



# Progress in personalized immunotherapy for patients with brain metastasis



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Brain metastasis leads to poor outcomes and CNS injury, significantly reducing quality of life and survival rates. Advances in understanding the tumor immune microenvironment have revealed the promise of immunotherapies, which, alongside surgery, chemotherapy, and radiation, offer improved survival for some patients. However, resistance to immunotherapy remains a critical challenge. This review explores the immune landscape of brain metastases, current therapies, clinical trials, and the need for personalized, biomarker-driven approaches to optimize outcomes.

## Brain metastases formation co-opts and reconfigures the brain microenvironment

The human brain was long considered an immune-privileged organ, and the role of the immune system in brain pathology was not given adequate consideration until evidence demonstrated the brain's potential to elicit an immunogenic response<sup>1</sup>, which is suppressed by primary CNS tumors during progression<sup>2,3</sup>. Brain metastasis (BrM) frequently originates from disseminated melanomas, lung cancers, or breast tumors<sup>4</sup>. The tumor microenvironment (TME) at secondary sites largely regulates the growth of metastases from disseminated tumor cells. The significance of immune cells in the TME to therapy has been established by the success of immune checkpoint blockade<sup>5</sup>.

The brain TME is unique, containing cells not found outside the CNS. This includes but is not limited to astrocytes, cortical neurons, oligodendrocytes, and microglia that found in extracranial tissue at cell densities observed in the brain. This unique ecosystem exhibits limited inflammatory responses from infiltrating lymphocytes under normal physiological conditions and is remodeled by pro-tumorigenic macrophages in BrM<sup>6</sup>. The TME of BrM further differs from the ecosystem of cells in primary CNS tumors by including a more prominent population of molecularly diverse tumor-infiltrating leukocytes (TILs), less tumor-associated macrophages (TAMs), the presence of regulatory T-cells (Tregs), and the presence of plasma cells<sup>7,8</sup>. While the underlying mechanisms responsible for these differences are an active topic of investigation, the findings signify that BrM forms in part by modifying the brain's immune milieu. This is supported by clinical evidence of increased survival among Glioma patients with increased monocytes and monocyte derived macrophages without a similar patient survival benefit from the presence of these two populations in BrM<sup>7</sup>. The implication of this observation is that these cells behave differently in BrM than primary CNS tumors. Likewise, transcriptomic analysis of flow-sorted CD45+ immune

cells from tumors found a larger signature of differentially expressed genes in tumor-derived microglia from BrM than in tumor-derived microglia from primary CNS gliomas<sup>8</sup>. Further exploration in this study of the gene expression changes underlying these signatures found enrichment for type I IFN signaling in BrM-derived microglia but not in glioma-derived microglia. This is noteworthy given reports of a relationship between IFN signaling, immune suppression, and evasion of immune checkpoint blockade<sup>9</sup>. Conditioned media experiments demonstrating that secreted factors from resuspended BrM cells cause treated monocyte-derived macrophage cultures to upregulate genes in this signature<sup>8</sup> further supports the notion of BrM reprogramming the immune components of the brain TME to permit the immune evasion necessary for BrM growth.

Formation of BrM is a complex multistage process that co-opts diverse signaling pathways and is influenced by the intrinsic molecular subtypes of primary tumors, transcriptional and epigenetic changes accumulated by cancer cells, and the formation of a permissive microenvironment in the brain to seed metastases<sup>10</sup>. The primary tumor is understood to disseminate circulating tumor cells (CTCs) that traverse the blood-brain barrier (BBB), migrate by severing junctional adhesion molecule B (JAM-B) in response to Cathepsin S, and seed secondary tumors in the brain<sup>11</sup>. Once seeded, these cells are stimulated by signal transducer and activator of transcription 3 (STAT3), L1 cell adhesion molecule (LICAM), and Cathepsin S signaling<sup>12,13</sup>. During this phase, the brain mounts an innate anti-tumor response by secreting plasmin from the brain stroma to transform the tumor necrosis factor (TNF) receptor family member Fas ligand (FasL), which triggers the caspase cascade and apoptosis<sup>14</sup>. CTCs seeding BrM overcome this by expressing serpins, which function as anti-plasminogen activators that inhibit plasmin and prevent FasL-induced apoptosis of the invasive cancer cells<sup>14,15</sup>. Reactive astrocytes equipped with STAT3 contribute to BrM formation by upregulating transforming growth factor  $\alpha$  (TGF- $\alpha$ ),

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epidermal growth factor (EGF), and macrophage migration inhibitor factor (MIF) to form astrospheres. Astrospheres are suppressive of CD8+ T cells in the brain, enabling immune evasion and growth of BrM<sup>12</sup>. The BrM niche is also primed for metastasis by molecular cues originating from extracellular vesicles (EVs) released from primary tumors and various tumor-associated stromal cells, including microglia, astrocytes, fibroblasts, and endothelial cells. These EVs play a significant role in facilitating the migration of cancer cells across the BBB and promoting brain tropism in addition to contributing to immune evasion<sup>16</sup>. Thus, understanding the key metastatic drivers and their crosstalk in the complex ecosystem of the brain TME is crucial for developing targeted immunotherapies and optimizing personalized medicine approaches to effectively and safely treat BrM (Fig. 1).

### Adaptation to the Brain Confers a CNS-Specific Phenotype to Brain Metastases

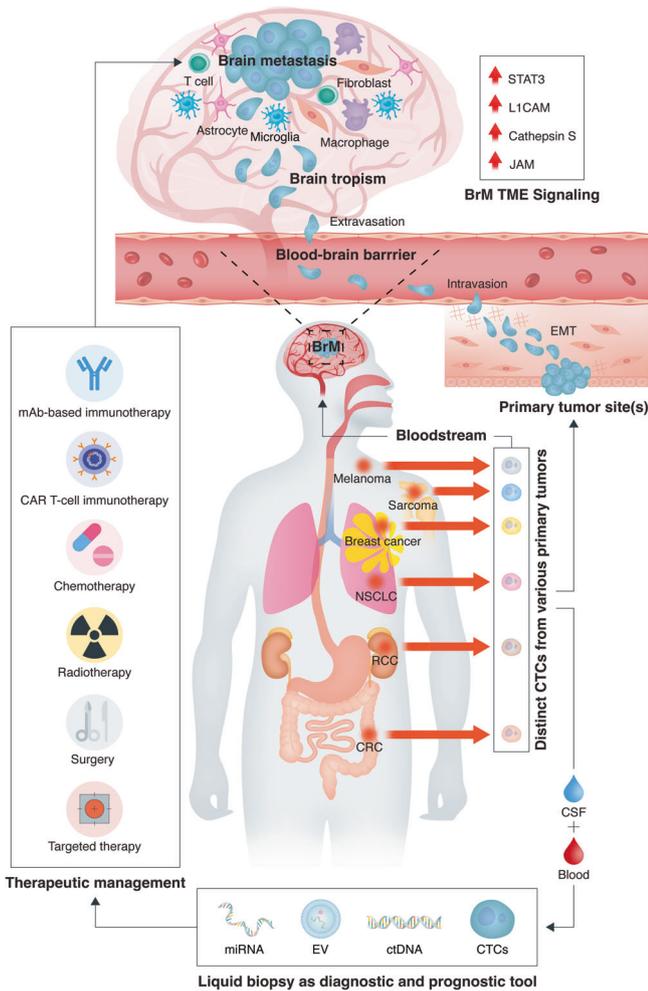
Secondary site-specific colonization and immune evasion lead to systemic and CNS metastases possessing different immune profiles and genetic signatures when compared to the primary tumor, including differences in mutations and heritable epigenetic modifications<sup>16</sup>. To communicate within the TME and cross the BBB, metastatic cancer cells express a set of genes that facilitate BrM formation under hypoxic and hypoglycemic conditions<sup>17</sup>. Transcriptome profiling in situ using an animal model of early metastatic colonization by breast cancer cells reveals that these expression programs are secondary site-specific, with cells seeding BrM demonstrating lower levels of oxidative stress and antioxidative response relative to cells forming lung metastases and mammary tumors<sup>18</sup>. These results suggest adaptation and/or selection of BrM cells for fitness under the unique metabolic conditions present in the brain<sup>19</sup>, a prospect supported by profiling of patient specimens demonstrating that metastatic brain lesions harbor mutations distinct from those present in the same patient's primary tumor, regional lymph nodes, bloodstream, and extracranial metastases<sup>20</sup>.

In addition to metabolic adaptation, cancer cells seeding BrM depend heavily on interactions with endothelial cells to metastatically colonize the brain<sup>14</sup>. An elegant study by Karimi et al.<sup>21</sup> highlights the role of differential gene expression in microvascular proliferation and the expression of tight junction protein claudin 5, which increases vascular permeability in BrM. Their findings reveal that cancer cell-associated endothelial cells show increased expression of the proliferative biomarker Ki67 in both the core and margins of BrM, indicating multiple foci of aggressive pro-angiogenic tumor cells. Moreover, the authors demonstrate the suppression of endothelial cell proliferation in BrM occurs via CD8+ T-cells interactions, suggesting that the immune system plays a role in limiting BrM progression by constraining tumor angiogenesis. This is particularly noteworthy given the established role of the vascular basement membrane (VBM) in BrM as a pro-growth substrate or 'niche' for disseminated tumor cells that supports their adhesion and provides proliferative signaling<sup>22</sup>.

Tumorigenesis under these conditions produces BrM that are biologically distinct from both the primary tumor that seeds BrM and their sibling metastases in extracranial organs. These differences are partly attributable to intrinsic molecular features enriched among CTCs that survive and successfully colonize the brain. However, the extrinsic influence of the neurovascular, astrocytic, and immune components of the brain TME is considerable.

### Diverse Immunological Signatures of BrMs Based on the Primary Tumor Type

The degree of BrM-associated inflammation varies by primary tumor, with the most prominent inflammation observed in BrMs originating from melanoma, and less severe inflammatory responses in BrMs originated from breast carcinomas<sup>21</sup>. Melanoma BrMs are particularly rich in monocytes, microglia, and accumulated CD8+ T-cells compared to lung and breast BrM. Tumor-infiltrating macrophages (TIMs) constitute the main component of the BrM immune signature, regardless of the primary tumor of origin, followed by infiltrating T-cells. Resident myeloid cells and microglia are also generally present in low numbers in lung and skin BrMs, but microglial infiltration is almost absent in breast BrM<sup>23</sup>. Furthermore, the TME of BrM has significantly higher levels of TILs compared to primary glioblastoma (GBM), which are considered immunologically 'cold'. This suggests a higher priming rate for anti-tumor T-cell responses in tumors of extracranial origin, likely due to the higher mutational burden found in melanoma and lung cancer. BrMs may also attract more T cells due to the release of immunomodulatory chemokines and activation of pathways that promote T-cell trafficking and extravasation into these secondary tumors<sup>24</sup>. Despite the increased T-cell numbers in BrM, there is limited T-cell-mediated anti-tumor response due to the upregulation of immune checkpoint molecules such as PD-1, LAG-3, and TIGIT. Therefore, analyzing the presence of inhibitory receptors in BrM in the context of the primary tumor



**Fig. 1 | The origin of brain metastases (BrMs) and their highly heterogeneous tumor microenvironment (TME), including the application of liquid biopsy as a diagnostic and prognostic tool in BrMs that allows better tumor management and more personalized therapeutic approaches.** Circulating tumor cells (CTCs) are shed from various primary carcinomas or sarcomas into the bloodstream, after undergoing epithelial-mesenchymal transition (EMT). These tumor cells acquire the ability to cross the blood-brain barrier (BBB), responding to biochemical and mechanical cues that facilitate their invasion of brain tissue. Once in the brain, they interact with stromal cells, evading immune detection and promoting tumor progression through the expression of molecules that enhance metastatic behavior. Liquid biopsy technique, which isolates CTCs, circulating tumor DNA (ctDNA), extracellular vesicles (EVs), and non-coding RNA species from peripheral blood or cerebrospinal fluid (CSF), can improve the accuracy of BrM diagnosis and guide therapeutic strategies. These strategies may include antibody-based or cell-based immunotherapy (CAR T cells), chemotherapy, surgery, radiotherapy, targeted therapy, or combinations of these approaches.

type is essential to optimizing the application of immune checkpoint inhibitors (ICI), especially for melanoma<sup>25</sup>.

Spatial analysis demonstrates an association between the distribution of extracellular matrix (ECM) components and the preferential localization of different immune cells to ECM-rich regions<sup>26</sup>. TIMs serve as the major immune cell type influencing brain TME remodeling across both stromal and neoplastic cell (NC)-rich regions<sup>27</sup>. analyzed the leukocyte landscape of brain tumors using high-dimensional single-cell profiling (CyTOF) and revealed differences between tissue-resident and invading immune cells within the TME of gliomas and BrMs. The glioma TME predominantly featured tissue-resident reactive microglia, whereas BrMs were enriched with accumulated TILs.

Recently, Wischniewski et al.<sup>28</sup> used transcriptomics to elucidate the composition and role of T-cells in both primary and BrM tumors. Their analysis of circulating and tumor-infiltrating T-cells from 84 individuals with brain cancer (36 with GBM and 48 with BrM) along with 44 individuals with extracranial tumors originating from lung and breast cancer, identified a subgroup of BrM patients with clonally expanded, potentially tumor-reactive T cells (pTRT)<sup>28</sup>. The study also found that CXCL13<sup>+</sup>CD39<sup>+</sup>CD8<sup>+</sup> TILs were enriched in pTRT cell-high BrM, similar to primary non-small cell lung cancer NSCLC<sup>29</sup>. The authors propose that individuals with pTRT cell-high BrM may benefit from immune checkpoint blockade, paving the way for individualized ICI treatments based on immunogenic expression profiling<sup>29</sup>.

Other factors that may influence the choice of ICI include the abundance of CXCL13-expressing (CD39<sup>+</sup>) CD8<sup>+</sup> T cells and the expression of CXCL9 in the TME, both of which are predictors of immunotherapy response in several primary malignancies, including lung and triple-negative breast cancer<sup>30</sup>. Myeloid cells in the TME may also impact ICI susceptibility. In a study of primary human gliomas, bone marrow-derived myeloid cells (BMDMs) were distinguished from brain-resident microglia using CD49D positivity, showing an association between BMDM infiltration and increased immunosuppression in analyzed tumors<sup>31</sup>. A separate study by Guldner et al.<sup>32</sup> found that human lung, breast, and skin BrMs possess myeloid cells recruited both locally from the brain parenchyma and from BMDMs. Their experiments using a murine BrM model further demonstrated a role for myeloid cells of both origins in promoting BrM through immunosuppression.

Together, these findings suggest that tumor resident T-cells in BrM are primed for an anti-tumor response but are rendered inactive due to the effects of other TILs within the TME. Nevertheless, BrMs, particularly those originating from melanoma, have shown vulnerability to ICI. An open-label, multicenter, phase 2 study with Nivolumab plus Ipilimumab demonstrated clinical benefits, with stable or reduced intracranial disease in 57% of BrM patients<sup>33</sup>. More recently combined ICI targeting both LAG-3 and PD-1 using Relatlimab in combination with Nivolumab increased progression-free survival to nearly 48%, compared to 36% with Nivolumab monotherapy, in a Phase II/III study of melanoma BrM patients<sup>34</sup>.

## Breast cancer BrM

Human epidermal growth factor receptor 2 positive (HER2-positive) and triple-negative (estrogen receptor/progesterone receptor/HER2 negative, TNBC) breast cancers are the most aggressive molecular subtypes of this disease, with TNBC having the lowest overall survival rates<sup>35</sup>. TNBC exhibits high rates of BrM, with estimates suggesting that up to one-third of women third of women diagnosed with TNBC will develop BrM<sup>36</sup>. Pathogenic *BRCA1* mutations in TNBC further predispose its progression to BrM<sup>37</sup>. A comparison of gene expression profiles of primary breast cancer metastasizing to the brain and those metastasizing to extracranial organs reveals that T cell activation is more prominent in BrM-forming primary tumors. T lymphocytes promote breast cancer cell invasion and metastasis to the brain by increasing vascular permeability, thus facilitating passage across the BBB. These effects have been attributed to the increased transcription and translation of guanylate-binding protein 1 (GBP1)<sup>38</sup>. Additionally, significant downregulation of MHC class I antigen presentation proteins has

been observed on metastatic TNBC cells, suggesting that their metastatic potential is linked to immune evasion<sup>39</sup>.

Giannoudis et al.<sup>40</sup> characterized the immune microenvironment of primary breast cancers and compared it to that of breast cancer BrM. Their study reveals a significant reduction of TILs in BrM compared to primary breast cancers. The authors also identified changes in 112 genes, including those involved in ECM remodeling, cytokine and chemokine signaling, lymphocyte regulation, antigen presentation, and immune cell adhesion and migration. Notably, PD-L1 (CD274), CTLA4, TIGIT and CD276 (B7H3) were downregulated in most BrMs, except in those originating from TNBC, where higher PD-L1 and CTLA4 expression were reported. CTLA4 expression was also reported to be higher in HER 2-positive versus estrogen receptor-positive breast cancer BrM. Increased ARG2 expression was also observed in breast cancer BrM.

These findings demonstrate that metastatic breast cancer exhibits an immune TME distinct from that primary breast cancer. BrM from breast cancer also display heterogeneity in the expression of immune modulators and conventional immune checkpoint targets, making it difficult to pinpoint a singular approach for immunotherapeutic targeting of these metastases. A personalized approach using ICI in combination with other therapeutic modalities may offer the most promising strategy for targeting breast cancer BrM.

## Lung cancer BrM

Approximately 16–60% of NSCLC patients develop BrM<sup>41</sup>. Recent single-cell transcriptomic analysis by Wang et al.<sup>42</sup> characterized the TME of NSCLC BrM as a remodeled, immunosuppressive, and fibrogenic niche that supports metastatic growth. This is accomplished by a combination of decreased antigen presentation by tumor cells and TAMs, diminished B and T cell activation, alongside an increase in neutrophils, M2 macrophages, and reactive astrocytes. The authors also identified disruptions in fibrosis and immune regulation as common features between the primary lung tumor and BrM TMEs formed by NSCLC.

Using bulk tumor DNA profiling and immunohistochemical (IHC) methods, Song et al.<sup>43</sup> also analyzed primary and BrM NSCLC. Their study found a higher tumor mutational burden (TMB) in primary NSCLC compared to BrM, which may reflect the early dissemination of metastatic clones, with the primary tumor continuing to accumulate mutations at higher rates. The authors also detected increased oligoclonal T-cell expansion by T-cell receptor repertoire (TCR) sequencing and equivalent PDL1 expression by IHC in BrM compared to primary NSCLC. Notably, differences in co-mutations were identified between EGFR-mutant BrM and EGFR-WT BrMs, with immune-related pathways enriched in EGFR-mutant BrMs. The study further demonstrated that increased CD8<sup>+</sup> TILs in NSCLC BrM predicted better overall survival following brain surgery. This association, along with Wang et al.<sup>42</sup> findings on immune dysregulation in the BrM TME, provides hope that NSCLC BrMs may respond to ICI. However, the differences observed in BrM arising from distinct NSCLC mutational subtypes support the need for personalized ICI approaches within the lung BrM patient cohort.

## Renal cell carcinoma BrM

Renal cell carcinoma (RCC) is an immunogenic tumor that accumulates dysfunctional immune cell infiltrates, which fail to control tumor growth effectively<sup>44</sup>. Between 25–30% of patients progress to develop metastatic RCC after treatment of their primary tumor<sup>45</sup>. The median overall survival (OS) for untreated patients with RCC BrM is poor, averaging between 3–4 months<sup>46</sup>.

As demonstrated by Braun et al.<sup>44</sup>, RCC BrM has significant infiltration of TILs, consistent with the tumor's immunogenic nature. However, the immunosuppressive characteristics of metastatic RCC cells attenuate the function of effector T-cells residing in the BrM TME<sup>38</sup>. Using single-cell RNA and T-cell receptor sequencing, the authors identified an enrichment of terminally exhausted CD8<sup>+</sup> T cells, M2-like immunosuppressive macrophages, and the expression of several ligand-receptor pairs by the

infiltrating leukocytes that support T-cell suppression. In primary RCC, co-expression of PD-1 and Tim-3 on TILs has been associated with advanced disease stages and poor clinical outcomes<sup>47</sup>. Additionally, immunosuppressive cytokines and stromal cell secretions have been reported to promote T cells exhaustion and impair cytotoxic function against cancer cells<sup>48</sup>. These findings provide a rationale for combination approaches to ICI therapy in RCC BrM, with a focus on addressing changes in the TME that greatly contribute to immunotherapy resistance.

### Melanoma BrM

Metastatic melanoma evades detection and elimination by the immune system by manipulating TILs. Tumor cell secretion of TGF $\beta$  activates immunosuppressive Tregs and the enrichment of activated CD8<sup>+</sup> T-cells with upregulated PD-L1 expression in the TME suppresses the anti-tumor immune response<sup>49</sup>. While soluble PD-L1 has been identified as a biomarker for melanoma, its expression being limited to certain melanoma subtypes restricts its broad application across all melanomas<sup>50</sup>. Emerging biomarkers of anti-tumor immunity in melanoma metastases include measures such as cytotoxic T-cell turnover and tumor infiltration by CD8<sup>+</sup> memory effector T-cells<sup>51</sup>. Additionally, novel molecular screening tools are distinguishing between naïve, early, and late dysfunctional states of intratumoral CD8<sup>+</sup> T-cells, which may prove valuable in guiding decisions regarding immunotherapies for patients with metastatic melanoma<sup>52</sup>.

A recent study expanded these insights by deploying single-cell analysis to characterize the immune microenvironment of human melanoma BrM in response to immune checkpoint inhibition<sup>53</sup>. The authors observed heterogeneity in the myeloid compartment of the melanoma BrM TME, including distinct neutrophil states, such as IL8-expressing population that correlated with tumor cell epithelial-to-mesenchymal transition (EMT). Additionally, the phenotype, clonotype, and overall quantity of infiltrating T cells in melanoma BrM were associated with response to ICI. The study also reported an expansion of exhausted and cycling T-cell clones, alongside non-clonally expanded CD4<sup>+</sup> FOXP3<sup>+</sup> T cells, memory CD8<sup>+</sup> T cells, and IFN-responsive CD8<sup>+</sup> T cells. Phenotypic differences between blood-overlapping and tumor-exclusive T-cell clones implied crosstalk between peripheral and intratumoral immune compartments in melanoma BrM.

It is generally agreed that an effective strategy for targeting melanoma BrM involves converting the brain's immunologically 'cold' environment into an immunoreactive TME<sup>54,55</sup>. A retrospective analysis of more than 2700 patients with stage 4 melanoma enrolled in the US National Cancer Database between 2010 and 2015 who presented with BrM supports this approach, showing that patients receiving ICI had a median survival of 12.4 months, compared to 5.2 months for those receiving other treatments<sup>56</sup>. Additional avenues for transforming the melanoma BrM TME from cold to hot include the development of antibodies targeting immunosuppressive cytokines like TGF- $\beta$ , or IL-10<sup>55</sup>. This could be particularly relevant in melanoma BrM, given the known role of elevated TGF- $\beta$ 2 in the expansion of immunosuppressive Tregs<sup>57</sup> and preclinical evidence supporting this approach in murine models of melanoma BrM<sup>58</sup>.

### Colorectal cancer BrM

BrM in patients with colorectal cancer (CRC) are infrequent and portend a poor prognosis when they occur<sup>59,60</sup>. Studies with small sample sizes have identified various mutations in *PIK3CA* and *BRAF*<sup>61</sup>, overexpression of *EGFR*<sup>62</sup> and *CXCR4*<sup>63</sup>, methylation of *MGMT*<sup>64</sup>, and elevated CA19-9<sup>65</sup> and carcinoembryonic antigen (CEA) levels as risk factors associated with CRC BrM<sup>66,67</sup>. Furthermore, correlations between *KRAS* mutations and *BRAF* mutations, as well as the role of chemokine receptor type 4 (*CXCR4*) have been identified in the context of BrM<sup>63</sup>. These findings suggest that the accumulation of heterogeneous tumor cell-intrinsic events in primary CRC may play role in determining the rare seeding of BrM, a notion supported by a recent study of 17 metastatic CRC (mCRC) patients, which found that most metastases arose from independent subclones within the primary tumor<sup>68</sup>.

In another study, Sun et al.<sup>69</sup> have also demonstrated mutations in CRC BrM that are distinct from those present in primary CRC in a cohort of 19

mCRC patients, with homologous recombination deficiency (HRD) and mismatch repair deficiency (MMRd) mutations elevated in BrM but not in respective primary tumors. Similarly, Roussille et al.<sup>70</sup> evaluated CRC BrM in comparison to primary CRC tumors and found lower levels of immune infiltrates in BrM. This aligns with findings from Harter et al.<sup>71</sup>, who reported low tumor expression of PD-L1+ and PD-1+ tumors alongside low rates of CD3+ and CD8+ T-cells in CRC BrM samples. These results highlight a consistent pattern observed across BrM from different tumor types. BrM are more immunologically cold than their respective primary tumors. As such, methods aimed at increasing immune reactivity in the TME of CRC BrM may confer clinical benefit to patients. However, limited data exist on the use of ICIs in CRC BrM due to the lack of adequately powered clinical studies to assess their efficacy in this setting.

### Sarcoma BrM

Sarcomas are a molecularly and phenotypically diverse group of malignancies that are rare, can develop in any organ, and arise from the neoplastic progression of mesenchymal cells<sup>72</sup>. While outcomes are generally favorable for patients with resectable organ-confined disease, those presenting with metastases have a median overall survival of only 18 months<sup>73</sup>. Combination chemotherapy and signal transduction inhibitors offer initial clinical benefits in patients with metastatic sarcoma who have the appropriate performance status and relevant tumor mutations, but acquired resistance remains a persistent treatment challenge<sup>74</sup>. Sarcoma BrM are rare but highly lethal, occurring in 1-8% of sarcoma patients, with mortality typically within 1 year of diagnosis<sup>73</sup>. Given that sarcoma BrM patients are usually oligometastatic, systemic therapy remains a mainstay<sup>75</sup> despite the poor CNS penetration of many agents, which is considered to contribute to the increased incidence of BrM in heavily treated metastatic sarcoma patients<sup>76</sup>.

The potential for immunotherapy to provide a therapeutic breakthrough for patients with sarcoma BrM is still uncertain. Both tumor cells and tumor-infiltrating white blood cells in primary sarcomas express immune checkpoint molecules, particularly PD1/PD-L1<sup>77</sup>. The observation has led to a series of clinical trials in both primary and metastatic sarcoma patients, including trials combining VEGF inhibitors with ICIs, given the known role of VEGF in suppressing immune response in sarcomas<sup>78</sup>. Although deep single-cell profiling of immune infiltrates in sarcoma BrM, similar to studies in NSCLC and melanoma BrM are not currently available, correlative studies from clinical trials suggest a role for PD-L1-expressing macrophages, tumor resident Tregs, and tumor resident memory effector T cells in predicting immunotherapy response in metastatic or unresectable sarcomas<sup>77</sup>. Given the rarity of sarcoma BrM within an already uncommon tumor type, powering deeper studies to identify biomarkers for personalized immunotherapy will likely require a consortium-scale effort.

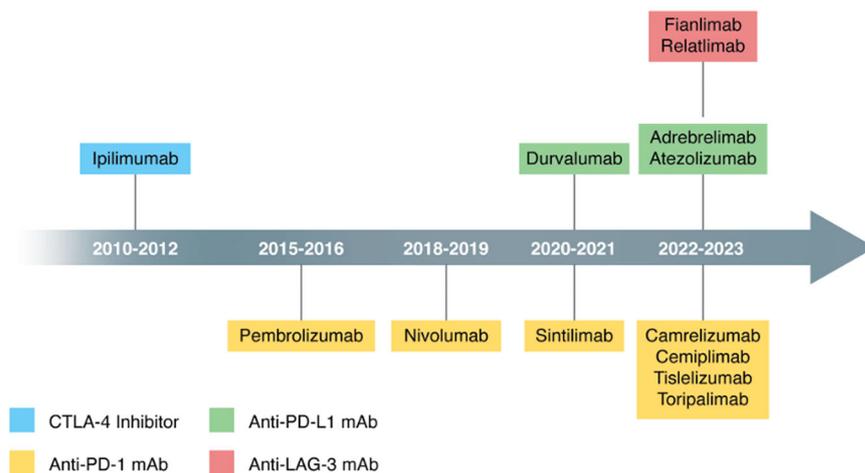
### Liquid biopsy as a powerful tool for BrM prognosis and treatment management

While not the core focus of this review, it is worth mentioning that due to the high heterogeneity of BrMs, the application of sophisticated cancer care tools such as liquid biopsy provides a more accurate assessment of cancer progression and patient outcomes<sup>78</sup>. Several recent studies in solid tumor management imply that this technique substantially improves early metastasis diagnosis and supports precision and personalized medicine approaches, especially in tailoring immunotherapeutic strategies<sup>79-81</sup>. As a diagnostic and prognostic tool in oncology, liquid biopsy allows the evaluation of different body fluids at cellular and molecular levels to differentiate and stratify biomarker candidates associated with metastasis. In the context of BrM, peripheral whole blood and cerebrospinal fluid (CSF) are the two most valuable fluids for analysis<sup>79,82</sup>.

Liquid biopsy typically analyzes CTCs, circulating tumor DNA (ctDNA), microRNAs (miRNAs), long non-coding RNAs (lncRNA), circular RNAs (cirRNA), and tumor-associated EVs<sup>81</sup>. Blood-derived CTCs provide a valuable source for identifying BrM-specific biomarkers. Nevertheless, as these cells disseminate from primary tumor sites, they often acquire novel mutations and distinct epigenetic signatures that in turn determine

**Fig. 2 | Historical timeline of FDA-approved immunotherapies used in the treatment of brain metastases (BrMs) over the last 13 years.** The monoclonal antibodies (mAbs) are color-coded based on their targets: PD-1 mAbs are marked in yellow, PD-L1 in green, LAG-3 in red, and CTLA-4 inhibitors in blue.

### Pharmacological Landscape of FDA-Approved Immunotherapies Relevant to BrMs



their metastatic organotropism<sup>79</sup>. Consequently, these alterations may lead to either dormancy or the development of specific immune evasion mechanisms at new metastatic sites in the brain, including upregulation of *CXCL8*, *CXCR4*, and *CD86*, as demonstrated by Herbst et al.<sup>83</sup>. On the other hand, CTCs isolated from CSF seem to have a unique molecular profile compared to CTCs from peripheral blood or extracranial CTCs<sup>84</sup>, making them more informative for therapeutic management and patient stratification<sup>85</sup>.

Additionally, ctDNA isolated from patients with BrM possesses specific methylation patterns not identified in other primary or secondary tumor sites, and these patterns such as EGFR variants could serve as primary prognostic markers<sup>86</sup>. An illustrative study by Zuccato et al.<sup>87</sup> working with lung adenocarcinoma patients developed a glmnet classifier informed by methylation signatures from cell-free DNA in the plasma of patients with intracranial masses that span the entities commonly considered in a differential diagnosis: glioma, CNS lymphoma, and brain metastasis. Their classifier accurately discerned with an AUC of 0.8 when plasma samples were from BrM patients in a validation set that included glioma, CNS lymphoma, and non-CNS control cases. The group achieved median accuracy of 74.2% when the classifier was tested in a second cohort. It is noteworthy that this performance was achieved using solely methylome analysis of liquid biopsies, highlighting the potential for this method as non-invasive diagnostic tool. In addition to DNA, differential expression of non-coding RNA species, particularly miRNAs (e.g., those targeting PTEN) and lncRNAs (e.g., XIST downregulation), shows significant potential as a diagnostic aid in BrM<sup>88,89</sup>.

Lastly, EVs are potent modulators of the BrM TME. Thus, their isolation and high-resolution molecular characterization hold great promise for managing secondary cancers in the brain. For instance, Serrati et al.<sup>90</sup> recently showed that PD-L1-positive exosomes derived from metastatic melanoma cells induced resistance to anti-PD-1 immunotherapy. Although challenges remain in the exploitation of liquid biopsy for BrM management, this method, together with advanced molecular analyses such as genomic sequencing and epigenetic profiling, offers an invaluable and complementary tool for understanding the complex molecular landscape of BrM. A deeper understanding of these mechanisms could enable the development of personalized therapeutic strategies, ultimately leading to improved patient outcomes.

### Immune checkpoints and ICIs

As reviewed above, co-opting immunosuppressive mechanisms to establish and maintain tumors is a common theme across BrMs originating from various primary malignancies. This is frequently achieved by exploiting immune checkpoint biology. In some cases, metastatic tumor cells express the necessary ligands, while in others, infiltrating cells within the BrM TME perform this function. Regardless, ICIs disrupt interactions between

checkpoint ligands and their receptors on effector T cells, restoring CD8+ T-cell-mediated clearance of tumor cells in BrM. The sections below describe current ICI by the specific checkpoint targeted. This list will likely grow as additional checkpoints and strategies for targeting them are developed (Fig. 2).

### Anti-PD-1 Immunotherapies

The programmed cell death protein-1 (PD-1)/programmed death-ligand 1 (PD-L1) checkpoint regulates the induction and maintenance of immune tolerance within the TME. Activation of PD-1 on T cells by its ligands, PD-L1 or PD-L2, impairs activation, proliferation, and cytotoxic activity necessary for an effective anti-tumor response. Nivolumab, a fully human monoclonal antibody (mAb) against the PD-1 cell surface receptor, inhibits its interaction with PD-L1. It has received FDA approval for several indications including metastatic melanoma, disease progression following Ipilimumab therapy, and metastatic squamous NSCLC with progression on or after platinum-based chemotherapy<sup>79</sup>. A key advantage of Nivolumab is its durable response, observed in as many as 68% of patients who meet specific predictive serum biomarker criteria<sup>80</sup>. Tables 1–3 list the clinical trials that have investigated the use of Nivolumab in BrMs.

Pembrolizumab, another humanized IgG4 mAb targeting PD-1, recently received its 40<sup>th</sup> FDA approval for advanced endometrial cancer patients based on clinical trials supporting its safety and efficacy<sup>81</sup>. This mAb is also used in solid tumors, including BrM and primary CNS malignancies, that have high microsatellite instability (MSI-H), DNA mismatch repair deficiency (dMMR), or high tumor mutational burden (TMB-H) based on data across 30 cancer types from the KEYNOTE trials (KEYNOTE-051, -158, and -164) [NCT02332668, NCT02628067, NCT02460198]. Another PD-1 inhibitor, Cemiplimab, is currently under limited investigation in patients with BrM.

In an open label phase 2 study by Goldberg et al.<sup>91</sup> on NSCLC and melanoma patients with BrM, 29.7% of patients with PD-L1 expression of at least 1% demonstrated BrM response to Pembrolizumab. It is noteworthy that PD-L1 expression was the only stratifying criteria in this study, suggesting a role for MSI, dMMR, and TMB in improving response rates. An independent study also investigated the value of optimizing assessments for the proportion of the tumor expressing PD-L1 (TPS score), finding a 54.7% response rate to ICI regimens using pembrolizumab among NSCLC BrM whose tumors had a median TPS of 7.5%<sup>92</sup>. The study demonstrated that patients with higher PD-L1 expression (TPS > 40%) had significantly longer intracranial progression-free survival, suggesting a role for checkpoint ligand expression as a predictor of both therapeutic response and response duration for patients receiving PD1 targeted ICI. A challenge when implementing PD-L1 expression as a predictive biomarker is the need to

**Table 1 | Outcomes of immune checkpoint inhibitors in the treatment of NSCLC with BrM**

Author	Study type	Treatment	Patients with brain metastasis	Outcomes
Ready et al. <sup>117</sup> CheckMate 817 NCT02869789	Phase IIIB	Nivolumab + Ipilimumab	49	mOS: 12.8 m 3-years OS rate: 21.0% mPFS: 2.8 m 3-years PFS rate: 14.2%
Reck et al. <sup>118</sup> CheckMate 227 Part 1 NCT02477826	Phase III	Nivolumab + Ipilimumab versus chemotherapy	68 66	mOS: 17.4 vs 13.7 m 5-year OS rates: 20% vs. 6% 5-year intracranial PFS rates: 16%
Carbone et al. <sup>119</sup> CheckMate 9LA NCT03215706	Phase III	Nivolumab + ipilimumab Chemotherapy versus chemotherapy	51 50	mOS: 19.3 vs. 6.8 m 2-year OS rates: 35% vs. 12% mPFS: 10.6 vs. 4.1 m
Nadal et al. <sup>95</sup> NCT03526900	Phase II	Atezolizumab + chemotherapy, followed by maintenance with Pemetrexed +	40	mOS: 13.6 m 2-year OS rate: 30.5% mPFS: 8.9 m Intracranial mPFS: 6.9 m
Hou et al. <sup>120</sup> NCT04211090	Phase II	Camrelizumab + chemotherapy, followed by maintenance with Camrelizumab	45	mOS: 21.0 mo mPFS: 7.4 mo Intracranial mPFS: 7.6 m
Powell et al. <sup>121</sup>	Pooled analysis	Pembrolizumab + Chemotherapy versus	171	mOS: 18.8 m vs. 7.6 m mPFS: 6.9 m vs. 4.1 m
Ozguroglu et al. <sup>122</sup> NCT03088540	Phase III	Pembrolizumab	68	mOS: 18.7 vs. 11.7 m mPFS: 10.4 vs. 5.3 m
Goldberg et al. <sup>91</sup> NCT02085070	Phase II	Cemiplimab versus chemotherapy	42	Patients with NSCLC and PD-L1 ≥ 1%: mPFS 1.9 m
Mansfield et al. <sup>123</sup>	Pooled analysis	Pembrolizumab versus chemotherapy	293	Patients with NSCLC and PD-L1 ≥ 1%: mOS: 13.4 vs. 10.3 m Pts with NSCLC and PD-L1 ≥ 50%:
Wang et al. <sup>124</sup> CAPSTONE-1 study NCT03711305	Phase III	Adebrelimab (SHR-1316) + chemotherapy Placebo + chemotherapy (placebo group)	232	mOS: 15.3 m vs 12.8 m for Placebo HR 0.72
Huang et al. <sup>125</sup> NCT04507217	Phase II	Efficacy and Safety of Tislelizumab combined with Pemetrexed/ Carboplatin in Patients with BrM of Non-squamous NSCLC	36 (29 with untreated, asymptomatic BrM)	PFS: 36.8% (95% CI, 18.0-55.7), mPFS: 7.5 m ORR: 50.0% 1-year OS: 70.5% 1-year iPFS: 56.6% (95% CI, 31.7-75.5), with 45.5% (95% CI, 19.4, 68.5) and 100% in pts with untreated and pretreated BrM Intracranial ORR: 56.7% with 53.8% in patients with untreated BrM and

BrM brain metastasis, NSCLC non-small cell lung cancer, IDCR intracranial disease control rate, iPFS intracranial PFS, m months, mOS median overall survival, mPFS median progression-free survival, HR hazard ratio, NR not reached, pts patients, PFS progression free survival, OS overall survival, IRR infusion related reaction, CI confidence interval.

**Table 2 | Clinical trials with renal cell carcinoma and brain metastasis**

Author	Study type	Treatment	Patients with brain metastasis	Outcomes
Masini et al. <sup>126</sup> NCT03469713	Phase II	Nivolumab + Stereotactic body radiotherapy in II and III line of patients with metastatic renal cell carcinoma	68	ORR: 17%  DCR: 55%.  mPFS: 5.6 m  mOS: 20 m  mDOR: 14 m
Emamekhoo et al. <sup>127</sup> CHECKMATE 920	Phase IIIB/IV	Nivolumab combined with Ipilimumab in subjects with previously untreated, advanced or metastatic renal cell carcinoma	Cohort 3: 28	ORR: 32%  mDOR: 24.0 m; 4 of 8 responders remained without progression mPFS: 9.0 m (95% CI, 2.9-12.0 months) mOS not reached (95% CI, 14.1 m to not estimable)
Wong et al. <sup>128</sup> NCT02978404	Phase II	Combining radiosurgery and Nivolumab in the treatment of brain metastases from NSCLC and renal cell	4	1-yr iPFS: 45.2% (95% CI 29.3-69.6%)  1-yr OS: 61.3% (95% CI 45.1-83.3%)

IDCR intracranial disease control rate, iPFS intracranial PFS, m months, mOS median overall survival, mPFS median progression-free survival, HR hazard ratio, NR Not reached, PFS Progression Free Survival, OS Overall Survival, IRR infusion-related reaction.

**Table 3 | Clinical trials with immunotherapy in patients with melanoma and brain metastasis**

Author	Study type	Treatment	Patients with brain metastasis	Outcomes			
Margolin et al. <sup>129</sup> NCT00623766	Phase II	Ipilimumab	Cohort A: (neurologically asymptomatic) 51	IDCR: 25%			
				mOS: 7.0 m			
				mPFS: 2.7 m			
			Cohort B: (symptomatic and on a stable dose of corticosteroids) 21	Intracranial mPFS: 1.9 m			
				IDCR: 10%			
				mOS: 3.7 m			
				mPFS: 1.3 m			
				Intracranial mPFS: 1.2 m			
Di Giacomo et al. <sup>130</sup> NCT01654692	Phase II	Ipilimumab + Fotemustine	20	IDCR: 50.0%			
				mOS: 13.4 m			
				mPFS: 4.5 m			
Goldberg et al. <sup>131</sup>	Phase II	Pembrolizumab					
Kluger et al. <sup>132</sup> NCT020850170	Phase II	Pembrolizumab	23	IRR: 26%			
				mOS: 17 m			
				mPFS: 2 m			
Long et al. <sup>133</sup> NCT02374242	Phase II	Nivolumab + Ipilimumab, followed by Nivolumab	Cohort A: 35	IRR: 46%			
				mOS: NR			
				mPFS: 13.8 m			
						Intracranial mPFS: NR	
		Nivolumab	Cohort B: 25	IRR: 20%			
				mOS: 13.8 m			
				mPFS: 2.6 m			
						Intracranial mPFS: 2.6 m	
		Nivolumab	Cohort C: Patients neurologically symptomatic 16	IRR: 6%			
mOS: 5.1 m							
mPFS: 2.6 m							
				Intracranial mPFS: 2.3 m			
Tawbi et al. <sup>34</sup> NCT02320058	Phase II	Nivolumab + Ipilimumab, followed by	94	IDCR: 57%			
				1-year OS rate: 81.5%			
				1-year PFS rate: 56.6%			
Tawbi et al. <sup>34</sup> NCT03470922	Phase II	Relatlimab + Nivolumab versus Nivolumab in participants with previously untreated metastatic or unresectable melanoma	Relatlimab + Nivolumab: 355  Nivolumab: 359	ImPFS: 10.1 m (95%[CI], 6.4 to 15.7) with Relatlimab-Nivolumab vs. 4.6 m (95% CI, 3.4 to 5.6) with Nivolumab (HR for progression or death, 0.75 [95% CI, 0.62 to 0.92]; <i>P</i> = 0.006 by the log-rank test			
				1-yr PFS rate: 47.7% (95% CI, 41.8 to 53.2) with Relatlimab-Nivolumab vs. 36.0% (95% CI, 30.5 to 41.6)			

IDCR intracranial disease control rate, *IPFS* intracranial PFS, *m* months, *mOS* median overall survival, *mPFS* median progression-free survival, *HR* hazard ratio, *NR* Not reached, *PFS* Progression Free Survival, *OS* Overall Survival, *IRR* infusion-related reaction.

assess expression in BrM at the start of therapy. Analysis of 73 primary-BrM pairs of lung cancer found discordance in tumor cell expression between primary and BrM specimens, with most discordant pairs having biopsies that were obtained more than 6-month apart<sup>93</sup>. The observation both discourages the use of the primary tumor for such assessments and highlights the need to account for temporal heterogeneity. Spatial heterogeneity presents a similar challenge, as tumor cells expressing PD-L1 with no nearby CD8+ T-cells will be unencumbered by PD1 targeted ICI. Immunoscoring is an in vitro diagnostics assay that addresses this by computing a score that accounts for both the densities of and distance between CD8+ T-cells and PD-L1+ tumor cells and performs well as a predictor of progression-

free survival in metastatic NSCLCs patients treated with anti-PD-1 or anti-PD-L1 therapy<sup>94</sup>. The study was not limited to BrM patients, but nevertheless illustrates the need to account for complexity in the BrM TME when developing response biomarkers for ICI.

**PD-L1 inhibitors**

PD-1 has two ligands, PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2). The expression of PD-L1, whether by immune cells in the TME or tumor cells, serves as a predictive biomarker for response to therapies targeting the PD-1/PD-L1 checkpoint<sup>92</sup>. Beyond PD-1, PD-L1 can also inhibit T-cell activation by binding to the negative regulator CD80 (B7.1)<sup>83</sup>. Therefore,

PD-L1's effect on T cells includes molecular processes beyond what PD-1 inhibition alone addresses. Antibodies targeting the PD-L1, such as Adbrelimab, Atezolizumab, and Durvalumab, may address this concern. A recent single arm phase 2 study of Atezolizumab in combination with chemotherapy established median overall survival of 11.8 months and 2-year overall survival rate of 27.5% in NSCLC patients with previously untreated BrM on this regimen<sup>95</sup>. While the single-arm design of this study limits assessment of Atezolizumab's contribution to survival in this cohort of combined chemotherapy-treated patients, the regimen was well tolerated with an overall response rate of 45% and median progression-free survival of 8.9 months.

### CTLA-4 inhibitors

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is expressed by T cells, and constitutively present on the surface of Treg cells. Upon activation by TCR or CD28 signaling, it is induced to localize on naïve T cells. CTLA-4 binds B7-1 and B7-2 on antigen-presenting cells (APCs), leading to T cell anergy. Tumors expressing B7-1 and B7-2 exploit this mechanism to suppress T-cell<sup>84</sup>. mAbs targeting CTLA-4, such as Ipilimumab, interfere with this interaction and have shown efficacy in clinical trials of melanoma<sup>85</sup>. Ipilimumab, an IgG1k anti-CTLA4 mAb, gained FDA approval in 2011 for non-resectable stage III/IV melanoma. It has been extensively tested in patients with melanoma BrM and NSCLC BrM, with success observed in combination with Nivolumab<sup>86</sup>.

### Anti-lymphocyte activation gene-3 antibody

Lymphocyte Activation Gene-3 or LAG-3 (CD223) is an inhibitory receptor that upregulates during physiologic immune responses to infections, serving as a checkpoint to prevent tissue damage and auto-immunity. LAG-3 binds to MHC class II molecules with greater affinity than CD4, attenuating cytotoxic T-cell responses. Tumor cells and the TME in BrM often co-opt this mechanism by overexpressing LAG-3<sup>88</sup>. Anti-LAG-3 therapies include LAG-3-targeting mAbs, bispecific LAG-3 antibodies, and fusion proteins. These immunotherapies suppress anti-inflammatory cytokines such as IL-12 and IFN- $\gamma$ <sup>89</sup>. Clinically, LAG-3 antibodies are used in combination with PD-1 inhibitors, as LAG-3 and PD-1 are co-expressed on tumor-infiltrating CD8 + T-cells. Relatlimab is the first LAG-3 inhibitor approved by the FDA for use in combination with Nivolumab, for treating unresectable or metastatic melanoma based on the RELATIVITY-047 trial<sup>34</sup>. Another LAG-3 antibody, Fianlimab, is currently being tested in combination with Cemiplimab in a phase III trial (NCT05352672) for patients with previously untreated locally advanced or metastatic melanoma.

### Combinatorial approaches to immune checkpoint inhibition

Combinatorial approaches involving LAG-3 inhibitors, such as Relatlimab, in combination with PD-1 inhibitors like Nivolumab, have shown promising outcomes in advanced melanoma, as demonstrated by improved progression-free survival in the RELATIVITY-047 trial<sup>34</sup>. While this trial did not focus specifically on brain metastases, its results highlight the potential efficacy of dual checkpoint blockade in immunologically suppressed tumor microenvironments, such as those found in brain metastases. Ongoing clinical trials, such as NCT05352672 evaluating Fianlimab with Cemiplimab in advanced melanoma, will further inform on the potential of dual checkpoint inhibition strategies to optimize immunotherapy responses. The rationale for combining LAG-3 and PD-1/PD-L1 blockade lies in the co-expression of these pathways on exhausted CD8 + T cells, where dual checkpoint inhibition can effectively reinvigorate T-cell function<sup>96</sup>.

While these registrational phase 3 trials are not specific to brain metastases, a multi-center open label phase 2 study by Long et al.<sup>97</sup> focused on melanoma brain metastasis evaluated therapeutic responses to the combination of nivolumab and ipilimumab and to monotherapy with nivolumab alone. The study found intracranial responses in 46% of patients in the combination therapy arm, nearly twice the 20% response rate to nivolumab monotherapy. Although this study has considerably smaller

enrollment than the phase 3 studies mentioned, it highlights the extent to which therapeutic benefits of combination ICI reported by registrational studies that are not enriched for BrM patients may nevertheless carry-over when tested in this setting.

### Immune checkpoint inhibition across tumors of differing primary origin

More granular insights into tumor microenvironment (TME) adaptations across different primary tumor origins, such as breast cancer and renal cell carcinoma, reveal significant heterogeneity in immune composition and suppressive mechanisms<sup>98</sup>. In breast cancer BrM, particularly from triple-negative and HER2-positive subtypes, reduced T-cell infiltration and upregulation of immune checkpoint molecules such as PD-L1 and CTLA-4 are observed, contributing to immune evasion<sup>99</sup>. In contrast, renal cell carcinoma BrM are characterized by infiltrating exhausted CD8+ T cells and M2-like macrophages, creating a profoundly immunosuppressive environment<sup>100</sup>. Aligning therapeutic strategies, such as immune checkpoint inhibitors or combination therapies, with these tumor-origin-specific immune adaptations could further optimize immunotherapy responses and improve clinical outcomes.

### Targeting BrMs with chimeric antigen receptor T-cell therapy

Over the past decade, researchers have developed chimeric antigen receptor (CAR) T-cell therapy through various innovative methods and techniques, including leukapheresis, immunomagnetic enrichment, genetic engineering, and ex vivo T-cell expansion<sup>90</sup>. This immunotherapy involves modifying patients' T effector cells to target malignant cells via their specific epitopes, such as CD19 and BCMA, primarily in hematological cancers, thus significantly suppressing tumor growth<sup>101</sup>.

Since CAR T-cell therapy is a cell-based approach, its native form is autologous, using the patient's own T cells to minimize the risk of immune rejection. However, cutting-edge technologies like induced pluripotent stem cells (iPSCs) are now opening new avenues towards scalable, allogeneic 'off-the-shelf' immune cell therapeutics. These cells are engineered to be non-immunogenic through specific genetic modifications during their manufacturing process<sup>102,103</sup>. Furthermore, these artificial iPSC-derived cells could be genetically engineered to overcome tumor resistance, enhance on-target efficacy, and reduce off-target cytotoxicity in situ<sup>104</sup>. Despite its success in hematologic malignancies, CAR T-cell therapy for solid tumors, particularly complex malignancies such as BrMs, is currently restricted in scope due to several key obstacles<sup>105</sup>. These include antigen heterogeneity and epitope downmodulation, on-target off-tumor cytotoxicity and related safety concerns, poor trafficking across the BBB and reduced intratumor infiltration, T cell exhaustion, and immunosuppressive TME<sup>106</sup>. As discussed earlier, the TME of BrMs is highly heterogeneous, with extensive antigenic variation often depending on the primary tumor type and the mutation status of distinct CTCs. Therefore, selecting appropriate BrM-specific antigens with sufficient antigen density for CAR recognition is crucial for the effective eradication of secondary malignancies while preventing tumor-induced immunosuppression and minimizing cytotoxicity to local healthy tissue<sup>107,108</sup>. A recent preclinical study using a human xenograft model demonstrated the effective application of HER2-BB $\zeta$  CAR T-cell immunotherapy against multifocal breast carcinoma metastasis to the brain, leading to significant tumor regression<sup>105</sup>. An important aspect of its efficacy was the route of administration. Regional intraventricular delivery significantly enhanced T-cell persistence and likely supported antigen-dependent trafficking, whereas systemic approaches showed a significant reduction in T-cell anti-tumor activity<sup>105,109</sup>.

In conclusion, cell-based immunotherapy, particularly allogeneic CAR T-cell therapy, holds great promise for the targeted and efficient reduction of solid tumors, including BrMs. Local injection of cell therapeutics into the brain appears to be crucial for overcoming the challenges posed by the BBB and/or poor cell trafficking within the hostile BrM TME. Complementarily, more efficient screening pipelines

for antigen candidates and advanced engineering of CAR constructs could be key to mitigating on-target, off-tumor toxicity<sup>106</sup>.

## Perspectives

TME analysis of different types of cancer and metastasis to the brain has revealed several promising biomarkers and therapeutic targets using state-of-the-art techniques such as single-cell sequencing technologies, multiplex imaging, spatial profiling, and bulk tumor profiling. In the case of BrM, a common theme across the spectrum of primary tumors of origin is a suppressed immune response in the BrM TME and a mutational profile unique to BrM when compared to the primary and extracranial “sibling” metastases. Analysis of patient cohorts with matched primary tumor samples also reveals differences in biomarker profiles for PD-1, PD-L1, and tumor-infiltrating CD8 + T cells.

Data on clinical response rates to ICI in patients with BrM are limited, as progressing to BrM is often an exclusion criterion for larger clinical trials evaluating the efficacy and safety of ICIs. However, smaller studies show efficacy in the form of intracranial tumor necrosis of BrM after only a single cycle of ICI treatment combined with chemotherapy in patients with NSCLC BrM<sup>110</sup>. Findings such as these call for study designs that include BrM patients in ICI efficacy trials and nest validation of predictive and response biomarkers. Molecular information such as tumor mutational burden, mismatch repair system status, and PD-L1 expression, in addition to the cellular density of tumor-infiltrating CD8 + T-cells, should also be collected in BrM patients to facilitate better prediction of response to ICIs by intracranial tumors. Additionally, understanding the impact of mutations in BrM that differ from those present in the primary tumor on BrM-specific tumor biology will prove essential to adequately caring for patients with this lethal diagnosis.

A recent meta-analysis of 56 studies with 2739 patients has shown a high level of disparity in PD-L1, PD-1, PD-L2, and TIL levels alongside a lesser likelihood of disparity in the tumor mutational burden and microsatellite instability between primary tumors and patient-matched metastases<sup>111</sup>. Therefore, evaluating frequently altered biomarkers in primary and metastatic tumors should be a priority before deciding on the immune checkpoint treatment to use in a particular BrM patient. The TME landscape<sup>112</sup>, genomic alterations<sup>113,114</sup>, and specific mutations involved in resistance to immune checkpoint therapies<sup>115</sup> must also be taken into consideration to maximize therapeutic benefit from ICIs in the setting of BrM. Promising results seen in CAR T cell-based treatments of patients with hematologic cancers, as well as early clinical development in treating brain tumors, pave the way for more effective and precise immunotherapy.

In the final analysis, personalization is a true clinical need. New holistic and innovative approaches for BrM treatment using bispecific monoclonal antibodies and combination therapy may provide better response rates and survival benefits than those achieved by traditional single treatment strategies. Methods in the preclinical phase of the therapeutic pipeline such as targeting the promotion of M1 properties in macrophages within the TME and the recruitment of tumor-infiltrating CD8+ T cells, may also prove potent once translated to the clinic<sup>116</sup>. Meanwhile, emerging insights from the elegant immune landscape studies reviewed here are bound to impact our ongoing effort to convert BrMs from immunosuppressed “cold” to immunologically “hot” environments<sup>55</sup>.

## Data availability

No datasets were generated or analyzed during the current study.

## Code availability

No custom code was developed for this review article as it solely analyzes and summarizes existing published research; therefore, no code is available.

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## References

1. Abedalthagafi, M., Barakeh, D. & Foshay, K. M. Immunogenetics of glioblastoma: the future of personalized patient management. *NPJ Precis. Oncol.* **2**, 27 (2018).
2. Nduom, E. K., Weller, M. & Heimberger, A. B. Immunosuppressive mechanisms in glioblastoma. *Neuro-Oncology* **17**, vii9–vii14 (2015). **Suppl 7**.
3. Peng, P. & Lim, M. Immunosuppressive mechanisms of malignant gliomas: parallels at non-CNS sites. *Front. Oncol.* **5**, 153 (2015).
4. Barnholtz-Sloan, J. S. et al. Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. *J. Clin. Oncol.* **22**, 2865–2872 (2004).
5. Binnewies, M. et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat. Med.* **24**, 541–550 (2018).
6. Quail, D. F. & Joyce, J. A. The microenvironmental landscape of brain tumors. *Cancer Cell* **31**, 326–341 (2017).
7. Friebel, E. et al. Single-cell mapping of human brain cancer reveals tumor-specific instruction of tissue-invading leukocytes. *Cell* **181**, 1626–1642.e20 (2020).
8. Klemm, F. et al. Interrogation of the microenvironmental landscape in brain tumors reveals disease-specific alterations of immune cells. *Cell* **181**, 1643–1660.e17 (2020).
9. Benci, J. L. et al. Tumor interferon signaling regulates a multigenic resistance program to immune checkpoint blockade. *Cell* **167**, 1540–1554.e12 (2016).
10. Achrol, A. S. et al. Brain metastases. *Nat. Rev. Dis. Primers* **5**, 5 (2019).
11. Sevenich, L. et al. Analysis of tumour- and stroma-supplied proteolytic networks reveals a brain-metastasis-promoting role for cathepsin S. *Nat. Cell Biol.* **16**, 876–888 (2014).
12. Priego, N. et al. STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. *Nat. Med.* **24**, 1024–1035 (2018).
13. Gao, Y. et al. Metastasis organotropism: redefining the congenial soil. *Dev. Cell* **49**, 375–391 (2019).
14. Valiente, M. et al. Serpins promote cancer cell survival and vascular co-option in brain metastasis. *Cell* **156**, 1002–1016 (2014).
15. Rehman, A. U. et al. Liquid biopsies to occult brain metastasis. *Mol. Cancer* **21**, 113 (2022).
16. Barakeh, D. H. et al. Clinicopathologic and genomic characterizations of brain metastases using a comprehensive genomic panel. *Front. Med.* **9**, 947456 (2022).
17. Lowery, F. J. & Yu, D. Brain metastasis: unique challenges and open opportunities. *Biochim. Biophys. Acta Rev. Cancer* **1867**, 49–57 (2017).
18. Basnet, H. et al. Flura-seq identifies organ-specific metabolic adaptations during early metastatic colonization. *Elife* **8**, e43627 (2019).
19. Flavahan, W. A. et al. Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. *Nat. Neurosci.* **16**, 1373–1382 (2013).
20. Brastianos, P. K. et al. Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets. *Cancer Discov.* **5**, 1164–1177 (2015).
21. Karimi, E. et al. Single-cell spatial immune landscapes of primary and metastatic brain tumours. *Nature* **614**, 555–563 (2023).
22. Carbonell, W. S., Anson, O., Sibson, N. & Muschel, R. The vascular basement membrane as “soil” in brain metastasis. *PLoS One* **4**, e5857 (2009).
23. Bowman, R. L. et al. Macrophage ontogeny underlies differences in tumor-specific education in brain malignancies. *Cell Rep.* **17**, 2445–2459 (2016).
24. Schulz, M., Salamero-Boix, A., Niesel, K., Alekseeva, T. & Sevenich, L. Microenvironmental regulation of tumor progression and

- therapeutic response in brain metastasis. *Front. Immunol.* **10**, 1713 (2019).
25. Di Giacomo, A. M. et al. Immunotherapy of brain metastases: breaking a “dogma”. *J. Exp. Clin. Cancer Res.* **38**, 419 (2019).
  26. Widodo, S. S. et al. Spatial analysis of the metastatic brain tumor immune and extracellular matrix microenvironment. *Adv. Cancer Biol. Metastasis* **7**, 100096 (2023).
  27. Musca, B. et al. The immune cell landscape of glioblastoma patients highlights a myeloid-enriched and immune suppressed microenvironment compared to metastatic brain tumors. *Front. Immunol.* **14**, 1236824 (2023).
  28. Wischnewski, V. et al. Phenotypic diversity of T cells in human primary and metastatic brain tumors revealed by multiomic interrogation. *Nat. Cancer* **4**, 908–924 (2023).
  29. Chowell, D. et al. Improved prediction of immune checkpoint blockade efficacy across multiple cancer types. *Nat. Biotechnol.* **40**, 499–506 (2022).
  30. Zhang, Y. et al. Single-cell analyses reveal key immune cell subsets associated with response to PD-L1 blockade in triple-negative breast cancer. *Cancer Cell* **39**, 1578–1593.e8 (2021).
  31. Pinton, L. et al. The immune suppressive microenvironment of human gliomas depends on the accumulation of bone marrow-derived macrophages in the center of the lesion. *J. Immunother. Cancer* **7**, 58 (2019).
  32. Guldner, I. H. et al. CNS-native myeloid cells drive immune suppression in the brain metastatic niche through Cxcl10. *Cell* **183**, 1234–1248.e25 (2020).
  33. Tawbi, H. A. et al. Combined nivolumab and ipilimumab in melanoma metastatic to the brain. *N. Engl. J. Med.* **379**, 722–730 (2018).
  34. Tawbi, H. A. et al. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N. Engl. J. Med.* **386**, 24–34 (2022).
  35. Jin, J. et al. Incidence, pattern and prognosis of brain metastases in patients with metastatic triple negative breast cancer. *BMC Cancer* **18**, 446 (2018).
  36. Syriac, A. K., Nandu, N. S. & Leone, J. P. Central nervous system metastases from triple-negative breast cancer: current treatments and future prospective. *Breast Cancer (Dove Med. Press)* **14**, 1–13 (2022).
  37. Garber, H. R. et al. Incidence and impact of brain metastasis in patients with hereditary BRCA1 or BRCA2 mutated invasive breast cancer. *npj Breast Cancer* **8**, 46 (2022).
  38. Mustafa, D. A. M. et al. T lymphocytes facilitate brain metastasis of breast cancer by inducing Guanylate-Binding Protein 1 expression. *Acta Neuropathol* **135**, 581–599 (2018).
  39. Pedersen, M. H. et al. Downregulation of antigen presentation-associated pathway proteins is linked to poor outcome in triple-negative breast cancer patient tumors. *Oncoimmunology* **6**, e1305531 (2017).
  40. Giannoudis, A. et al. Characterisation of the immune microenvironment of primary breast cancer and brain metastasis reveals depleted T-cell response associated to ARG2 expression. *ESMO Open* **7**, 100636 (2022).
  41. Buriolla, S. et al. Immunotherapy in NSCLC patients with brain metastases. *Int. J. Mol. Sci.* **23**, 7068 (2022).
  42. Wang, Z. et al. Single-cell transcriptomic analyses provide insights into the cellular origins and drivers of brain metastasis from lung adenocarcinoma. *Neuro-Oncology* **25**, 1262–1274 (2023).
  43. Song, S. G. et al. Comparative analysis of the tumor immune-microenvironment of primary and brain metastases of non-small-cell lung cancer reveals organ-specific and EGFR mutation-dependent unique immune landscape. *Cancer Immunol. Immunother.* **70**, 2035–2048 (2021).
  44. Braun, D. A. et al. Progressive immune dysfunction with advancing disease stage in renal cell carcinoma. *Cancer Cell* **39**, 632–648.e8 (2021).
  45. Berghoff, A. S. & Preusser, M. The inflammatory microenvironment in brain metastases: potential treatment target? *Chin. Clin. Oncol.* **4**, 21 (2015).
  46. Kattan, J., Rassy, E. E., Assi, T., Bakouny, Z. & Pavlidis, N. A comprehensive review of the role of immune checkpoint inhibitors in brain metastasis of renal cell carcinoma origin. *Crit. Rev. Oncol. Hematol.* **130**, 60–69 (2018).
  47. Granier, C. et al. Tim-3 expression on tumor-infiltrating PD-1(+) CD8(+) T cells correlates with poor clinical outcome in renal cell carcinoma. *Cancer Res.* **77**, 1075–1082 (2017).
  48. Jiang, Y., Li, Y. & Zhu, B. T-cell exhaustion in the tumor microenvironment. *Cell Death Dis.* **6**, e1792 (2015).
  49. Spranger, S. et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. *Sci. Transl. Med.* **5**, 200ra116 (2013).
  50. Redmer, T. Deciphering mechanisms of brain metastasis in melanoma - the gist of the matter. *Mol. Cancer* **17**, 106 (2018).
  51. Valpione, S. et al. Immune-awakening revealed by peripheral T cell dynamics after one cycle of immunotherapy. *Nat. Cancer* **1**, 210–221 (2020).
  52. van der Leun, A. M., Thommen, D. S. & Schumacher, T. N. CD8(+) T cell states in human cancer: insights from single-cell analysis. *Nat. Rev. Cancer* **20**, 218–232 (2020).
  53. Alvarez-Breckenridge, C. et al. Microenvironmental landscape of human melanoma brain metastases in response to immune checkpoint inhibition. *Cancer Immunol. Res.* **10**, 996–1012 (2022).
  54. Louveau, A., Harris, T. H. & Kipnis, J. Revisiting the mechanisms of CNS immune privilege. *Trends Immunol* **36**, 569–577 (2015).
  55. Sevenich, L. Turning “cold” into “hot” tumors-opportunities and challenges for radio-immunotherapy against primary and metastatic brain cancers. *Front. Oncol.* **9**, 163 (2019).
  56. Iorgulescu, J. B. et al. Improved risk-adjusted survival for melanoma brain metastases in the era of checkpoint blockade immunotherapies: results from a national cohort. *Cancer Immunol. Res.* **6**, 1039–1045 (2018).
  57. Huang, L. et al. Targeting regulatory T cells for immunotherapy in melanoma. *Mol. Biomed.* **2**, 11 (2021).
  58. Orozco, J. I., Manughian-Peter, A. O., Salomon, M. P. & Marzese, D. M. Epigenetic classifiers for precision diagnosis of brain tumors. *Epigenet Insights* **12**, 2516865719840284 (2019).
  59. Shindorf, M. L., Jafferji, M. S. & Goff, S. L. Incidence of asymptomatic brain metastases in metastatic colorectal cancer. *Clin. Colorectal Cancer* **19**, 263–269 (2020).
  60. Muller, S. et al. Brain metastases from colorectal cancer: a systematic review of the literature and meta-analysis to establish a guideline for daily treatment. *Cancers* **13**, 900 (2021).
  61. Tran, B. et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer* **117**, 4623–4632 (2011).
  62. Scartozzi, M. et al. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. *J. Clin. Oncol.* **22**, 4772–4778 (2004).
  63. Mongan, J. P. et al. Brain metastases from colorectal cancer: risk factors, incidence, and the possible role of chemokines. *Clin. Colorectal Cancer* **8**, 100–105 (2009).
  64. De Maglio, G. et al. MGMT promoter methylation status in brain metastases from colorectal cancer and corresponding primary tumors. *Future Oncol* **11**, 1201–1209 (2015).
  65. Tanriverdi, O. et al. The clinical and pathological features of 133 colorectal cancer patients with brain metastasis: a multicenter retrospective analysis of the Gastrointestinal Tumors Working Committee of the Turkish Oncology Group (TOG). *Med. Oncol.* **31**, 152 (2014).

66. Higashiyama, M. et al. Surgery for pulmonary metastases from colorectal cancer: the importance of prethoracotomy serum carcinoembryonic antigen as an indicator of prognosis. *Jpn. J. Thorac. Cardiovasc. Surg.* **51**, 289–296 (2003).
67. Yang, X.-H. Risk factor analysis for brain metastasis after radical resection of colorectal cancer within 5 years. *Acad. J. Second Mil. Med. Univ.* 993–996 (2017).
68. Naxerova, K. et al. Origins of lymphatic and distant metastases in human colorectal cancer. *Science* **357**, 55–60 (2017).
69. Sun, J. et al. Genomic signatures reveal DNA damage response deficiency in colorectal cancer brain metastases. *Nat. Commun.* **10**, 3190 (2019).
70. Roussille, P. et al. Pathological and molecular characteristics of colorectal cancer with brain metastases. *Cancers* **10**, 504 (2018).
71. Harter, P. N. et al. Distribution and prognostic relevance of tumor-infiltrating lymphocytes (TILs) and PD-1/PD-L1 immune checkpoints in human brain metastases. *Oncotarget* **6**, 40836–40849 (2015).
72. Kallen, M. E. & Hornick, J. L. The 2020 WHO classification: what's new in soft tissue tumor pathology? *Am. J. Surg. Pathol.* **45**, e1–e23 (2021).
73. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2020. *CA Cancer J. Clin.* **70**, 7–30 (2020).
74. von Mehren, M. et al. NCCN guidelines insights: soft tissue sarcoma, version 1. 2021. *J. Natl Compr. Cancer Netw.* **18**, 1604–1612 (2020).
75. Gronchi, A. et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **32**, 1348–1365 (2021).
76. Sobczuk, P. et al. Systemic treatment for advanced and metastatic malignant peripheral nerve sheath tumors—a sarcoma reference center experience. *J. Clin. Med.* **9**, 3157 (2020).
77. Keung, E. Z. et al. Correlative analyses of the SARC028 trial reveal an association between sarcoma-associated immune infiltrate and response to pembrolizumab. *Clin Cancer Res.* **26**, 1258–1266 (2020).
78. Fazel, M. et al. Immunotherapy for soft tissue sarcomas: anti-PD1/PDL1 and beyond. *Cancers* **15**, 1643 (2023).
79. Delmas, D., Hermetet, F. & Aires, V. PD-1/PD-L1 checkpoints and resveratrol: a controversial new way for a therapeutic strategy. *Cancers* **13**, 4509 (2021).
80. Schiwitza, A. et al. Monitoring efficacy of checkpoint inhibitor therapy in patients with non-small-cell lung cancer. *Immunotherapy* **11**, 769–782 (2019).
81. Eskander, R. N. et al. Pembrolizumab plus chemotherapy in advanced endometrial cancer. *N. Engl. J. Med.* **388**, 2159–2170 (2023).
82. Linhares, A. D. S. et al. Therapeutic PD-L1 antibodies are more effective than PD-1 antibodies in blocking PD-1/PD-L1 signaling. *Sci. Rep.* **9**, 11472 (2019).
83. Herbst, R. S. et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **515**, 563–567 (2014).
84. Waldman, A. D., Fritz, J. M. & Lenardo, M. J. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat. Rev. Immunol.* **20**, 651–668 (2020).
85. Grosso, J. F. & Jure-Kunkel, M. N. CTLA-4 blockade in tumor models: an overview of preclinical and translational research. *Cancer Immun* **13**, 5 (2013).
86. Tawbi, H. A. et al. Long-term outcomes of patients with active melanoma brain metastases treated with combination nivolumab plus ipilimumab (CheckMate 204): final results of an open-label, multicentre, phase 2 study. *Lancet Oncol* **22**, 1692–1704 (2021).
87. Zuccato, J. A. et al. Prediction of brain metastasis development with DNA methylation signatures. *Nat. Med.* <https://doi.org/10.1038/s41591-024-03286-y> (2024).
88. Andrews, L. P., Marciscano, A. E., Drake, C. G. & Vignali, D. A. LAG3 (CD223) as a cancer immunotherapy target. *Immunol. Rev.* **276**, 80–96 (2017).
89. Avice, M. N., Sarfati, M., Triebel, F., Delespesse, G. & Demeure, C. E. Lymphocyte activation gene-3, a MHC class II ligand expressed on activated T cells, stimulates TNF-alpha and IL-12 production by monocytes and dendritic cells. *J. Immunol.* **162**, 2748–2753 (1999).
90. Serrati, S. et al. Circulating extracellular vesicles expressing PD1 and PD-L1 predict response and mediate resistance to checkpoint inhibitors immunotherapy in metastatic melanoma. *Mol Cancer* **21**, 20 (2022).
91. Goldberg, S. B. et al. Pembrolizumab for management of patients with NSCLC and brain metastases: long-term results and biomarker analysis from a non-randomised, open-label, phase 2 trial. *Lancet Oncol* **21**, 655–663 (2020).
92. Wasilewski, D. et al. Predictive role of intracranial PD-L1 expression in a real-world cohort of NSCLC patients treated with immune checkpoint inhibition following brain metastasis resection. *J Neurooncol* **167**, 155–167 (2024).
93. Mansfield, A. S. et al. Temporal and spatial discordance of programmed cell death-ligand 1 expression and lymphocyte tumor infiltration between paired primary lesions and brain metastases in lung cancer. *Ann. Oncol.* **27**, 1953–1958 (2016).
94. Ghiringhelli, F. et al. Immunoscore immune checkpoint using spatial quantitative analysis of CD8 and PD-L1 markers is predictive of the efficacy of anti-PD1/PD-L1 immunotherapy in non-small cell lung cancer. *EBioMedicine* **92**, 104633 (2023).
95. Nadal, E. et al. Phase II trial of atezolizumab combined with carboplatin and pemetrexed for patients with advanced nonsquamous non-small-cell lung cancer with untreated brain metastases (Atezo-Brain, GECP17/05). *J. Clin. Oncol.* **41**, 4478–4485 (2023).
96. Woo, S. R. et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res.* **72**, 917–927 (2012).
97. Long, G. V. et al. Combination nivolumab and ipilimumab or nivolumab alone in melanoma brain metastases: a multicentre randomised phase 2 study. *Lancet Oncol.* **19**, 672–681 (2018).
98. Ciriello, G. et al. Cancer evolution: a multifaceted affair. *Cancer Discov.* **14**, 36–48 (2024).
99. Jin, M. et al. PD-1/PD-L1 immune checkpoint blockade in breast cancer: research insights and sensitization strategies. *Mol. Cancer* **23**, 266 (2024).
100. Liu, H. et al. Molecular understanding and clinical aspects of tumor-associated macrophages in the immunotherapy of renal cell carcinoma. *J. Exp. Clin. Cancer Res.* **43**, 242 (2024).
101. Haslauer, T., Greil, R., Zaborsky, N. & Geisberger, R. CAR T-cell therapy in hematological malignancies. *Int. J. Mol. Sci.* **22**, 8996 (2021).
102. Nezhad, M. S., Abdollahpour-Alitappeh, M., Rezaei, B., Yazdanifar, M. & Seifalian, A. M. Induced Pluripotent Stem Cells (iPSCs) provide a potentially unlimited T cell source for CAR-T cell development and off-the-shelf products. *Pharm. Res.* **38**, 931–945 (2021).
103. Themeli, M. et al. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. *Nat. Biotechnol.* **31**, 928–933 (2013).
104. Mishra, H. K. & Kalyuzhny, A. Revolutionizing cancer treatments through stem cell-derived CAR T cells for immunotherapy: opening new horizons for the future of oncology. *Cells* **13**, 1516 (2024).
105. Priceman, S. J. et al. Regional delivery of chimeric antigen receptor-engineered T cells effectively targets HER2(+) breast cancer metastasis to the brain. *Clin. Cancer Res.* **24**, 95–105 (2018).

106. Sirini, C., De Rossi, L., Moresco, M. A. & Casucci, M. CAR T cells in solid tumors and metastasis: paving the way forward. *Cancer Metastasis Rev.* **43**, 1279–1296 (2024).
107. Walker, A. J. et al. Tumor antigen and receptor densities regulate efficacy of a chimeric antigen receptor targeting anaplastic lymphoma kinase. *Mol. Ther.* **25**, 2189–2201 (2017).
108. Martinez, M. & Moon, E. K. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. *Front. Immunol.* **10**, 128–128 (2019).
109. Ahmed, N. et al. HER2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma: a phase 1 dose-escalation trial. *JAMA Oncol.* **3**, 1094–1101 (2017).
110. Niu, L. et al. Case report: brain metastasis necrosis with immune checkpoint inhibitors plus chemotherapy for advanced non-small cell lung cancer. *Front. Immunol.* **13**, 1064596 (2022).
111. Zou, Y. et al. Discordance of immunotherapy response predictive biomarkers between primary lesions and paired metastases in tumours: a systematic review and meta-analysis. *EBioMedicine* **63**, 103137 (2021).
112. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **175**, 998–1013.e20 (2018).
113. Skoulidis, F. et al. STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov.* **8**, 822–835 (2018).
114. Braun, D. A. et al. Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. *Nat. Med.* **26**, 909–918 (2020).
115. Patel, S. J. et al. Identification of essential genes for cancer immunotherapy. *Nature* **548**, 537–542 (2017).
116. Van Woensel, M. et al. Sensitization of glioblastoma tumor microenvironment to chemo- and immunotherapy by Galectin-1 intranasal knock-down strategy. *Sci. Rep.* **7**, 1217 (2017).
117. Ready, N. E. et al. First-line nivolumab plus ipilimumab for metastatic non-small cell lung cancer, including patients with ECOG performance status 2 and other special populations: CheckMate 817. *J. Immunother Cancer* **11** (2023).
118. Reck, M. et al. Systemic and intracranial outcomes with first-line nivolumab plus ipilimumab in patients with metastatic NSCLC and baseline brain metastases from CheckMate 227 Part 1. *J. Thorac. Oncol.* **18**, 1055–1069 (2023).
119. Carbone, D. et al. OA09.01 first-line nivolumab + ipilimumab + chemo in patients with advanced NSCLC and brain metastases: results from CheckMate 9LA. *J. Thorac. Oncol.* **16**, S862 (2021).
120. Hou, X. et al. Efficacy, safety, and health-related quality of life with camrelizumab plus pemetrexed and carboplatin as first-line treatment for advanced nonsquamous NSCLC with brain metastases (CAP-BRAIN): a multicenter, open-label, single-arm, phase 2 study. *J. Thorac. Oncol.* **18**, 769–779 (2023).
121. Powell, S. F. et al. Outcomes with pembrolizumab plus platinum-based chemotherapy for patients with NSCLC and stable brain metastases: pooled analysis of KEYNOTE-021, -189, and -407. *J. Thorac. Oncol.* **16**, 1883–1892 (2021).
122. Ozguroglu, M. et al. Cemiplimab monotherapy as first-line (1L) treatment of patients with brain metastases from advanced non-small cell lung cancer (NSCLC) with programmed cell death-ligand 1 (PD-L1)  $\geq 50\%$ : EMPOWER-Lung 1 subgroup analysis. *J. Clin. Oncol.* **39**, 9085–9085 (2021).
123. Mansfield, A. S. et al. Outcomes with pembrolizumab monotherapy in patients with programmed death-ligand 1-positive NSCLC with brain metastases: pooled analysis of KEYNOTE-001, 010, 024, and 042. *JTO Clin. Res.* **2**, 100205 (2021).
124. Wang, J. et al. Adebrelimab or placebo plus carboplatin and etoposide as first-line treatment for extensive-stage small-cell lung cancer (CAPSTONE-1): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* **23**, 739–747 (2022).
125. Huang, Y. et al. Tiselizumab plus chemotherapy in patients with advanced brain metastases of non-squamous non-small cell lung cancer: a multicenter, prospective, open-label phase 2 study. *J. Clin. Oncol.* **41**, 9080–9080 (2023).
126. Masini, C. et al. Nivolumab in combination with stereotactic body radiotherapy in pretreated patients with metastatic renal cell carcinoma. results of the phase II NIVES Study. *Eur Urol.* **81**, 274–282 (2022).
127. Emamekhoo, H. et al. Safety and efficacy of nivolumab plus ipilimumab in patients with advanced renal cell carcinoma with brain metastases: CheckMate 920. *Cancer* **128**, 966–974 (2022).
128. Wong, P. et al. Phase II multicenter trial combining nivolumab and radiosurgery for NSCLC and RCC brain metastases. *Neurooncol Adv.* **5**, vdad018 (2023).
129. Margolin, K. et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. *Lancet Oncol.* **13**, 459–465 (2012).
130. Di Giacomo, A. M. et al. Three-year follow-up of advanced melanoma patients who received ipilimumab plus fotemustine in the Italian Network for Tumor Biotherapy (NIBIT)-M1 phase II study. *Ann Oncol.* **26**, 798–803 (2015).
131. Goldberg, S. B. et al. Pembrolizumab for patients with melanoma or non-small-cell lung cancer and untreated brain metastases: early analysis of a non-randomised, open-label, phase 2 trial. *Lancet Oncol.* **17**, 976–983 (2016).
132. Kluger, H. M. et al. Long-term survival of patients with melanoma with active brain metastases treated with pembrolizumab on a phase II trial. *J. Clin. Oncol.* **37**, 52–60 (2019).
133. Long, G. V. et al. 1311O - Long-term outcomes from the randomized phase II study of nivolumab (nivo) or nivo+ipilimumab (ipi) in patients (pts) with melanoma brain metastases (mets): Anti-PD1 brain collaboration (ABC). *Ann. Oncol.* **30**, v534 (2019).

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## Author contributions

M.A. conceptualized and wrote the manuscript. L.P. and N.K. reviewed the manuscript and provided detailed revisions. Figures 1 and 2 in this manuscript were created and customized specifically for this topic by co-author and biomedical illustrator NK. These figures, including all their elements, were designed using Adobe Illustrator (version 2024) and are copyrighted by NK (all rights reserved). No elements of these figures were adapted from or sourced from other works, and therefore, no additional permissions are required. The figures have been properly credited in their respective captions. All authors have revised, read, and agreed to the final version of the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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