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Immunity in malignant brain tumors – Tumor entities, role of immunotherapy, and specific contribution of myeloid cells to the brain tumor microenvironment

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Summary

Malignant brain tumors lack effective treatment, that can improve their poor overall survival achieved with standard of care. Advancement in different cancer treatments, has shifted the focus in brain tumor research and clinical trials towards immunotherapy-based approaches. The investigation of the immune cell landscape revealed a dominance of myeloid cells in tumor microenvironment. Their exact role und functions are subject of ongoing research. Current evidence suggests a complex interplay of tumor cells and myeloid cells with competing functions towards support vs. control of tumor growth.

Here, we provide a brief overview on the three most abundant brain tumor entities: meningioma, glioma, and brain metastases. We also describe the field of ongoing immunotherapy trials and their results, including immune checkpoint inhibitors, vaccination studies, oncolytic viral therapy, and CAR-T cells. Finally, we summarize the phenotypes of microglia, monocyte-derived macrophages, border associated macrophages, neutrophils, and potential novel therapy targets.

Introduction

A variety of central nervous system (CNS) tumors exists, and they can be mainly divided into benign and malignant entities. In adults, the most common ones are meningioma, glioma and brain metastases. Especially malignant brain tumors are associated with a poor prognosis.[1] Recently, the immune landscape of these tumors has been resolved at the single cell level. These studies revealed that malignant brain tumors are dominated by tissue resident and monocyte-derived macrophages (MoMACS) which are proposed to be predominantly pro-tumorigenic.[2, 3] Due to identified PDL1 expression of brain tumor cells, immune checkpoint inhibitor treatment was used in clinical trials.[4] Unfortunately, they lack success in glioma and showed only limited efficacy in brain metastases.[5] The reasons for treatment failure are thought to be found within he tumor immune microenvironment, which is poorly infiltrated by cytotoxic T cells, harbors a low mutational burden, lack of neoantigens and the 'corruption' of local macrophages towards establishing an immune suppressive milieu.[6] Other treatments that are currently under investigation are dendritic cell (DC) vaccination, oncolytic

viral therapy, CAR-T cells, and cytokine antibody fusions with the aim to increase the local infiltrate and anti-tumor activity.

Our review gives a brief overview on the most frequent tumor types, immunotherapeutic advancements and describes the canonical myeloid populations in the brain tumor environment and their proposed functional properties.

Origin of different tumor cell types

Meningioma

Meningioma stem from the meninges, more specifically from the arachnoid cap cell layer.[7] They usually grow slowly[8], and can reach extensive diameters until the occurrence of symptoms. Although, they are generally considered benign (WHO Grade 1), approximately 7% present as atypical (Grade 2) and 2% as malignant (Grade 3) meningiomas.[9, 10] Grade 1 meningiomas can be treated curative by surgical resection or non-invasively with stereotactic radiation.[11] Atypical meningiomas show a recurrence rate of 40% within 4 years,[12] and for malignant meningiomas this rate is 80% within 5 years.[13] Resection of the tumor is part of the treatment and also includes stereotactic radiation and systemic therapies.[14] The overall survival rate for grade 2 and 3 meningiomas is 50% to 79%[15, 16], and 14–34%[17] at 10 years, respectively.

Glioma

Glioma occur as either low grade (Grade 1 and 2) or high grade glioma (grade 3 and 4). Tumors of grade 2 and 3 transform into a higher grade and malignant glioma over a median interval of 16 - 56 months.[18] The survival rate of low grade gliomas is approximately 7 - 15 years.[19, 20] Glioma, glioneural tumors and neuronal tumors have recently been reclassified in the World Health Organization 2021 Classification of Tumors.[21] The adult-type diffuse gliomas entity entails 1) Astrocytoma IDH mutant, 2) Oligodendroglioma, IDH mutant 1p/19q co-deleted and 3) Glioblastoma IDH wildtype.[21] Astrocytomas are of astrocytic origin, isocitrate dehydrogenase (IDH) mutated and are divided in

WHO grade 2 – 4. IDH mutant Oligodendrogliomas stem from oligodendroglial precursor cells and are classified in WHO Grade 2 and 3. The IDH wildtype glioblastoma is the most frequent adult diffuse glioma with a poor prognosis. The incidence increased during the last years and is approximately 2-5 per 100,000 inhabitants per year.[10, 22] Despite maximal safe resection and concurrent chemotherapy with temozolomide and radiation, glioblastoma progress or recur with limited treatment options at this stage.[23] Median overall survival with standard therapy including maximal safe resection and adjuvant radio-chemotherapy is about 15 months.[24]

Brain metastases

Brain metastases arise in 10-30% of patients diagnosed with systemic tumor burden, typically at stage IV of the disease.[25] Most common primary tumors that metastasize to the brain are melanoma, lung and breast cancer.[26] This number is expected to increase with more surveillance, individualized therapies and improvement of patients overall survival.[27] Each year, there are about 10 times as many brain cancer patients diagnosed with a metastatic brain tumor than with a primary brain tumor.[28] Current therapies for brain metastases include local treatments (surgery, stereotactic radiation),[29, 30] chemotherapy and newer systemic approaches such as targeted therapies and immune checkpoint inhibitors.[31] Brain metastases respond well to these treatments, but can progress, recur or increase in numbers. The median overall survival for patients with brain metastases is less than one year, and in addition to the number of brain metastases, also depends on age, clinical performance and extracranial disease stage.[32, 33]

Current progress in immunotherapy treatment of malignant brain cancers

Immunotherapy has revolutionized the treatment of several types of cancers. Immune checkpoints (IC) regulate the immune response strength and avoid killing of healthy cells by turning “off” T cells. Immune checkpoint inhibitors (ICI) block co-inhibitory checkpoint receptors typically on T cells. This blocking halts binding of the receptor with the ligand on tumor cells and allows T cells to kill cancer cells by preventing the inactivation cascade on T cells.

In high-grade glioma, ICI have been combined with surgical resection and this combination showed promising results in hypermutated recurrent tumors.[34, 35] The median survival in preoperative anti-PD1 treated patients was 417 days compared to 228.5 days in the postoperative treated group.[4] These trials included small numbers of patients and the fact, that the neoadjuvant group performed better, means that ICI works specifically when tumor antigens are present an can be recognized by T cells that expand afterwards. The CheckMate 143 phase 3 randomized trial compared the effect of anti-PD1 with bevacizumab (anti-VEGF) in patients with recurrent glioblastoma in a large, randomized trial.[5] A total of 369 patients were randomized at first recurrence in a multicenter trial. The median overall survival was comparable between the groups (9.8 months in the anti-PD1 group vs. 10 months in the anti-VEGF treated), and therefore could not confirm the benefit of single ICI treatment. Other studies investigate combinations of single ICI treatment plus radiation therapy and or temozolomide (CheckMate 498 and 548), but have already announced, that the primary endpoint of overall survival and progression free survival was not met. Further trials are ongoing and explore other ICI such as the combination of anti-CTLA-4 and anti-PD1, anti-TIM-3, anti-LAG-3, anti-TIGIT, and anti-4-1BB. Table 1.

Numerous immunotherapeutic approaches that have been tested in glioblastoma (GBM) patients are vaccination trials. The results of a phase 3 prospective controlled trial with 331 patients receiving autologous tumor lysate-loaded dendritic cell vaccination (DCVax) have recently been published.[36] Patients were treated with DCVax and standard of care (soc) and compared to matched patients treated with soc. The median overall survival in the intervention group was 19.3 months for newly diagnosed glioblastoma compared to 16.5 months in the external controlled soc group. In the recurrent GBM group this difference was 13.2 months vs. 7.8 months.[36] Although the study group interpreted their findings as clinically meaningful, this trial was highly criticized for changing the comparison arm from a placebo treated cohort to an external cohort and it was hypothesized that the non-randomization in this study could have resulted in an unreal bias toward DCVax.

Another peptide vaccine called Rindopepimut targeting EGFR variant III was explored in the ACT IV phase 3 trial. Only a subset of patients' tumors express EGFR variant III and are therefore suitable for this vaccine. The study was terminated, after the interim analysis revealed no benefit of the treatment.[37] Recently, a multicenter phase 1 trial (NOA-16) included 33 patients with newly diagnosed WHO grade 3 and 4 IDH1 mutated tumors with an IDH1 specific peptide vaccine.[38] The

trial met the primary safety and immunogenicity endpoint and further phase studies are expected with great interest.

For mounting of an antitumor immune response, oncolytic viral therapies have shown to be beneficial. Typical viruses that are used for these treatments are replication competent, and include polioviruses, adenovirus, herpes simplex viruses, retroviruses, and measles viruses.[39, 40] Data from a phase 2/3 trial are available for vocimagene amiretrorepvec with flucytosine (Toca 511 and Toca FC), a non-lytic replicating retrovirus that specifically infects tumor cells with virus encoding an optimized yeast cytosine deaminase, the enzyme that converts 5-fluorocytosine into 5-fluorouracil.[41] The overall survival of Toca 511/FC (201 patients) was compared to standard treatment (202 patients). The virus was injected in the resection cavity wall during tumor resection in first or second recurrence of GBM or anaplastic astrocytoma followed by 6 weeks of oral Toca FC.[42] The median overall survival did not show any difference between the groups.[42] Adenovirus based immunotherapy was used in a phase 2 multicenter study using the adenoviral vector containing the herpes simplex virus thymidine kinase gene, aglatimagene besadenovec (AdVtk) followed by treatment with valacyclovir in combination with standard of care for newly diagnosed GBM patients.[43] Forty-eight patients were included. The results were positive with a median overall survival of 17.1 months in the AdV-tk group compared to 13.5 months in the soc alone.[43] The difference was most prominent in the gross total resection group (median overall survival 25 months in AdV-tk group vs. 16.9 months in soc group). Nevertheless, phase 3 data is lacking to confirm the positive trend.

Several clinical trials taking advantage of engineered Chimeric antigen receptor (CAR) -T cells for high-grade gliomas are ongoing.[44] CAR-T cells are personalized T cells which are taken from the patient's blood, and have been genetically engineered in the laboratory to have a specific T cell receptor which recognizes a precise tumor antigen. After infusion the CAR-T cells can bind the antigen and destroy the cancer cells.

An early study of CAR-T cells targeting EGFR variant III in 10 patients with recurrent GBM demonstrated that the manufacturing and infusion of autologous CAR-T cells is feasible and safe.[45] No cytokine release syndrome or any off-tumor toxicity occurred. Noteworthy, that one patient depicted stable disease over an 18-months follow-up period.[45] Other currently investigated CAR-T cells directed targets are, among others HER2[46], IL-13 Ra2[47], GD2[48], and B7-H3.[49]

In summary, so far all immunotherapeutic trials for glioblastoma have been disappointing in larger scale studies. Newer approached such as immunocytokines for example fused to IL12 or CAR-Macrophages offer some hope on the horizon.

For brain metastases, ICI treatment is more effective. Multiple trials investigated the role of anti-CTLA4 and anti-PD1 in melanoma brain metastases. A randomized phase 3 trial included 676 patients with metastatic melanoma and compared anti-CTLA4 treatment to glycoprotein 100 (gp100) peptide vaccine.[50] Median survival with anti-CTLA4 and gp100 alone was 10.1 months and 6.4 months, respectively.[50] Another phase 2 trial with 23 patients with new or progressing melanoma brain metastases revealed that anti-PD1 treatment resulted in 2 months progression free and 17 months overall survival.[51] Combination of anti-PD1 and anti-CTLA4, was investigated in patients with at least one melanoma brain metastasis.[52] The rate of intracranial clinical benefit was 57%, with a complete response rate of 26%.[52] Anti-PD1 treatment was also evaluated in NSCLC brain metastases and a response was achieved in 33% of patients.[53] Further studies for brain metastases are ongoing and also include DC vaccination trials and ICI treatment in combination with stereotactic radiation for melanoma, lung and breast cancer brain metastases.[54]

A similar trend is seen in the field of recurrent meningioma, where trials are ongoing with ICI. Particularly anti-PD1, anti-PDL1, and combination with anti-CTLA4 or stereotactic radiation are explored.[55]

Role of tissue resident macrophages in the malignant brain tumor microenvironment

Microglia

Brain resident microglia located in the brain parenchyma are of embryonic origin with self-renewal capacity, without replenishment through monocyte-derived macrophages (MoMacs).[56] The role of CNS-resident microglia in the glioma microenvironment has not been definitely clarified and might be multifaceted. Figure 1. In healthy brain they typically express homeostatic markers *SALL1, TMEM119,* and *P2RY12,* which can be downregulated in brain tumors.[2, 57] A recent study revealed

that high-grade glioma associated microglia are activated by tumor derived TGFb1.[58] Depletion of TGFb1 also resulted in reduced tumor growth. These activated microglia expressed *CX3CR1, NLRP1, IL1B, APOE, PDGFRA, SOX2,* and promoted tumor growth via IL1B.[58] Secretion of IL-1b in glioma associated microglia was mediated by apolipoprotein E and the NLRP1 inflammasome.[58] Microglia expressing *CX3CR1* and *PDGFRA* were mainly detected in IHD-WT GBM and showed an increased response to TGFb1 reflecting the proliferative, $Ki67^+$ phenotype.[58]

In human brain cancer samples, microglia also displayed a reactive phenotype with upregulation of CD14 and CD64.[2] They additionally express *HMOX1*, and secrete IL10, which -in neocortical slice cultures- resulted in CD8 T cell exhaustion through the STAT3-BLIMP-1 axis.[59] Depletion of *HMOX1* microglia on the contrary resulted in re-activated effector T cells.[59]

Brain tumor cells express inhibiting markers such as *CD47* "don't eat me" signal that binds to the ligand *SIRPA*. It has been shown, that anti-*CD47* antibodies disrupting *SIRPA* anti-phagocytosis inhibit tumor growth, by increased macrophage phagocytosis.[60] Further dissection revealed that anti-*CD47* treatment in mice lacking *CCR2* recruited macrophages provide *CX3CR1* expressing microglia that were able to reduce tumor growth which resulted in prolonged survival.[61] *TREM2* is an additional marker highly expressed by microglia and MoMacs, and is also associated with a worse survival in glioma patients.[62] Knockdown of *TREM2* inhibited tumor growth in preclinical models, increased interferon gamma levels and induced a proinflammatory polarization.[63] In mouse and human brain metastases *TREM2 was* upregulated on tumor associated microglia and even more pronounced on MoMacs and reflects a potential myeloid target.[3, 57, 64, 65]

In a NSCLC brain metastases model, IL6 was identified to drive so-termed anti-inflammatory microglia expressing *CD206* and *ARG1* via JAK2/STAT3, and was also associated with higher risk of brain metastases and poor prognosis in patient samples.[66] Interestingly, in human and mouse samples, brain metastases MoMacs preferred a localization in the tumor center, whereas microglia located more frequent in the adjacent brain tissue.[2, 57]

S100A proteins are associated with regulation of inflammation and migration/invasion of macrophages.[67] Investigation of their role in human brain metastases samples showed high expression levels of S100A4, S100A6 and S100A10 in TAM-microglia.[3, 68]

Overall, there is mounting evidence, that microglia support tumor growth and limit anti-tumor immunity.

Border associated macrophages

So called border associated macrophages (BAMs) which reside at CNS interfaces consist of meningeal macrophages, perivascular macrophages and choroid plexus macrophages.[69, 70] Their role in the malignant brain tumor microenvironment is still unclear. Figure 1. In the mouse brain, border associated macrophages (BAMs) and their ontogeny have been better studied. A recent single-cell atlas of mouse brain macrophages identified six major BAMs subsets.[71] Fate mapping revealed that a small subset of choroid plexus epithelium macrophages resembling a unique microglia subset, and subdural BAMs are of embryonic origin. Other BAMs were infiltrated and replaced by precursors derived from the bone-marrow.[71] They typically express among other markers *CD206, CD163, Folr2, Lyve1, P2RX7, NRP1, CD63, MHCII, CLEC12A*, and signature genes included *Apoe, Ms4a7, Ms4a6c, Lyz2 and Tgfbi.*[71] Different functions have been assigned to perivascular macrophages (PVMs) in the TME of other tumor types. These are support of tumor angiogenesis, intravasation of cancer cells, and seeding of distant metastasis.[72] The frequency of PVMs has been correlated with the density of tumor microvessels.[73] Preclinical intravital imaging studies showed that PVMs are regulating the escape of breast tumor cells in the local microenvironment and regulate vascular permeability by vascular endothelial growth factor A.[74-76]

A recent study revealed in ICI treated human samples of brain metastases and recurrent GBM, that an accumulation of PVMs occurred in the perivascular space of recurrent GBM, while in brain metastases PVMs infiltrated into the tumor tissue.[77]

Meningeal macrophages and T cells are colocalized in the dural layer surrounding the brain. The role of meningeal macrophages in antigen presentation and T cell activation was shown in experimental autoimmune encephalomyelitis models and this fact might be useful for prospective immunotherapy purposes.[78]

Invading myeloid cells – foe or friend?

Monocyte derived macrophages

The ratio of microglia and MoMacs may differ between different types of brain tumors. MoMacs are derived from the bone marrow and infiltrate malignant brain tumors to become the dominant population, especially in IDH wildtype glioma, whereas the IDH mutant present higher frequencies of microglia.[2, 3] Brain metastases are more heterogeneous due to different histological origins. In common is their higher percentage of MoMac infiltration comparable in the tumor core and margin with lower frequencies of microglia.[3, 79] The abundance of MoMacs increased from melanoma to breast and lung brain metastases and was vice versa for microglia.[2, 3]

MoMacs origin from monocytes and are recruited by chemokine receptor such as *CX3CR1* or *C3AR1* involved in migration in brain metastases.[64]

The current evidence suggests, that these MoMacs are shaped by the tumor type and also become immune-suppressive.[2] Figure 1. They express markers such as *CD206, CD209, CD204, LILRB2, CD163*, *CD169*, and *PDL1*.[2, 3, 6] *PDL1-PD1* axis was targeted by immune checkpoint inhibitors, but failed to result in improved survival, as described above. *LILRB2* is another immune checkpoint receptor that can be explored as a target in the future.

In a GBM mouse model, CSF-1 receptor inhibitors were used as immunotherapy approach and resulted in survival benefit.[80] Notably such inhibitors may also affect microglia. In another preclinical experiment, using GL261 glioma cell line, reduced CSF1R inhibition only mature tumor associated macrophages (TAMs), but increased the percentage of monocytes, assuming that the monocyte to macrophage transformation is altered.[81] This study showed also, that microglia and MoMacs compete for space and that impairing monocyte influx results in compensatory mechanisms that maintain TAMs numbers by increasing the number of microglia in the tumor. In a breast cancer brain metastases model, tumor recurrence and TAMs activation following CSF1R inhibition was driven by compensatory CSF2R-STAT5 pathway.[82] Unfortunately, so far clinical trials with CSF1R inhibition in GBM have not shown any efficacy.[83]

In a preclinical model of lung brain metastases, bulk and single cell RNA sequencing expression profile revealed that tumor associated microglia induced a pro-inflammatory phenotype, whereas

Mo-Macs developed towards signatures of alternative activation including antigen presentation and wound healing.[57]

The stratification of MoMacs into so called M1 and M2 has proven to be largely misleading and not supported by the actual data in that a complex and plastic structure of overlapping macrophage phenotypes exist.[84, 85] In mouse models, different markers expressed on putative anti-inflammatory MoMacs have been tested as therapeutic target. For example, *MerTK* inhibition in GL261 model decreased vascular formation and TAMs in numbers and prolonged survival.[86] Another immunotherapy target on MoMacs is *S100A4,* and TAMs with *S100A4* depletion showed increased phagocytic activity.[87] *S100A4* is a small calcium binding protein and revealed to avoid apoptosis in TAMs in different tumor models.[88] On the other hand, *CD169+* MoMac-TAMs were depicted to promote anti-tumor inflammation in GBM and reflect a beneficial subset of MoMacs.[89] These MoMacs support T-cell accumulation by enhancing phagocytosis of glioma cells and secretion of pro-inflammatory chemokines.[89] *MHC class II* antigen presentation on MoMacs was necessary for functional T-cell toxicity and its loss lead to CD8 T cell dysfunction via *osteopontin*.[90] Supporting the important anti-tumor effect of MoMac-TAMs, *MPO+* macrophages were recently associated with significant prolongation of survival in GBM patients.[79] *MPO+* MoMacs increase depicted more interactions between endothelial cells, and MoMacs with T cells aiming at tumor t-cell infiltration and stimulation. [79]

Neutrophils

Neutrophils are derived from the bone marrow and are the most frequent type of granulocytes circulating in the blood.[91] In the brain tumor setting have elevated levels of circulating neutrophils have been identified as a poor prognostic marker.[92] Especially increased neutrophil to lymphocyte ratio was correlated with reduced overall survival.[93, 94] Their function in the tumor microenvironment is still unclear. Figure 1. Some studies have reported, that activated neutrophils in glioma belong to the myeloid-derived suppressor cells and contribute to the local immune suppression by secretion of *nitric oxide* and *arginase*.[95] It was also shown that glioma can remotely regulate systemic myeloid differentiation in the bone marrow to generate neutrophils pre-committed to a tumor supportive phenotype.[96] In a preclinical metastatic model, reduction of *Arg1+PDL1+* neutrophils inhibited brain

metastases formation.[97] For functional assignment of neutrophils, the term tumor associated neutrophils (TANs) as either N1 (anti-) or N2, (protumorigenic) has been introduced.[98] This concept is certainly not reflected by a "black" and "white" approach and future research will define more markers to distinguish between different subgroups of neutrophils.

Conclusions

Malignant brain tumors are associated with a reduced quality of life and short survival. The immune microenvironment of these tumors has recently been characterized and is dominated by myeloid cells which are clearly shaped by tumor types. It is becoming increasingly clear that the myeloid landscape in the TME is the key to understand treatment failures and tumor progress. Clinical immunotherapy trials have largely failed so far, and there is urgent need for profound research to reinvigorate the use of the myeloid cells for immunotherapeutic options.

Future perspectives

We believe that there is a future role for immunotherapy for malignant brain tumors. The focus of most immunotherapy trials so far was on the T cell compartment with the aim to reinvigorate the microenvironment with additional T cells (CAR-T cells, viral therapies, DCVax) or to re-activate exhausted T cells. The innate immune compartment in brain cancers has however not yet been systematically targeted and remains somewhat understudied. Myeloid cells resemble the most frequent immune cell population in malignant brain tumors, and we should take advantage of this fact. Antibody cytokine fusions can be delivered by targeting myeloid cells. Important is the better understanding of the function of each myeloid subgroup in the tumor environment. For example, in the MoMacs population, identification of the "good" and "bad" ones could help us to target und re-educate the immune environment. BAMs might be involved in the blood brain barrier regulation, and antigen-presentation at the border regions, which are two important features of immune response regulation. Tumor vessel recruitment by perivascular macrophages could reveal new targets for anti-angiogenic drugs. More in-depth and systematic research on the myeloid compartment is needed to design new drugs for the treatment of brain cancers.

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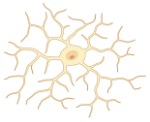
Figure legend

Figure 1: Tumor invading and tissue resident myeloid cells in the brain tumor microenvironment.

The role and interactions of different canonical myeloid cells in the malignant brain tumor landscape are not yet well understood. Tissue resident microglia express homeostatic marker such as TEM119, which can be used to differentiate them from other brain tumor associated macrophages. They could be used for brain tumor immunotherapy, but current evidence suggests a tumor induced shift to a protumorigenic phenotype. Similar results were found for the MoMacs that invade the brain from the periphery (express CD49d) and were primarily shown to gain immune-suppressive

functions. BAMs contribution to the brain tumor microenvironment is currently completely unexplored.

- Symbol indicating Receptor or Ligand expressed on immune cells.
- Arrow width reflects the frequency of the populations.
- Box with markers that identify the population or are expressed by the Population
- Drawing in the center: Malignant brain tumor cells  and different types of immune cells in the brain tumor microenvironment such as T cells and myeloid cells Microglia  T cells MoMacs  and BAMs 
- Microglia 
- BAMs 
- MoMacs 
- Neutrophils 

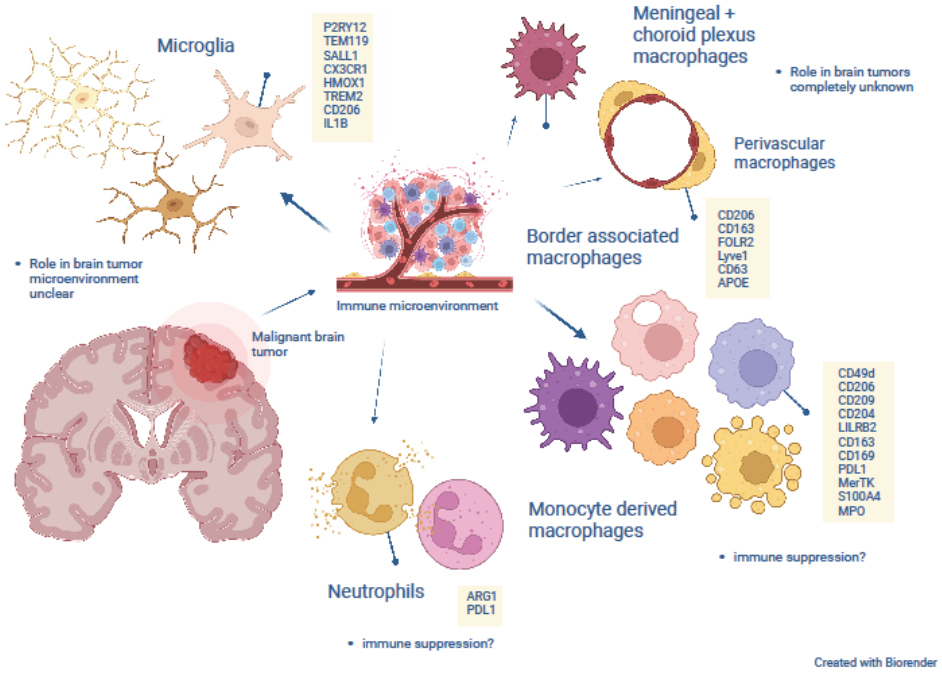


Table legend

Table 1: Overview of a selection of completed and ongoing phase 2 and 3 immunotherapy trials for glioblastoma and brain metastases.

CMV = Cytomegalovirus, DCs = dendritic cells, EGFFR = Epidermal Growth Factor Receptor, GBM = Glioblastoma multiforme, GM-CSF = Granulocyte-macrophage colony-stimulating factor, GCV = Ganciclovir, KLH = keyhole limpet hemocyanin, MGMT methylation = correlated with survival and sensitivity to temozolomide in glioblastoma, MGMT methylated responds tumor show a higher efficacy to TMZ OS = overall survival, ICI = Immune checkpoint inhibitors, standard of care = TMZ + RT, TMZ = temozolomide, RT = radiotherapy

High grade glioma						
Trial ID	Drug	Phase	Inclusion criteria	Patients	Survival	Conclusion
NCT02017717 CheckMate 143	anti-PD1 vs. Anti-VEGF-A	Phase 3 randomized	Recurrent GBM	369	median OS 9.8 months in anti-PD1 vs. 10 months in anti-VEGF	ICI did not improve survival
NCT02617589 CheckMate 498	anti-PD1 + RT vs. standard of care	Phase 3 randomized	Newly diagnosed GBM with unmethylated MGMT promotor	550	median OS 13.4 months in anti-PD1 + RT vs. 14.9 months standard of care	ICI did not improve survival
NCT02667587 CheckMate 548	anti-PD1 + standard of care vs. placebo + standard of care	Phase 3 randomized	Newly diagnosed GBM with methylated MGMT promotor	693	median OS 28.9 months in anti-PD1 + standard of care vs. 32.1 months standard of care + placebo	ICI did not improve survival
NCT00807027	standard of care + activated T lymphocyte (Immuncell-LC) vs. standard of care	Phase 3 randomized	Newly diagnosed GBM	180	median OS 22.5 months in the immune-LC group vs. 16.9 months standard of care	Immune-LC did not improve survival
NCT01480479 (ACT IV)	Rindopepimut (peptide vaccine that targets EGFRvIII) + GM-CSF + TMZ + vs KLH + TMZ	Phase 3 randomized	Newly diagnosed GBM expressing EGFRvIII + standard of care	745	median OS 20.1 months in rindopepimut group vs. 20.0 months in KLH group	Rindopepimut did not improve survival
NCT02573324 (Intellance1)	ABT-414 (antibody-drug conjugate binding to EGFR amplification) + standard of care vs. placebo + standard of	Phase 3 randomized	Newly diagnosed GBM with EGFR amplification	720	median OS 18.9 months ABT-414 group vs. 18.7 months placebo group	ABT-414 did not improve survival

	care					
NCT03149003	DSP-7888 (vaccine targeting Wilms tumor gene 1) + anti-VEGF-A vs. anti-VEGF-A	Phase 3 randomized	Recurrent GBM	221	median OS 10.2 months in DSP-7888 group vs. 9.4 months in anti-VEGF-A group	DSP-7888 did not improve survival
NCT00045968	DCVax-L (autologous tumor lysate loaded dendritic cells vaccine) vs. placebo	Phase 3 randomized trial	Newly diagnosed GBM + standard of care	331	median OS 19.3 months in DCVax-L group vs 16.5 months in control group	For patients with recurrent GBM median OS 13.2 months vs. 7.8 months in control patients DCVax-L improved survival, but results are highly criticized due to use of use of external control arm
NCT04396860	anti-PD1 + anti-CTLA4 + RT vs. standard of care	Phase 2/3 randomized	Newly diagnosed GBM with unmethylated MGMT promotor	485	Completion 2024	
NCT05685004	Activated autologous T-cells (TVI-Brain-1) + standard of care vs. standard of care	Phase 2/3 randomized	Newly diagnosed GBM with unmethylated MGMT promotor	96	Completion 2026	
NCT05100641	AV-GBM-1 (autologous dendritic cells vaccine) + GM-CSF vs. autologous monocytes + GM-CSF	Phase 3 randomized	Newly diagnosed GBM + standard of care	672	Completion 2029	

NCT01454596	EGFRvIII CAR T Cells + Aldesleukin + Fludarabine + Cyclophosphamide	Phase 1/2	Recurrent GBM	18	Results from 18 patients from phase 1 trial: median OS 6.9 months	Phase 2/3 needed, so far CAR T cells did not improve survival, phase 3 needed
NCT02798406 (Captive)	DNX-2401 (replicative oncolytic adenovirus) + anti-PD1	Phase 2	Recurrent GBM	49	median OS 12.5 months	treatment was safe with notable survival benefit in selected patients, phase 3 needed
NCT00870181 (HGG-01)	ADV-TK (Adenovirus-Mediated Delivery of herpes simplex virus thymidine kinase)/GCV vs. surgery or chemotherapy	Phase 2 randomized	Recurrent GBM	47	median OS 45.7 weeks in ADV-TK group vs. 8.6 weeks control group	Survival improvement in ADV-TK group, phase 3 needed

NCT00589875 (BrTK02)	ADV-TK (adenovirus expressing herpes simplex thymidine kinase) + valacyclovir + standard of care	Phase 2	Newly diagnosed GBM	52	median OS 17.1 months in ADV-TK vs. 13.5 months standard of care	ADV-TK + valacyclovir did not improve survival
NCT01582516	Delta24-RGD (oncolytic replication adenovirus)	Phase 1/2	Recurrent GBM	20	mean survival 15.9 months	Delta24-RGD did not improve survival
NCT03291314 (GliAvAx)	Axitinib (VEGFR 1-3 inhibitor) + anti-PDL1 (cohort 1) vs. Axitinib + anti-PDL1 after 6 weeks (cohort 2)	Phase 2	Recurrent GBM	54	median OS 26.6 weeks in cohort 1 and 18.0 weeks in Cohort 2	Anti-PDL1 + Axitinib did not improve survival
NCT02858895	MDNA55 (IL4 fused to pseudomonas Exotoxin delivered with stereotactically placed catheter)	Phase 2	Recurrent GBM	44	median OS 11.64 months	MDNA55 did not improve survival
NCT01280552	ICT-107 (autologous dendritic cells pulsed with synthetic tumor antigens) vs. unpulsed autologous dendritic cells	Phase 2 randomized	Newly diagnosed GBM + standard of care	124	median OS 17 months for ICT-107 group vs. 15 months control group	ICT 107 did not improve survival
NCT01006044	autologous dendritic cells vaccine tumor lysate loaded + standard of care	Phase 2	Newly diagnosed GBM	26	median OS 23.4 months	OS improved compared to standard of care, phase 3 needed
NCT00323115	autologous dendritic cells vaccine co-cultured with tumor cells and injected in cervical lymph nodes + standard of care	Phase 2	Newly diagnosed GBM	11	median OS 28 months	OS improved compared to standard of care, phase 3 needed
NCT01213407 (GBM-Vax)	autologous dendritic cells secreting IL-12 tumor lysate loaded + standard of care vs. standard of care	Phase 2	Newly diagnosed GBM	87	median OS 564 days in dendritic cells group vs. 568 days in control group	Autologous dendritic cell vaccine did not improve survival
NCT02366728 (Elevate)	autologous unpulsed DCs + autologous CMV mRNA pulsed DCs (CMV-DC) + labeled DCs for migration study (I) vs. tetanus diptheria toxoid (Td) + CMV-DC + labeled DCs + TMZ (II) vs. Basiliximab (anti-IL2R) + CMV-DC + Td + TMZ (III)	Phase 2 randomized	Newly diagnosed GBM + standard of care	64	Td-mediated increased migration of DCs median OS 16 months in group I, 20 months in group II and 19 months in group III	Increase in migration of DCs to the draining lymph nodes as a result of Td preconditioning Autologous dendritic cell vaccine group II and III did not show improved survival

NCT01920191	IMA950 (multi tumor associated peptide vaccine) + poly ICLC (TLR3 agonist)	Phase 1/2	Newly diagnosed GBM + standard of care	19	median OS 19 months	IMA950 + poly ICLC did not improve survival
NCT00643097 (Activate + ACT II)	Vaccination with EGFRvIII peptide - KLH + GM-CSF + TMZ	Phase 2	Newly diagnosed GBM, EGFRvIII-positive tumor + standard of care	40	median OS 26.0 months	Phase 3 needed
NCT03047473 (SEJ)	anti-PDL1 + standard of care	Phase 2	Newly diagnosed GBM	30	median OS 15.3 months	Anti-PDL1 did not improve survival
NCT02550249 (Neo-nivo)	anti-PD1	Phase 2	Primary or recurrent GBM	29	median OS 7.3 months	Neoadjuvant anti-PD1 did not improve survival
NCT03452579	anti-PD1 + anti-VEGF-A vs. anti-PD1 + low dose anti-VEGF-A	Phase 2 randomized	Recurrent GBM	90	age > 60 median OS 10.6 months vs 5.9 months; age ≤ 60 years median OS 8.0 months vs 12.4 months	Anti-PD1 + anti-VEGF-A in different doses did not improve survival
NCT02337491	anti-PD1 + anti-VEGF-A vs. anti-PD1	Phase 2 randomized	Recurrent GBM	80	median OS 8.8 months vs. 10.3 months	Anti-PD1 +/- anti-VEGF-A did not improve survival
NCT03367715	anti-PD1 + anti-CTLA4 + RT	Phase 2	Newly diagnosed GBM with unmethylated MGMT promotor	10	median OS 16.85 months	Anti-PD1 + anti-CTLA4 did not improve survival
NCT02794883	anti-PDL1 vs. anti-CTLA4 vs. anti-PDL1 + anti-CTLA4	Phase 2 randomized	Recurrent GBM	36	median survival 11.71 months, vs. 7.246 months, vs. 7.703 months	Anti-PDL1 in combination with anti-CTLA4 did not improve survival
NCT00895180	Ramucirumab (VEGFR2 inhibitor) vs. anti-PDGFR alpha antibody (IMC-3G3)	Phase 2	Recurrent GBM	80	median OS 49.5 weeks vs. 34.3 weeks	Ramucirumab and anti-PDGFR alpha did not improve survival
NCT01071837	APG101 (CD95 ligand binding fusion protein) + RT vs. RT	Phase 2 randomized	Recurrent GBM	84	median OS 11.5 months vs. 11.5 months	APG101 did not improve survival
NCT01301430 (ParvOryx01)	H-1PV (Oncolytic H-1 parvovirus intratumoral or/and intravenous)	Phase 1/2	Recurrent GBM	18	median OS 464 days	H-1PV did not improve survival
NCT03750071	VXM01 (Salmonella typhi carrying plasmid encoding	Phase 1/2	Recurrent GBM	30	No survival data available	

	for VEGFR-2) + anti-PDL1					
NCT03618667	GC1118 (human anti-epidermal growth factor receptor antibody)	Phase 2	Recurrent GBM with high EGFR amplification	21	median overall survival 5.7 months	GC1118 did not improve survival
NCT02343406	ABT-414 (Depatuxizumab mafodotin, antibody (EGFR IGg1)-drug conjugate (tubulin inhibitor monomethyl auristatin F) (I) vs. ABT-414 + TMZ (II) vs. Lomustine or TMZ (III)	Phase 2 randomized	Recurrent GBM with EGFR amplification, adult and children	266	median OS 7.9 months (I) vs. 9.6 months (II) vs. 8.2 months (III) in adults	ABT-414 did not improve survival
NCT01648348	anti-VEGF-A + TRC105 (anti-CD105 monoclonal antibody) vs. Anti-VEGF-A	Phase 1/2 randomized	Recurrent GBM	116	median OS 9.7 months vs. 7.4 months	TRC105 did not improve survival
NCT01498328 (ReACT)	anti-VEGF-A + Rindopepimut (peptide vaccine that targets EGFRvIII) + GM-CSF vs. KLH (Keyhole limpet hemocyanin) + anti-VEGF-A	Phase 2 randomized	Recurrent GBM EGFRvIII positive	127	24-month survival rate 20% for rindopepimut vs. 3% for control median OS 12.0 months vs. 8.8 months	Rindopepimut did not improve survival, phase 3 needed
NCT02540161	Sym004 (mixture of antibodies directed against distinct epitopes on the extracellular domain of EGFR)	Phase 2	Recurrent GBM +/- EGFR-amplification	43	median OS 9.95 months	Use of Sym004 did not improve survival
NCT02335918	anti-CD27 + anti-PD1	Phase 1/2	Recurrent GBM	22	median OS 10 months	Use of anti-CD27 + anti-PD1 did not improve survival
NCT01290692	TVI Brain 1 (cancer vaccine plus immune adjuvant plus activated white blood cells)	Phase 2	Recurrent GBM	86	No survival data available	
NCT00458601 (Act III)	Rindopepimut (peptide vaccine that targets EGFRvIII) + GM-CSF + TMZ	Phase 2	Newly diagnosed GBM expressing EGFRvIII + standard of care	82	median OS 24.6 months	Phase 3 needed
NCT01290263	Amgen 386 (elective angiopoietin 1/2-neutralizing peptibody) vs. Amgen 386 + anti-VEGF-A	Phase 1/2	Recurrent GBM	48	median OS 341 days vs. 285 days	Amgen 386 did not improve survival
NCT04006119	ad-RTS-hIL-12 (gene therapy expressing IL-12 after administration of activator ligand) + Veledimex + anti-PD1	Phase 2	Recurrent GBM	40	median OS from phase 1 16.9 months, phase 2 data pending	

NCT03018288	standard of care + anti-PD1 + autologous tumor-derived heat shock protein peptide-complex vaccine (HSPPC-96) vs. standard of care + anti-PD1 + placebo vaccine vs. standard of care + anti-PD1	Phase 2	Newly diagnosed GBM with unmethylated MGMT promotor, IDH wildtype	90	Completion 2022, results pending	
NCT01609790	AMG 386 (peptide inhibitor neutralizes Ang1 + Ang2 interaction with Tie2 receptor, reducing tumor angiogenesis) + anti-VEGF-A vs. anti-VEGF-A + placebo	Phase 2 randomized	Recurrent GBM	137	median OS 7.5 months vs. 11.5 months	AMG 386 did not improve survival
NCT04801147 (Dendr1)	autologous dendritic cells vaccine tumor lysate loaded	Phase 1/2	Newly diagnosed GBM + standard of care	76	Completion 2023	
NCT03400917	autologous dendritic cell vaccine after co-culture with tumor cells + GM-CSF	Phase 2	Newly diagnosed GBM + standard of care	55	Completion 2023	Preliminary results showed median OS of 14.7 months for IDH wildtype patients
NCT04115761	standard of care + autologous dendritic cell vaccine tumor lysate loaded (ADCV01) to subaxillary lymph nodes vs. standard of care	Phase 2 randomized	Newly diagnosed GBM	24	Completion 2023	
NCT04388033	dendritic + tumor cells fusion + IL12 + TMZ	Phase 1/2	Newly diagnosed GBM + standard of care	10	Completion 2023	
NCT05131711 (inSituVac2 + Csreig)	RT + GM-CSF + Sapylin (lyophilized mixture of group A Streptococcus pyogenes) + MnCL2 (Manganese dichloride) vs. FDA approved strategies	Phase 1/2 randomized	Recurrent GBM	60	Completion 2023	
NCT01903330	ERC1671 (tumor cells) vaccination + GM-CSF + cyclophosphamide + anti-VEGF-A vs. placebo + anti-VEGF-A	Phase 2	Recurrent GBM	84	Completion 2023	Preliminary results showed median OS of 10.5 months
NCT03890952	anti-PD1 + anti-VEGF-A	Phase 2	Recurrent GBM	40	Completion 2023	
NCT04479241 (Luminos-101)	PVSRIPO (oncolytic poliovirus vaccine targeting CD155) + anti-PD1	Phase 2	Recurrent GBM	30	Completion 2023	
NCT03661723	anti-PD1 + RT vs. anti-PD1 +	Phase 2	Recurrent GBM	60	median OS 11.8 months vs. 8.6	ICI + anti-VEGF-A did not improve

	anti-VEGF-A + RT				months	survival
NCT05053880	ACT001 (parthenolide derivative targeting NF-κB and STAT3 signaling pathways) + anti-PD1 vs. anti-PD1	Phase 1/2 randomized	Recurrent GBM	48	Completion 2023	
NCT03665545 (IMA950-106)	anti-PD1 + IMA950 (multipeptide vaccine with glioma associated antigens) + poly ICLC (TLR3 agonist)	Phase 1/2 randomized	Recurrent GBM	24	Completion 2023	
NCT02337686	Neoadjvant anti-PD1	Phase 2	Recurrent GBM	18	median OS 20 months	Subgroup of patients undergoing surgery with resectable tumors, promising results, phase 3 needed
NCT03491683	INO-5401 (DNA plasmids targeting Wilms tumor gene-1 antigen, prostate-specific membrane antigen and human telomerase reverse transcriptase) + INO-9012 (DNA plasmid for expression interleukin-12) + anti-PD1 + standard of care	Phase 1/2	Newly diagnosed GBM	52	Completion 2023	
NCT05033587	anti-PD1 + Anlotinib + RT	Phase 2	Newly diagnosed GBM with unmethylated MGMT promotor	28	Completion 2023	
NCT02658279	anti-PD1	N/A	Recurrent malignant glioma with a hypermutated phenotype (30 mutations)	27	Completion 2024	
NCT04145115	anti-PD1 anti-CTLA4	Phase 2 study	Somatically hypermutated recurrent WHO Grad IV Glioma	37	Completion 2024	
NCT02311582	anti-PD1 +/- MRI guided laser ablation	Phase 1/2 randomized	Recurrent malignant glioma	55	Completion 2024	
NCT03277638	anti-PD1 + MRI guided laser ablation	Phase 1/2 randomized	Recurrent GBM	34	Completion 2024	
NCT04406272	VB 111 (adenovirus for targeted apoptosis of tumor vessels) pre- and/or postop + anti-VEGF-A vs. placebo +	Phase 2 randomized	Recurrent GBM	45	Completion 2024	

	standard of care					
NCT03879512	Treg depletion (metronomic cyclophosphamide), autologous dendritic cell immunotherapy + anti-PD1/anti-CTLA4	Phase 1/2 trial	Children with recurrent high grade glioma	25	Completion 2024	
NCT02649582 (addit-Glio)	autologous dendritic cell vaccine loaded with Wilms' tumor 1 mRNA + TMZ	Phase 1/2 trial	Newly diagnosed GBM + standard of care	20	Completion 2024	
NCT02465268 (Attac-II)	CMV mRNA pulsed DCs + GM-CSF + tetanus diptheria toxoid + TMZ vs. unpulsed PBMC + TMZ	Phase 2 randomized	Newly diagnosed GBM + standard of care	175	Completion 2024	
NCT04888611	anti-PD1 + autologous dendritic cells vaccine tumor stell-like cell antigen loaded vs. anti-PD1 + placebo	Phase 2 randomized	Recurrent GBM	40	Completion 2024	
NCT02974621	Cediranib Maleate (anti-VEGFR-TK) + Olaparib (PARP inhibitor) vs. anti-VEGF-A	Phase 2 randomized	Recurrent GBM	70	Completion 2024	
NCT03782415	MN-166 (phosphodiesterase inhibitor) + TMZ	Phase 1/2	Newly diagnosed and recurrent GBM + standard of care	50	Completion 2024	
NCT04977375	anti-PD1 + RT	Phase 1/2	Recurrent GBM	10	Completion 2024	
NCT03743662	RT + anti-PD1 + anti-VEGF-A	Phase 2	Recurrent IDH wildtype, MGMT methylated GBM	39	Completion 2024	
NCT04121455 (Gloria)	Olaptesed pegol (RNA aptamer that targets CXCL12) + RT + anti-PD1 vs. Olaptesed pegol + RT vs. Olaptesed pegol + RT + anti-VEGF-A	Phase 1/2	Newly diagnosed GBM with unmethylated MGMT promotor	27	Completion 2024	
NCT03405792 (2-the-top)	TMZ + tumor treating fields + anti-PD1	Phase 2	Newly diagnosed GBM + standard of care	31	Completion 2024	
NCT04013672	anti-PD1 + SurVaxM (peptide vaccine conjugate targets survivin) + GM-CSF + Motanide ISA 51 (vaccine adjuvant)	Phase 2	Recurrent GBM	51	Completion 2024	
NCT03158389 (Noa-20)	RT + targeted therapy (Alectinib, Idasanutlin, Visomodegib, Temsirolismus, Palbociclib)	Phase 1/2	Newly diagnosed GBM with unmethylated	350	Completion 2024	

	vs. RT + APG101 (CD95 ligand inhibitor) vs. RT + anti-PDL1 vs. standard of care		MGMT promotor			
NCT03899857 (Pergola)	standard of care + anti-PD1	Phase 2	Newly diagnosed GBM, IDH wildtype	56	Completion 2024	
NCT05191784	GX-17 (efineptakin alfa; interleukin-7 fused to hyFc) + anti-VEGF-A	Phase 2	Recurrent GBM	20	Completion 2024	
NCT04077866	TMZ vs. TMZ + CD276 CAR-T cells via intracranial injection	Phase 1/2 randomized	Recurrent GBM, B7-H3 (CD276) positive tumor expression	40	Completion 2025	
NCT05084430 (NSC 733972)	M032 (oncolytic herpes simplex virus) + anti-PD1	Phase 1/2	Recurrent GBM	28	Completion 2025	
NCT03548571 (Den-Stem)	autologous dendritic cell vaccine loaded with mRNA mRNA of survivin, hTERT from autologous tumor stem cells + standard of care vs. standard of care	Phase 2/3 randomized	Newly diagnosed GBM, IDH wildtype, MGMT-promotor unmethylated	60	Completion 2025	
NCT04523688 (Combi G-Vax)	autologous dendritic cell vaccine tumor lysate loaded + TMZ	Phase 2	Newly diagnosed GBM + standard of care	28	Completion 2025	
NCT03395587 (GlioVax)	autologous dendritic cell vaccine tumor lysate loaded + standard of care vs. standard of care	Phase 2 randomized	Newly diagnosed GBM, IDH wildtype	136	Completion 2025	
NCT01204684	autologous dendritic cell vaccine tumor lysate loaded (DC) + resiquimod (TLR7+8 agonist) vs. DC + poly ICLC (TLR3 agonist) vs. DC + placebo	Phase 2 randomized	Newly diagnosed or recurrent high grade glioma	60	Completion 2025	
NCT03688178 (Derive)	TMZ + autologous CMV mRNA pulsed DCs (CMV-DC) + autologous unpulsed DCs vs. TMZ + CMV-DC + tetanus diptheria toxoid (Td) vs. TMZ + CMV-DC + Td + Varlilumab (anti-CD27)	Phase 2 randomized	Newly diagnosed GBM + standard of care	112	Completion 2025	
NCT04573192 (Gliostar)	L19TNF (antibody-cytokine fusion) + Lomustine vs. Lomustine	Phase 1/2 randomized	Recurrent GBM, IDH wildtype	142	Completion 2025	

NCT05463848	anti-PD1 + Olaparib + TMZ vs. anti-PD1	Phase 2 randomized	Recurrent GBM, IDH wildtype	78	Completion 2025	
NCT04195139 (Nutmeg)	anti-PD1 + TMZ vs. TMZ	Phase 2 randomized	Newly diagnosed GBM + standard of care	103	Completion 2025	
NCT03174197	anti-PDL1 + standard of care	Phase 1/2	Newly diagnosed GBM	80	Completion 2025	
NCT05084430 (NSC 733972)	M032 (oncolytic herpes simplex virus expressing IL12 intracranially injected) + anti-PD1	Phase 1/2	Recurrent and newly diagnosed GBM + standard of care	28	Completion 2025	
NCT05039281	anti-PDL1 + Cabozantinib	Phase 1/2	Recurrent GBM	6	Completion 2025	
NCT04225039	anti-GITR (promotes effector T cells and inhibits Tregs) + anti-PD1 +/- RT	Phase 2	Recurrent GBM	32	Completion 2025	
NCT03382977	VBI-1901 (gB/pp65 CMV protein vaccine) + GM-CSF vs. Carmustine or Lomustine	Phase 1/2	Recurrent GBM	98	Completion 2025	
NCT02800486	Intraarterial Cetuximab (monoclonal antibody epidermal growth factor receptor inhibitor) + RT	Phase 2	Recurrent high grade glioma, EGFR overexpression	37	Completion 2025	
NCT03532295	anti-PD1 in combination with RT + anti-VEGF-A +/- IDO1 inhibitor	Phase 2	Recurrent high grade glioma	49	Completion 2026	
NCT04443010 (Gliosun)	L19TNF (antibody-cytokine fusion TNFa + anti-fibronectin) + standard of care. vs. standard of care	Phase 1/2 randomized	Newly diagnosed GBM	226	Completion 2026	
NCT04817254	anti-PD1 + anti-CTLA4 + TMZ vs. anti-PD1 + anti-CTLA4 (higher dose) + TMZ	Phase randomized	Newly diagnosed GBM + standard of care	48	Completion 2026	
NCT05879120	anti-PD1 vs. MRI-guided Focused Ultrasound (to open blood brain barrier) + anti-PD1	Phase 2 randomized	Recurrent GBM, IDH wildtype	10	Completion 2026	
NCT03718767	anti-PD1	Phase 2	Recurrent GBM, IDH mutant	70	Completion 2026	
NCT05973903	Lenvatinib + anti-PD1 + tumor treating fields	Phase 1/2	Recurrent GBM	47	Completion 2027	
NCT05502991	anti-PD1 + low dose anti-VEGF-A	Phase 2	Recurrent GBM	60	Completion 2027	
NCT04482933	HSV-1 G207 (oncolytic	Phase 2 trial	Reccurent high	40	Completion 2028	

	herpes simplex virus type 1) + RT		grade glioma in children			
Brain metastases						
Trial ID	Drug	Phase	Inclusion criteria	Patients	Survival	Conclusion
NCT04768075	anti-PD1 + RT + Chemotherapy (Cisplatin, Carboplatin, Pemetrexed Paclitaxel, Albumin paclitaxel) vs. Placebo + RT + Chemotherapy	Phase 3 randomized	NSCLC brain metastases	200	Completion 2024	
NCT04674683	Hbi-8000 (selective histone deacetylase inhibitor) + anti-PD1 vs. Anti-PD1 + Placebo	Phase 3 randomized	Melanoma brain metastases	480	Completion 2025	
NCT05807893 (Super brain)	anti-PD1 + anti-VEGF-A + first line chemotherapy	Phase 2/3	NSCLC brain metastases	30	Completion 2025	
NCT05522660 (Usz-strike)	RT + anti-PD1 1+/- anti-CTLA4 +/- chemotherapy +/- targeted therapy vs. anti-PD1 1 +/- anti-CTLA4 +/- chemotherapy +/- targeted therapy	Phase 3 randomized	Melanoma and NSCLC brain metastases	190	Completion 2026	

NCT02662725 (Ipi + rts)	anti-CTLA4 + RT	Phase 2	Melanoma brain metastases	57	median OS 13.2 months	Increase in survival with synergy of ICI and RT, phase 3 needed
NCT02085070	anti-PD1	Phase 2	Melanoma and NSCLC brain metastases	65	median OS 17 months for melanoma group and 8.9 months for NSCLC group	Better results for melanoma subgroup, anti-PD1 can result in benefit for OS, phase 3 needed
NCT03526900 (Atezo-brain)	anti-PDL1 + Carboplatin + Pemetrexed	Phase 2	NSCLC brain metastases	43	median OS 13.6 months	anti-PDL1 + carboplatin + pemetrexed yields promising OS rate, phase 3 needed
NCT00623766	anti-CTLA4 + Corticosteroids vs. anti-CTLA4	Phase 2	Melanoma brain metastases	72	median OS 3.75 months vs. 6.97 months	anti-CTLA4 did not improve survival, but showed better efficacy without steroids, phase 3 and comparison to anti-PD1 + anti-CTLA combination needed

NCT02115139 (Gray-b)	anti-CTLA4 + RT	Phase 2	Melanoma brain metastases	58	median OS 4.73 months	anti-CTLA did not improve survival
NCT02886585	anti-PD1	Phase 2	Brain metastases from solid tumors	101	Completion 2023	Preliminary results: median OS 8.0 months
NCT04610684	anti-PDL1 + Carboplatin + Etoposide	Phase 2	SCLC brain metastases	60	Completion 2023	
NCT02978404	RT + anti-PD1	Phase 2	NSCLC, SCLC, melanoma or renal cell carcinoma brain metastases	26	median OS 21.4 months	Combination of 1 dose of stereotactic radiosurgery within 14 days of first anti-PD1 infusion results in improved OS rates, phase 3 needed
NCT04507217	anti-PD1 + Pemetrexed/ Carboplatin	Phase 2	NSCLC brain metastases	36	Completion 2023	Preliminary results: median OS was not yet reached, with 1-year OS rate of 70.5%
NCT05018702	ARX788 (antibody–drug conjugate with anti-HER2 antibody + potent tubulin inhibitor)	Phase 2	HER2+ breast brain metastases	32	Completion 2023	
NCT05048212	anti-PD1 + anti-CTLA4 + Cabozantinib (tyrosine kinase inhibitor)	Phase 2 randomized	Renal cell carcinoma brain metastases	40	Completion 2024	
NCT02681549	anti-PD1 + anti-VEGF-A	Phase 2	Melanoma and NSCLC brain metastases	53	Completion 2024	
NCT03175432	anti-PDL1 + anti-VEGF-A +/- Cobimetinib	Phase 2	Melanoma brain metastases	40	Completion 2024	
NCT03873818	anti-PD1 + anti-CTLA4	Phase 2	Melanoma brain metastases	30	Completion 2024	
NCT04348747	Dendritic cell vaccine against Her2/3 + anti-PD1	Phase 2	Triple negative breast cancer brain metastases	23	Completion 2025	
NCT05064280	anti-PD1 + Lenvatinib (VEGFR 1-3 inhibitor)	Phase 2 randomized	Brain metastases from solid tumors	104	Completion 2025	
NCT03483012	anti-PDL1 + RT	Phase 2	Triple negative breast cancer brain metastases	45	Completion 2025	
NCT02696993	anti-PD1 + RT. vs. anti-PD1 + RT + anti-CTLA4	Phase 1/2	NSCLC brain metastases	130	Completion 2025	

NCT04789668	Bintrafusp Alfa (Anti-PDL1/TGFb Trap) + Pimasertib	Phase 1/2	Melanoma, NSCLC, and breast brain metastases	36	Completion 2025	
NCT03340129 (ABC-X)	anti-PD1 + anti-CTLA4 vs. anti-PD1 + anti-CTLA4 + RT	Phase 2 randomized	Melanoma brain metastases	218	Completion 2025	
NCT05812534 (CBC)	Cadonilimab (anti-PD1/anti-CTLA4 tetravalent bispecific antibody) + anti-VEGF-A + Carboplatin + Pemetrexed	Phase 2	NSCLC brain metastases	36	Completion 2025	
NCT04889066	anti-PDL1 + RT	Phase 2 randomized	NSCLC brain metastases	46	Completion 2025	
NCT04647916	Sacituzumab Govitecan (Trop-2-directed antibody + topoisomerase inhibitor conjugate)	Phase 2	HER2 negative breast cancer brain metastases	44	Completion 2026	
NCT05704647	anti-PD1 + anti-LAG3	Phase 2	Melanoma brain metastases	30	Completion 2026	
NCT04955743	anti-PD1 + Lenvatinib	Phase 2	Melanoma and renal cell carcinoma brain metastases	56	Completion 2026	
NCT04129515	Tumor treating fields + anti-PD1	Phase 1/2	Melanoma brain metastases	30	Completion 2026	
NCT05012254 (Nivipi-brain)	anti-PD1 + anti-CLTA4 + Chemotherapy	Phase 2	NSCLC brain metastases	71	Completion 2026	
NCT05746481	anti-TIGIT + Carboplatin + Pemetrexed + anti-PLD1	Phase 2	NSCLC brain metastases	35	Completion 2027	
NCT04511013	Encorafenib + Binimetinib + anti-PD1 vs. anti-PD1 + anti-CTLA4	Phase 2 randomized	Melanoma brain metastases	112	Completion 2027	
NCT03449238	anti-PD1 + RT	Phase 1/2	Breast cancer brain metastases	41	Completion 2027	
NCT05669352	(IRAK-4) interleukin-1 receptor-associated kinase-4 + anti-PD1	Phase 1/2	RT treated melanoma brain metastases	29	Completion 2027	
NCT02374242	anti-PD1 vs. anti-PD1 +	Phase 2	Melanoma brain	76	Completion 2028	

(ABC)	anti-CTLA4	randomized	metastases			
NCT05840770	Anti-PD1	Phase 2	NSCLC brain metastases	34	Completion 2028	
NCT04074096 (Bepcome-Mb)	RT + Binimetinib + Encofafenib + anti-PD1 vs. Binimetinib + Encofafenib + anti-PD1	Phase 2 randomized	Melanoma brain metastases	150	Completion 2029	
NCT04700072 (MK-3475-02D/ Keymaker-U02)	anti-PD1/anti-CTLA4 + Lenvatinib vs. anti-PD1 + Lenvatinib	Phase 1/2 randomized	Melanoma brain metastases	300	Completion 2030	
NCT04964960	anti-PD1 + Chemotherapy (Nab paclitaxel, Paclitaxel, Pemetrexed, Carboplatin)	Phase 2	NSCLC brain metastases	45	Completion 2033	

Glioblastoma and brain metastases are associated with a poor prognosis and reduced overall survival. We summarize immunotherapy approaches which so far have mainly failed to improve standard of care. The predominant immune population are myeloid cells. Phenotype and targets are revealed for monocyte-derived macrophages, resident microglia and border associated macrophages.