Development of immunotherapy for brain metastasis (Review)

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Received January 30, 2020; Accepted June 22, 2020

DOI: 10.3892/ijo.2020.5091

Abstract. Brain metastasis (BM) is associated with a poor prognosis, with the typical overall survival rate ranging from weeks to months in the absence of treatment. Although the concept of immune privilege in the central nervous system has eroded over time, the advent of immunotherapy has opened a new set of potential therapeutic options for patients with BM. Recently, immunotherapy has been demonstrated to confer survival advantages to patients with multiple malignancies commonly associated with BMs. Data from a number of clinical trials have demonstrated that immune checkpoint inhibitors are effective for patients with BM. In addition, cellular therapies, including the application of chimeric antigen receptors T-cell therapy and dendritic cell vaccine, have also been utilized in the treatment of BM. In the present review, preclinical and clinical evidence supporting the applicability of immunotherapy for the treatment of BMs from melanoma, non-small cell lung cancer (NSCLC) and renal cell carcinoma (RCC) were examined, where the challenges and safety of this treatment modality were also discussed.

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Key words: immunotherapy, brain metastasis, immune checkpoint inhibitors, cellular therapy, review

1. Introduction

Brain metastases (BMs) occur in ≤20% adults with systemic malignancies, which represent a significant clinical challenge (1,2). Patients who develop BMs have a poor prognosis, with an average survival rate of <6 months (3). Although surgery, chemotherapy and radiotherapy (RT) represent the mainstay of treatment options for patients with BMs (4), such treatments are rarely curative and result in substantial toxicity owing to the sensitivity of the brain (5).

T-cell immunotherapy has demonstrated promising early results in the treatment of patients with BM, which has challenged the traditional paradigm that the brain is an organ with immune privilege (2). A number of approaches have been applied to stimulate or enhance endogenous T-cell immune responses for brain tumors or BMs, including tumor neoantigen vaccines (6), chimeric antigen receptor (CAR) T-cells (7) and immune checkpoint inhibitors (ICIs) (8,9). Among these, ICIs have become the protagonist in anticancer immunotherapy. The most clinically relevant ICIs are those targeting the programmed cell death protein 1 (PD-1) and its ligand programmed cell death ligand (PD-L)1, in addition to cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). CTLA-4 and PD-1 are both the members of the B7/CD28 family; however, their inhibitory effects are mediated via different pathways. The blockade of CTLA-4 prevents the reception of inhibitory signals from B7 during the priming phase of T cell activation. By contrast, anti-PD-1 therapies block the negative regulation of cytotoxic T lymphocytes during the effector phase by preventing their interaction with PD-L1 and PD-L2 (10-12). Clinical studies have previously suggested that ICIs are effective in improving the overall survival (OS) in patients with BMs (8,9), though these observations were never confirmed by large-scale prospective phase III clinical studies. Additionally, the engineering of new T-cell immunity using CARs is a cancer treatment strategy that is rapidly advancing in this field (13,14). For patients with brain tumors or BMs, preliminary results with CAR T-cell therapy have also shown promise.

Therefore, in the present review, existing preclinical and clinical evidence that supports the applicability of immunotherapy for the treatment of BMs, including that the use of ICIs and cell immunotherapy, were enumerated. Additionally,

the challenges and safety of this treatment modality were discussed.

2. Biology of brain metastases

The brain was previously considered to be an organ with immune privilege that is excluded from systemic immune surveillance (10). The presence of the blood-brain-barrier (BBB) and the absence of lymphatic vessels, which serve as a highway for antigen presenting cells (APC), may have contributed to this concept (4).

In the healthy brain, specialized tight junctions between endothelial cells in the BBB impede communication with the circulatory system (15), restricting the entry of immune cells from the peripheral circulation. However, it has recently been revealed that resting T-cells can migrate from the meningeal blood vessels into the cerebrospinal fluid (CSF) (16). In addition, under various pathophysiological conditions, the integrity of the BBB becomes compromised. The increased permeability of the BBB in association with pathologically impaired microvessels has been previously observed (17). Activated circulating $CD4^+$ T-cells have been demonstrated to cross the BBB and induce local T cell activation (18). Previously considered to be devoid of lymphatic vessels, a lymphatic system within the central nervous system (CNS) was discovered in 2015 (19). These findings demonstrate that T-cells can in principle cross the BBB where they can serve a role in BMs, which have been reported by previous preclinical studies (20,21).

To date, several studies have demonstrated that large quantities of tumor infiltrating lymphocytes (TILs) can be found in BMs of different primary malignancies. TIL subsets of 116 BM specimens were investigated in a study previously conducted by Berghoff *et al* (22) using immunohistochemistry, which revealed that $CD3^+$ and $CD8^+$ TILs were present in 115/116 (99.1%) and 112/116 (96.6%) of the tumor specimens, respectively (22). Furthermore, 19/67 (28.4%) of the specimens evaluated for PD-L1 expression had shown >5% membranous expression (22). In another study, Kluger *et al* previously demonstrated that greater $CD8^+$ T cell infiltration was associated with the delayed onset of melanoma brain metastasis (MBM) and an improved survival (23). Therefore, the observed concordance of a high TIL density with an improved OS supports the application of immunotherapy in treating patients with BMs (24).

Collectively, these aforementioned studies suggest that T-cells can move from the meningeal blood vessels into the CSF and cross the BBB in the presence of BMs. Significant TILs have been observed in BMs of different primary cancers, where high TIL density modulates the mode of immune response elicited by metastatic brain tumor cells in conjunction with other resident cell types in the brain.

3. Evidence of treatment with ICIs for patients with BMs

Immune checkpoint proteins, such as PD-1 and CTLA-4, are co-receptors that promote tumor cell survival through immune evasion. PD-1 is a receptor that is expressed on the surfaces of T-cells that binds to PD-L1 and PD-L2 expressed on (APCs) or tumor cells, in turn reducing T-cell activity and subsequently limiting cancer cell elimination (25). CTLA-4 is an inhibitory receptor that interacts with human leukocyte antigen (HLA)-B7-1 and HLA-B7-2 on T-cells to inhibit the initial stages of T-cell activation (2). ICIs were designed to interrupt these immunosuppressive processes and reinvigorate antitumor immune responses (26). Previous clinical trials performed, including that of EORTC 18071 (27), KEYNOTE-001 (28) and CheckMate 057 (29), revealed that using CTLA-4-based or PD-1-based ICIs can improve the survival of patients with various types of cancer, including melanoma, non-small cell lung cancer (NSCLC) and Hodgkin's lymphoma (30-32). Notably, a number of clinical studies have also been conducted on patients with BMs, which have demonstrated promising results.

Clinical data on ICIs for the treatment of BMs. There are numerous studies available that have retrospectively investigated patients with BMs who have been treated with ICIs. Among these, the largest study was conducted by Iorgulescu *et al* (8), which used the National Cancer Database to analyze data from 220,439 patients diagnosed with melanoma from 2010 to 2015. They found that ICIs could improve OS to 12.4 months, compared with 5.2 months for patients who did not receive ICIs. In addition, single-institution studies have also investigated the efficacy of ICIs for patients with BMs. In one such study, 128 patients with BMs secondary to NSCLC, renal cell carcinoma (RCC) and melanoma were included, where they were treated with ICIs. The 1-year survival rates for patients with NSCLC, melanoma and RCC were found to be 48.3, 54.5 and 55.4%, respectively (2). Lanier *et al* performed a retrospective study consisting of 271 patients with BMs who were treated with stereotactic radiosurgery (SRS) (33). Of these patients, the median OS of the 101 (37%) who received immunotherapy was 15.9 months, whilst that of the 170 (63%) who did not receive immunotherapy was 6.1 months (33).

By contrast, the number of prospective clinical trials in this field remains limited. Patients with untreated or active BMs have always been excluded from clinical trials, as in the majority of cases, the management of BM requires supportive approaches, such as corticosteroids to relieve intracranial pressure (34). Table I outlines a subset of prospective trials that investigated the use of ICIs for the treatment of BMs.

The first prospective clinical trial that specifically investigated the use of ICIs in BMs was a phase II trial (trial no. NCT00623766) conducted by Margolin *et al* (35). The study enrolled 72 patients with both symptomatic and asymptomatic MBMs, where all patients received 4 doses of ipilimumab, an anti-CTLA4 monoclonal antibody. After 12 weeks, 9/51 (18%) of the patients in the asymptomatic group exhibited disease control, compared with 1/21 patients (5%) in the symptomatic group (35). Ipilimumab appears to be more effective for patients with asymptomatic BMs. Another previous phase II study (NIBIT-M1) included 20 patients with asymptomatic BMs for investigating the efficacy of ipilimumab combined with fotemustine, which demonstrated that two patients achieved intracranial complete responses (CR) and 10/20 patients achieved disease control (36). Additionally, the median OS was 12.7 months, demonstrating the long-term efficacy of this combination therapy in patients with BMs (9). An expanded access program (EAP) in Italy analyzed 146 patients

Table I. Selected studies evaluating immune checkpoint inhibitors in brain metastases.

Authors (Refs.)	NTC no.	Phase	No. of patients	ICI target	Tumor type	Treatment group	Intracranial control rate	Median OS	Median PFS
Margolin *et al* (35)	00623766	II	72	CTLA-4	Melanoma	Ipilimumab alone i) asymptomatic ii) symptomatic	i) 24% ii) 10%	i) 7.0 months ii) 3.7 months	i) 1.9 months ii) 1.2 months
Di Giacomo *et al* (9,36)	01654692 (NIBIT-M1)	II	20*	CTLA-4	Melanoma	Ipilimumab plus fotemustine	50%	12.7 months	3.0 months
Goldberg *et al* (38)	02085070	II	36	PD-1	Melanoma and NSCLC	Pembrolizumab i) Melanoma ii) NSCLC	i) 22% ii) 33%	i) 17.0 months ii) NR	i) 2.0 months ii) NR
Tawbi *et al* (46)	02320058	II	90	CTLA-4/PD-1	Melanoma	Nivolumab plus ipilimumab	56%	6 months OS rate 92.3%	6 months PFS rate 64.2%
Long *et al* (42)	02374242	II	66	CTLA-4/PD-1	Melanoma	i) Asymptomatic treatment naïve treated with nivolumab plus ipilimumab ii) Asymptomatic treatment naïve with nivolumab iii) Symptomatic patients or failed previous local	i) 44% ii) 20% iii) 6%	6-month PFS rate i) 50% ii) 29% iii) 0%	NR
Gandhi *et al* (40)	02578680	III	108*	PD-1	NSCLC	i) Pembrolizumab plus chemotherapy ii) placebo plus chemotherapy	NR	NR	NR
Gadgeel *et al* (45)	02008227	III	123*	PD-1	NSCLC	i) Atezolizumab ii) docetaxel	NR	i) 16.0 months ii) 11.9 months	NR
Williams *et al* (63)	01703507	I	16	CTLA-4	Melanoma	Ipilimumab plus i) WBRT ii) SRS	7% for entire cohort	i) 8.0 months ii) NR	i) 2.5 months ii) 2.1 months

Number of patients who began trial with brain metastases in a large phase II/III study; NR, no report; OS, overall survival; PFS, progression-free survival; ICI, immune checkpoint inhibitor; CTLA-4, cytotoxic T lymphocyte-associated protein 4; PD-1, programmed cell death protein 1; NSCLC, non-small cell lung cancer; WBRT, whole brain radiotherapy; SRS, stereotactic radiosurgery.

with MBMs who were treated with ipilimumab (37). That study found that 4 patients achieved CR and 13 patients had a partial response (PR) yielding a disease control rate of 27%. In terms of prognosis, ~20% of the patients were alive 1 year after the initiation of ipilimumab treatment, where the median progression-free survival (PFS) and OS were revealed to be 2.8 and 4.3 months, respectively (37).

The first anti-PD-1 monoclonal antibody that clearly demonstrated efficacy against untreated BMs was pembrolizumab created by Merck & Co Inc. Patients with melanoma or NSCLC with measurable BMs (>5-20 mm) were enrolled into a phase II study to evaluate the efficacy of pembrolizumab in both cohorts (trial no. NCT02085070) (38). Brain metastasis responses were achieved in 4 of 18 (22%) patients with melanoma and 6 of 18 patients (33%) with NSCLC, according to the interim analysis of this study (38). The final results of the full melanoma cohort revealed that 26% patients exhibited a BM response, where the median PFS and OS were 2 and 17 months, respectively. The 2-year survival rate was similar compared with that in patients without BMs treated with the anti-PD-1 antibodies, suggesting that pembrolizumab is effective for treating MBMs with acceptable toxicity (39). The final results of the full NSCLC cohort of that study have not yet been reported.

Additional data supporting the use of pembrolizumab for untreated BMs are derived from a study funded by Merck & Co Inc. (KEYNOTE-189). KEYNOTE-189 (trial no. NCT02578680) compared the efficacy of pembrolizumab combined with chemotherapy and chemotherapy alone in patients with metastatic NSCLC, including 109 patients with BMs (17.5%). In the subgroup analysis of the BM patients, the 73 patients treated with combined pembrolizumab + chemotherapy exhibited markedly longer OS compared with the 35 patients treated with chemotherapy alone (40).

Similar to pembrolizumab, nivolumab has also demonstrated efficacy for untreated BMs. In a pool analysis of data from the CheckMate 017 (trial no. NCT01642004), CheckMate 057 (trial no. NCT01673867) and CheckMate 063 (trial no. NCT01721759) studies, patients with BM treated with nivolumab showed a longer median OS (8.4 months) compared with those treated with docetaxel (6.2 months) (41). In another phase II ABC study (trial no. NCT02374242) conducted previously, 27 patients with asymptomatic MBMs were treated with nivolumab alone (42). At the data cutoff, the intracranial response rate of this population was found to be 20%, where the median intracranial PFS and OS were calculated to be 2.5 and 18.5 months, respectively (42). In addition, an EAP in Italy included 409 patients with asymptomatic BMs associated with NSCLC, who were treated with nivolumab (43). The disease control rate of these patients was revealed to be 39%, which included 4 and 64 patients achieving CRs and PRs, respectively. The median OS was 8.6 months. The results of this Italian EAP suggested that nivolumab is effective in patients with BMs associated with NSCLC (44). These observations regarding nivolumab treatment were consistent with another Italian EAP previously conducted, which included 389 patients with RCC, 32 of whom had BMs. The intracranial response rate of the BM patients was 18.7% with a disease control rate of 53.1%, demonstrating that patients with BM-associated RCC may also benefit from nivolumab application (44).

The efficacy of atezolizumab, an inhibitor of PD-L1, in patients with and without a history of BMs was previously evaluated in the OAK trial (trial no. NCT02008227) (45). In patients with a history of BMs, median OS was longer in patients treated with atezolizumab compared with those treated with docetaxel (16.0 vs. 11.9 months). Furthermore, patients with asymptomatic BMs treated with atezolizumab had a lower probability of developing new symptomatic brain lesions compared with those treated with docetaxel (45).

The possibility of combining the two ICIs for the treatment of BMs has also been evaluated in several phase I/II clinical trials. CheckMate-204 (NCT02320058) is a phase II study combining nivolumab and ipilimumab to treat MBMs (46). Subsequent analysis revealed a 52% intracranial response rate, including 24 (26%) CRs. The intracranial clinical benefit rate was 57% (46), concordant with extracranial activity. A similar result was found in the phase II ABC study (trial no. NCT02374242) aforementioned (42). The intracranial response rate in patients treated with the nivolumab + ipilimumab combination was 46%, which was notably higher compared with those treated with nivolumab alone (20%). Following a median follow-up of 14 months, both median PFS and median OS were not reached in the combination group (42). These results indicate that the combination of these two kinds of ICIs may confer superior efficacy compared with the administration of either ICI alone, where the combination therapy can be considered as the first-line therapeutic option for patients with BM.

Overall, the data reviewed as aforementioned demonstrate that the use of ICIs has significant utility for the treatment of BMs. However, the majority of these trials were early clinical studies with small sample sizes. Larger studies will be required to verify the observations of these early findings. The majority of clinical trials had enrolled patients with BM who had at least one target intracranial lesion. Thus, the treatment strategies of immunotherapy for isolated and multiple BMs are the same currently. Further studies are warranted to determine the difference between isolated and multiple BMs treatment. Nevertheless, due to previous findings that treatment with ICIs combined with other therapies has demonstrated promising efficacy for solid tumors, this intervention strategy should also be considered for BMs.

Integration of radiation therapy with ICIs for the treatment of BMs. RT is currently the standard therapeutic intervention option for patients with non-resectable BMs. Whole brain radiotherapy (WBRT) and SRS are two of the most important forms of RT. Conventionally, WBRT is prescribed for patients with multiple BMs, poor prognosis and/or poor performance status (PS), whilst SRS would be considered for patients with fewer numbers of BMs and those with good PS (47). Since SRS can achieve high local tumor control without affecting healthy brain tissues, it is associated with a reduced risk of severe side-effects compared with WBRT, including neurocognitive damage and hair loss (48).

The integration of RT with the ICIs has been investigated in patients with BMs in previous studies. The rationale for combining ICIs with RT may be derived from the previously observed effects of RT on the immune system. RT has been demonstrated to induce tumor cell death (49), resulting in the

release of tumor-associated antigens (50), which activate APCs to then prime cytotoxic T-cells to kill tumor cells at distant locations (51,52). RT has also been shown to upregulate major histocompatibility complex (MHC) I expression on tumor cells (53). Previous studies have reported that RT can alter the tumor environment, induce the secretion of proinflammatory chemotactic factors by cancer cells that mediate strong immunostimulatory effects (54,55) and even upregulate PD-L1 expression in tumor cells (56), which could in theory enhance the antitumor effect of anti-PD-L1 antibodies (57).

Numerous previous studies have investigated the efficacy and safety of combining ICIs and RT for patients with BM. In particular, two similar studies retrospectively evaluated the outcomes of 46 patients with MBMs, who were treated with ipilimumab and SRS, where both revealed improved responses when SRS was administered concurrently with ipilimumab (58,59). Diao *et al* (60) conducted a single-institution study that retrospectively enrolled 72 patients with MBMs and found that the supplementation of ipilimumab with SRS not only improved tumor response, but also reduced edema volume. Furthermore, Acharya *et al* (61) demonstrated in another study that SRS treatment in combination with ICIs, regardless of whether ipilimumab or an anti-PD-1 agent was used, was associated with reduced local and distant intracranial failure compared with patients with MBMs treated with SRS alone.

In general, concurrent therapy, including RT and ICIs appears to be a promising treatment strategy for patients with BMs. However, the type of ICIs that results in the optimal treatment outcome when combined with RT has not been researched in detail. Choong *et al* (62) previously analyzed 108 patients with MBMs who were treated with SRS combined with various therapies, including anti-CTLA-4 (n=28), anti-PD-1 (n=11) and the B-Raf proto-oncogene inhibitor (n=39). The median OS for patients treated with the anti-PD-1 agent + SRS (27.4 months) was found to be markedly higher compared with that for patients treated with anti-CTLA-4 agents + SRS (7.5 months). However, due to the number of patients treated with this type of immunotherapy being relatively small, the results of that study may not be representative of all patients with BMs.

The only prospective evidence of this modality of treatement comes from a phase I study conducted at Thomas Jefferson University (trial no. NCT01703507) (63). That study enrolled 16 patients to receive WBRT + ipilimumab (n=5) or SRS + ipilimumab (n=11) treatments, selected based on the intracranial disease burden. The results revealed that although concurrent treatment with ipilimumab + SRS or WBRT was well-tolerated, the efficacy of this combination treatment was not as expected, as 14/16 either exhibited disease progression and/or did not survive during the follow-up period. This could be due to the nature of phase I studies, in which treatment safety took precedence over efficacy as the primary endpoint of that study. Additionally, insufficient numbers of patients for the assessment of efficacy may have contributed to these unexpected results.

Following a review of the previous aforementioned studies, the concurrent use of combined RT and ICIs in treating BMs remains at an exploratory stage, as the efficacy of this combination has not yet been confirmed by a prospective phase II/III study. Furthermore, the optimal timing at which radiotherapy and immunotherapy should be administered is also a question that remains to be resolved. Although several retrospective studies (58,59) have suggested that the concurrent application of RT and ICIs is associated with an improved OS compared with either treatment alone, the definition of 'concurrent' remains controversial. This is due to the fact that certain studies have reported this term to be the administration of ICIs as short as 2 weeks before or after RT (64), whilst others even extended this period to 4 weeks (65), even to as long as 5.5 months (66). However, a number of prospective trials are planned or are already underway to validate the use of ICIs combined with radiotherapy for the treatment of BMs, which should hopefully provide evidence to support the efficacy of this type of combinatorial therapy.

Safety of ICIs for the treatment of BMs. Immune-related adverse events (irAEs) are a series of inflammatory side effects caused by immunotherapy, most commonly affecting organs, including the gastrointestinal tract, endocrine glands and liver (67). In addition, the CNS can also be affected (68). Previous clinical trials have reported that irAEs of any grade can occur in 13-65% patients who are treated with anti-PD-1 antibodies or ipilimumab monotherapy (36,69-73). Among these, neurological adverse events (AEs) caused by ICIs, including forms of hypophysitis, encephalitis, demyelinating polyneuropathy and encephalomyelitis, occur in ~1% patients.

For patients with BMs, clinical data have demonstrated that the safety of ICIs was similar to that reported in patients with malignant tumors who did not have BMs (46). Furthermore, the most common AEs found were mainly systemic, including fatigue, diarrhea, headache and pruritus. In a clinical trial investigating the use of ipilimumab for the treatment of MBMs, the most commonly observed neurological AEs were grade 1/2 headache and dizziness, with causes possibly related to ipilimumab treatment (35). In another clinical study, safety analysis of the results from a phase II study using pembrolizumab to treat patients with BMs revealed that neurological AEs occurred in 65% patients, with the most common neurological AEs being grade 1/2 gait disturbance (22%) and headache (17%) (39).

The nivolumab/ipilimumab combination appears to demonstrate a higher degree of toxicity compared with either nivolumab or ipilimumab alone. In a phase II ABC study, 63% patients receiving the nivolumab/ipilimumab combination presented with AEs that were grade 3 or higher. By contrast, in patients receiving nivolumab alone, only 16% patients reported such AEs (42). Safety data from the CheckMate-204 trial revealed that grade 3/4 AEs occurred in 55% patients treated with combination therapy, including a patient who was afflicted with terminal immune-related myocarditis. Additionally, 8% patients experienced neurological AEs, half of which had to subsequently discontinue treatment as a result of the neurological AEs (46).

A common concern that is raised when combining ICIs and RT is the occurrence of possiby elevated or unexpected toxicity. Available clinical data suggest a weak association between the addition of brain radiotherapy to ICIs and the incidence or severity of irAEs. A recent large-scale retrospective study reported the outcomes of 260 patients with BMs who had

received SRS and ICIs. That study demonstrated that the concurrent or sequential administration of SRS and ICIs did not increase acute toxicity. The rates of irAEs between patients who received concurrent SRS and ICI or either alone were not found to differ significantly (64). Apart from irAEs, the addition of ICI to RT was observed to markedly increase toxicity associated with RT, particularly neurotoxicity. From the previous studies aforementioned, the observed toxicities that can be potentially associated with RT in combined RT and ICI therapy included radiation necrosis (RN), neurocognitive decline and intratumoral hemorrhage (74-77). Among these, the most common side-effect was RN. Colaco *et al* (78) previously evaluated the safety of radiosurgery combined with various systemic therapies, including chemotherapy, target therapy and/or ICIs, in 180 patients with BMs from a number of primary malignancies. That study found that ~37.5% patients who received ICIs developed RN, compared with 16.9% who received chemotherapy and 25.0% who received target therapy (78). In another study, Martin *et al* (79) evaluated the safety of 480 patients with BMs secondary to NSCLC, melanoma and RCC who were treated with SRS with/without ICIs. The results revealed that RN occurred in 20.0 and 6.85% patients who did and those who did not receive ICIs, respectively (79). These observations were consistent with those found in the studies conducted by Patel *et al* (80) and Kaidar-Person *et al* (81). These aforementioned studies raised significant concern that concurrent ICI and SRS treatment may increase the risk of RN. However, data from the subsequent studies did not exhibit the same trend, as neither Silk *et al* (82) nor Mathew *et al* (83) reported increased rates of RN in patients treated with the combination of RT and ipilimumab. Potential explanations for these disparities in results include the smaller cohort sizes among the studies and variations in the prescribed SRS doses, isodose line and number of fractions. Prospective studies that minimize these confounding factors are required to characterize the risks of combining RT and ICIs more comprehensively. The selected studies investigating the safety of ICI in BMs are presented in Table II.

Planned and ongoing clinical trials on ICIs on BMs. Although there is a paucity of prospective data validating the use of ICIs for the treatment of BMs, several prospective trials have been planned or are already underway (Table III). For example, NCT02460068 is a phase III trial that aims to investigate differences in the OS of patients with MBMs. Patients in that study will receive fotemustine, ipilimumab + fotemustine, or ipilimumab + nivolumab. In addition, NCT02681549 is a phase II study that will investigate the BM response rate in patients with BMs administered pembrolizumab and bevacizumab concurrently. NCT03903640 is a phase II study that will evaluate the efficacy of Optune, an FDA-approved device that delivers alternating electric fields to the tumor (84,85), in comparison with ICIs in patients with BMs. Additional evidence of the efficacy and safety of ICIs for the treatment of BMs will be acquired from these studies.

4. Cellular therapy for patients with BMs

CAR T-cell therapy. CAR T cell therapy is a promising approach in immunotherapy that has been considered for the treatment of brain tumors. Using this technique, CAR T-cell receptors are specifically engineered to eradicate tumors through the recognition of surface proteins expressed on tumor cells (5). The extracellular domain of the CAR is the antibody region, allowing for recognition of the specific antigen in a MHC-independent manner (86). Inside the cell, the CAR contains a co-stimulatory signaling domain that is important for antitumor activity in addition to CAR T-cell proliferation and persistence (87). First-generation CARs contained an antigen-binding domain that is directly fused to the intracellular portion of CD3ζ chain of the T-cell receptor complex. However, this design demonstrated minimal clinical success due to the very low levels of engraftment observed in patients (88,89). By contrast, second- and third-generation CARs included multiple co-stimulatory molecules from other receptors, including 4-1BB and CD28, in addition to the CD3ζ chain (5,90), which markedly increased the antitumor efficacy and persistence of the CAR T-cells.

The efficacy of CAR T-cells for the treatment of hematological malignancies is impressive, particularly for acute lymphoblastic leukemia (ALL), where a clinical response rate of 90% was observed (91). This has resulted in numerous clinical studies on generating CARs directed against various hematological antigens, including CD19, CD20 and CD22 (92). For solid tumors, candidate target antigens currently being investigated in clinical trials in this manner include mesothelin, carcinoembryonic antigen, interleukin 13 receptor α (IL-13Rα) and human epidermal growth factor receptor 2 (93). This modular design, which provides the flexibility for adjusting antigen recognition and signaling domains based on the targeted cancer types, is one of the advantages of CARs (5).

Lee *et al* (94) previously conducted a phase I study to investigate the safety and efficacy of CD19-CAR T cells in the treatment of pediatric patients with ALL, which found that CD19-CAR T cells could be detected in the CSF (94). In 2016, Brown *et al* (95) reported a patient with multifocal glioblastoma achieving a notable response after being administered with intraventricular infusions of IL-13Rα2 CAR T-cells (97). In addition, Abramson *et al* (96) described a patient with CNS diffuse large-B-cell lymphoma in 2017. After receiving CD19-CAR T-cell therapy, this patient achieved complete remission (96). These studies aforementioned suggest that systemically-administered CAR T-cells can reach the brain, which can be used in the treatment of brain tumors.

Initial clinical reports on the use of CAR T-cells for brain tumors have mainly focused on the treatment of recurrent or refractory glioblastoma. Several first-in-human studies CAR T-cell application in the treatment of glioblastoma have been published, including that of NCT00730613, which investigated the efficacy of intracranial IL13Rα2-CAR delivery on glioblastoma (97), NCT02209376, which determined the safety and efficacy of EGFRvIII CAR-T for patients with glioblastoma (98) and NCT01109095, which assessed the safety and activity of human epidermal growth factor receptor 2 (HER2) CAR-T in adult and pediatric glioblastoma (99).

Studies focusing on using CAR T-cells for the treatment of solid tumors that have metastasized to the brain remain scant according to the literature. A single study was found, which was a preclinical study conducted by Priceman *et al* (100).

Table II. Selected studies investigating the safety of immune checkpoint inhibitors (plus radiotherapy) in brain metastases.

Authors/(Refs.)	Study characteristics	Explore	Safety
Margolin *et al* (35)	Phase II study involving 72 patients with BMs from melanoma	Ipilimumab	There was no grade 4 irAEs and the most common grade 3 irAEs were diarrhoea and increased serum aspartate aminotransferase.
Kluger *et al* (39)	Phase II study involving 23 patients with BMs from melanoma	Pembrolizumab	The majority of AEs were grade 1 to 2. Grade 3 AEs affecting only one patient each included hepatitis, hyponatremia, acidosis, and rash. Most neurologic toxicities were grade 1 or 2
Long *et al* (42)	Phase II study involving 79 patients with BMs from melanoma	Nivolumab plus ipilimumab (cohort A) or nivolumab alone (cohort B)	Grade 3 AEs occurred in 19 (54%) patients in cohort A, 4 (16%) in cohort B, the most common of which were diarrhoea or colitis and hepatitis; 3 grade 4 events were all reported in cohort A and none was reported in cohort B.
Tawbi *et al* (46)	Phase II study involving 94 patients with BMs from melanoma	Nivolumab plus ipilimumab	Grade 3 to 4 AEs were reported in 55% of patients, the most common of which were increased serum alanine aminotransferase or aspartate aminotransferase. One patient died from immune-related myocarditis.
Williams *et al* (63)	Phase I study involving 16 patients with BMs from melanoma	WBRT vs. SRS + ipilimumab	A total of 21 grade 1-2 neurotoxic effects (headache, nausea, dizziness); no grade 4-5 toxicity or RN.
Chen *et al* (64)	Retrospective study involving 260 patients with BMs from melanoma, NSCLC, or RCC	SRS ± ICI (Ipilimumab, nivolumab or pembrolizumab)	Fatigue, headache, and nausea/vomiting occurred most commonly; there was no significant difference in rates of irAEs between patients who received concurrent or non-concurrent SRS and ICI
Colaco *et al* (78)	Retrospective study involving 180 patients with BMs from various tumor types	Radiotherapy + ICI, chemotherapy, and/or targeted therapy	In total, 37.5% of patients who received ICI developed RN, compared with 16.9% who received chemotherapy and 25.0% who received target therapy.
Martin *et al* (79)	Retrospective study involving 480 patients with BMs from melanoma, NSCLC, or RCC	SRS vs. SRS + ICI	Symptomatic RN in 7% of patients receiving SRS vs. 20% in patients receiving SRS + ICI.
Patel *et al* (80)	Retrospective study involving 54 patients with BMs from melanoma	SRS vs. SRS + Ipilimumab	RN in 21% of patients receiving SRS versus 30% of patients receiving SRS + ipilimumab; intratumoral hemorrhage in 14.7% of patients receiving SRS vs. 15.0% of patients receiving SRS + ipilimumab (P=1.00).
Kaidar-Person *et al* (81)	Retrospective study involving 58 patients with BMs from melanoma	SRS vs. SRS + ICI	RN in 0% of patients receiving SRS alone; RN in 28% of patients receiving SRS + ICI.

BMs, brain metastases; irAE, immune-related adverse events; WBRT, whole brain radiotherapy; SRS, stereotactic radiosurgery; RN, radiation necrosis; ICI, immune checkpoint inhibitor; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

Table III. Major ongoing clinical trials of immunotherapy (plus radiotherapy) in the treatment of brain metastases.

NCT no.	Phase	Tumor type	Country	Status	N (planned)	Arm(s)	Primary outcomes
02460068	III	Melanoma	Italy	Recruiting	168	Fotemustine alone Fotemustine plus ipilimumab Ipilimumab plus nivolumab	Overall survival
02681549	I	Melanoma NSCLCL	USA	Recruiting	53	Pembrolizumab plus bevacizumab	Brain metastasis response rate
01449279	I	Melanoma	USA	Active, not recruiting	20	Ipilimumab plus palliative radiation	Safety
02858869	I	Melanoma NSCLC	USA	Recruiting	30	Pembrolizumab plus SRS (30 Gy/5f) Pembrolizumab plus SRS (27 Gy/3f) Pembrolizumab plus SRS (18-21 Gy/1f)	Safety
02716948	I	Melanoma	USA	Recruiting	90	Nivolumab plus SRS	Safety
02696993	I/II	NSCLC	USA	Recruiting	88	Nivolumab plus SRS Nivolumab plus WBRT Nivolumab plus ipilimumab plus SRS Nivolumab plus ipilimumab plus WBRT	Recommended phase 2 dose; 4-month Intracranial progression free
02097732	II	Melanoma	USA	Active, not recruiting	40	Ipilimumab administrated before SRS Ipilimumab administrated after SRS	Local control rate
02978404	II	NSCLC/RCC	Canada	Recruiting	60	Nivolumab plus SRS	Progression-free survival
03340129	II	Melanoma	Australia	Recruiting	218	Nivolumab plus ipilimumab Nivolumab plus ipilimumab plus SRS	Neurological specific cause of death
03903640	II	Melanoma	USA	Recruiting	23	Optune + ipilimumab + nivolumab	Intracranial progression-free survival
03955198	II	Melanoma	France	Not yet recruiting	100	Radiotherapy alone Radiotherapy + durvalumab	Time to intracranial progression
03175432	II	Melanoma	USA	Recruiting	60	Atezolizumab + bevacizumab Atezolizumab + bevacizumab + cobimetinib	Objective intracranial response rate; Safety
03873818	I	Melanoma	USA	Active, not recruiting	10	Ipilimumab + pembrolizumab	Clinical benefit rate
03696030	I	HER2 positive tumor metastatic to the CNS	USA	Recruiting	39	HER2-CAR T-cells	Incidence of dose limiting toxicities
02442297	I	HER2 positive tumor metastatic to the CNS	USA	Recruiting	28	HER2-specific T-cells	Number of patients with dose limiting toxicity
03638765	I	Breast cancer NSCLC	USA	Not yet recruiting	24	Autologous dendritic cells (DCVax-Direct)	Safety

Table III. Continued.

NCT no.	Phase	Tumor type	Country	Status	N (planned)	Arm(s)	Primary outcomes
02808416	I	Any tumor	China	Active, not recruiting	10	DCs pulsed with mRNA-encoded tumor antigens	Safety and tolerability
01782274	II/III	Breast cancer	Russia	Enrolling by invitation	60	DCs plus allogeneic hematopoietic stem cells	All cause mortality
01782287	II/III	Lung cancer	Russia	Enrolling by invitation	60	DCs plus allogeneic hematopoietic stem cells	All cause mortality

WBRT, whole brain radiotherapy; SRS, stereotactic radiosurgery; DC, dendritic cell; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

They used human breast cancer xenograft models that had metastasized to brain and evaluated the efficacy of HER2-CAR T-cells, which demonstrated robust antitumor responses mediated by HER2 CAR T-cells following local intratumor or regional intraventricular delivery (100).

At present, several clinical trials aiming to investigate the efficacy of CAR T-cells in treating solid metastatic brain tumors are underway. For example, NCT03696030 is a phase I study that will investigate the possible side effects and find the optimal dose of HER2-CAR T-cells for treating 39 patients with solid tumors that spread to the brain or leptomeninges. Additionally, NCT02442297 is a study that will be conducted by Baylor College of Medicine. It plans to enroll patients with recurrent or refractory HER2-positive primary CNS tumors or HER2-positive solid tumors that metastasized to the CNS, following which the safety and efficacy of HER2-CAR T cells will be evaluated (Table III). For patients with BMs, the potential therapeutic effect of CAR T-cells will be elucidated in the future.

Dendritic cell (DC) immunotherapy. DCs are professional APCs of the immune system that can capture antigens in the periphery and present them to T-cells (101). DCs can also facilitate the killing of tumor cells without affecting normal cells. Therefore, DC-based immunotherapy has been proposed as a promising cancer treatment method in various types of malignancies, including brain tumors (102-104).

Numerous clinical studies have investigated the safety and efficacy of DC-based immunotherapy in patients with glioblastoma patients. Liau *et al* (105) treated 12 patients with glioblastoma using DC vaccines (105), where the patients received 3 biweekly injections of $\leq 1 \times 10^6$ DCs pulsed with 50-100 μg acid-eluted tumor peptides. These DC vaccinations were found to be well tolerated, where 6 patients developed measurable systemic antitumor CTL responses (105). Fadul *et al* (106) reported the outcomes of 10 patients with glioblastoma who were treated with tumor lysate-pulsed DCs (106). No severe adverse events were observed and the median OS was found to be 28 months for the vaccinated patients (106). Data involving patients with glioblastoma who were vaccinated with DCs utilizing specific tumor antigens have also been reported over the past number of years. Sampson *et al* (107) conducted a phase I dose escalation study to evaluate the safety and efficacy of DCs pulsed with an EGFRvIII peptide in patients with glioblastoma (107). Sakai *et al* (108) reported the results of 10 patients with recurrent malignant glioma who were treated with autologous DCs pulsed with peptides corresponding to the mutant Wilms' tumor 1 antigen. DCs pulsed with specific tumor antigens were demonstrated to be well tolerated, with promising results.

Although supportive data from clinical trials remain insufficient, a number of case studies have indicated the beneficial effects of DCs in patients with BMs. Laurell *et al* (109) conducted a phase I/II study to evaluate the ability of allogeneic DCs as immune enhancers for a patient with newly diagnosed metastatic RCC. In that study, a patient with brain and liver metastases was enrolled who responded well to the treatment, such that all brain and liver lesions completely disappeared (109). In another case study,

Karbach *et al* (110) reported a patient with BMs associated with melanoma treated with radiosurgery and autologous tumor lysate-loaded DCs, the patient remained in the CR status 10 years following treatment (110).

Several ongoing studies will provide additional information on the effects of DCs on patients with BMs. NCT03638765 is a phase I study that will aim to evaluate the efficacy of DCVax-Direct, which is an autologously activated DC designed for intratumoral injection, in 24 patients with unresectable BMs from breast cancer and NSCLC. NCT02808416 is a phase I study that will to treat patients with BMs using personalized cellular tumor vaccines. In total, ~10 patients will be immunized with DCs pulsed with mRNA-encoded tumor antigens. NCT01782274 and NCT01782287 are 2 clinical studies that will investigate the efficacy of proteome-based immunotherapy for treating breast and lung cancer BMs (Table III). In these 2 studies, a cohort of patients will receive a combination of the DC vaccine, allogeneic hematopoietic stem cells and cytotoxic lymphocytes, whilst the other cohort will be treated with either the DCs vaccine, autologous hematopoietic stem cells or cytotoxic lymphocytes alone.

5. Conclusions

Metastatic brain cancer involves several types of tumors and is typically associated with a poor prognosis. Since the concept of immune privilege in the CNS has been eroded, the advent of immunotherapy has opened a new avenue of potential therapeutic options for the treatment of patients with BMs. Although the BBB remains to be a significant obstacle, activated T cells can circumvent this barrier. Tumor-specific T cells can be activated de novo during the development of cancer vaccines, oncolytic viral therapy and cell therapies engineered *ex vivo*. ICIs have demonstrated efficacy in BMs. Additionally, cellular therapies, including CAR T-cell therapy and DC vaccines, have been applied for the treatment of BMs. However, larger scale clinical trials, particularly those of phase III trials remain lacking. Therefore, the consequence of immunotherapy and/or radiotherapy on BMs largely remains unknown. The present article only reviewed immunotherapy against the BMs from melanoma, NSCLC and RCC, due to an insufficient number of studies on immunotherapy for BMs originating from other tumors, including gastrointestinal tumors and ovarian carcinoma. Ongoing, planned prospective trials and additional data are required to explore and further validate the effect of immunotherapy on BMs from a wider variety of malignant tumors.

Acknowledgements

Not applicable.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Authors' contributions

RD, LC, ZS and TX made substantial contributions to the conception and design of the study and wrote the manuscript. QW, FJ and QP contributed to the study design and assisted in the literature search for this review article. All authors have read and approved the final version of the manuscript for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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