFR and ALK. Nevertheless, resistance mechanisms and the immunosuppressive nature of the BM microenvironment require further research to optimize treatment regimens. Novel agents, including bispecific antibodies, CAR T-cell therapies, and combination strategies targeting the TME, and vascular abnormalities are currently under investigation and show promising results. However, it is crucial to design specific preclinical studies and clinical trials to better characterize and assess CNS metastatic disease and treatment response.

Future efforts should focus on identifying reliable predictive biomarkers to improve therapeutic efficacy, patient stratification, and therapeutic efficacy. Additionally, liquid biopsy techniques such as ctDNA and CSF profiling will be crucial for real-time monitoring of immunotherapy efficacy and resistance in BM patients. Although tumor profiling is important, better characterization of the immune landscape of CNS metastasis is essential. A comprehensive assessment of each NSCLC BM patient will be key to ensuring personalized treatment and improving patient outcomes.

Data availability

No datasets were generated or analysed during the current study.

Abbreviations

ADC	Antibody-Drug Conjugate
APC	Antigen-Presenting Cell
BBB	Blood-Brain Barrier
BM	Brain Metastases
CAR	Chimeric Antigen Receptor
ChT	Chemotherapy
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CTLA-4	Cytotoxic T-Lymphocyte Associated Protein 4
ICIs	Immune Checkpoint Inhibitors
MDM	Monocyte-Derived Macrophages
MRI	Magnetic Resonance Imaging

NK cells	Natural Killer cells
NSCLC	Non-Small Cell Lung Cancer
ORR	Overall Response Rate
OS	Overall Survival
PFS	Progression-Free Survival
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Death-Ligand 1
pTRT-cells	potentially Tumor-Reactive T cells
RT	Radiotherapy
SRS	Stereotactic radiosurgery
TAMs	Tumor-Associated Macrophages
TKI	Tyrosine kinase inhibitor
TILs	Tumor-Infiltrating Lymphocytes
TMB	Tumor Mutational Burden
TME	Tumor Microenvironment
TANs	Tumor-Associated Neutrophils
WBRT	Whole-brain radiation therapy

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Author contributions

Conceptualization: E.P., R.C., and C.C.F. Drafting manuscript: E.P. Revising and editing manuscript content: R.C. and C.C.F. E.P., R.C., M.A., F.F., G.M., C.G., R.R., R.C., T.R.P., A.C.A., F.F., A.L.M., A.S.V., D.H., P.A., and C.C.F. have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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