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The current state of immunotherapy for primary and secondary brain tumors: similarities and differences

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Abstract

Treatment and resolution of primary and metastatic brain tumors have long presented a challenge to oncologists. In response to the dismal survival outcomes associated with conventional therapies, various immunotherapy modalities, such as checkpoint inhibitors, vaccine, cellular immunotherapy and viral immunotherapy have been actively explored over the past couple of decades. Although improved patient survival has been more frequently noted in treatment of brain metastases, little progress has been made in improving patient survival in cases of primary brain tumors, specifically glioblastoma, which is the representative primary brain tumor discussed in this review. Herein, we will first overview the findings of recent clinical studies for treatment of primary and metastatic brain tumors with immunotherapeutic interventions. The clinical efficacy of these immunotherapies will be discussed in the context of their ability or inability to overcome inherent characteristics of the tumor as well as restricted antigen presentation and its immunosuppressive microenvironment. Additionally, this review aims to briefly inform clinicians in the field of neuro-oncology on the relevant aspects of the immune system as it pertains to the central nervous system, with special focus on the differing modes of antigen presentation and tumor microenvironment of primary and metastatic brain tumors and the role these differences may play in the efficacy of immunotherapy in eradicating the tumor.

Key words: brain tumor, glioma, glioblastoma, brain metastasis, immunotherapy, immune checkpoint inhibitor, vaccine, cellular immunotherapy, viral immunotherapy, central nervous system, antigen presentation, tumor microenvironment

Introduction

Brain tumors can arise in any tissue of the central nervous system (CNS). These include both primary and secondary brain tumors, as well as malignant and non-malignant tumors. The incidence of all primary brain tumors is reported to be ~4–8 cases per 100 000 individuals annually, and estimated to be ~250 000 cases globally (1).

Diffuse gliomas, including the most aggressive form, glioblastoma (GBM), are the most common primary malignant CNS tumors, accounting for 25.5% of all primary brain tumors and 80.8% of all primary malignant tumors (2). They typically grow invasively, progress to higher grades and most patients eventually succumb to the disease (3). Moreover, gliomas can arise in all age groups. In children, ages 0 to 19 years old, they are especially devastating and indeed the leading cause of cancer-related mortality and morbidity (4). The prognosis for children with diffuse midline gliomas (DMG), including diffuse intrinsic pontine gliomas (DIPG), is markedly poor (5,6). As such, development of novel and effective treatment

modalities is urgently warranted in both adult and pediatric glioma patients.

On the other hand, secondary brain tumors, or brain metastases (BMs), are 10 times more common than primary brain tumors (7). Lung and breast cancers, as well as melanoma, are the principal primary diseases responsible for more than three-quarters of BMs (8,9). It is reported that 25–50% of all cancer patients develop BMs in their lifetime (10–12). Although primary disease can be controlled with improved therapeutics, BMs remain major obstacles that must be overcome with better treatment options to improve patient outcomes (13).

Immunotherapy still holds promise both in primary and secondary brain tumors. Although the CNS was long considered as 'immune-privileged', the concept has been revisited recently, with the discovery of a functional lymphatic vasculature (14,15). Of note, several clinical studies have reported excellent disease control in BMs treated with immune checkpoint inhibitors (ICIs) (16–18), while no phase III trials have succeeded in confirming a robust clinical benefit for gliomas thus far (19,20).

In this review, we first overview the recent preclinical/clinical advancement in immunotherapy for glioma, followed by those for BMs. As there are many excellent review articles addressing the advancements and challenges of immunotherapy for gliomas over the past several decades (21–28), here we focus primarily on specific topics, studies and articles reported in the last few years to share up-to-date insights, including therapeutic strategies with paradigm-shifting potential. Thereafter, we outline several unique components of CNS immunology crucial to understanding the similarities and differences in efficacy of immunotherapy between primary and secondary brain tumors.

Recent advances for glioma

The following section reviews recent studies investigating ICIs, vaccines, cellular immunotherapy, and viral immunotherapy for glioma. In particular, some intriguing therapeutic concepts have been emerging in various research areas, such as neoadjuvant ICI therapy, multiple peptide vaccines incorporating private neoantigens, chimeric antigen receptor (CAR)-T-cell therapy for novel targets, as well as various types of viral immunotherapy.

ICIs for gliomas

ICI therapy works by blocking inhibitory receptor–ligand interactions on immune cells, taking the brakes off the T-cells and freeing them to kill the malignancy. ICIs, especially those targeting the CTLA-4 and PD-1/PD-L1 axis, have shown dramatic and prolonged efficacy in many types of tumors, such as melanoma, lung and microsatellite instability-high colon cancers (29–33), but not for gliomas thus far. One explanation is thought to be the presence of the blood–brain barrier (BBB), as compounds > 400–600 Da cannot penetrate the BBB, such as nivolumab, an anti-PD-1 monoclonal antibody (mAb), with a molecular mass of 146 kDa (34,35). However, even though the integrity of the BBB is disrupted in tumor vasculature (36), there is evidence that antibody-mediated blockade of the PD-1/PD-L1 axis and consequent T-cell activation occurs outside of the CNS (37). Multiple preclinical and early phase clinical trials have demonstrated that tumor-specific effector T-cells bound to ICIs are capable of migrating across the BBB and exerting immune responses in the intraparenchymal tumor microenvironment (TME) of gliomas (37–39). Although a series of phase III trials have failed to show optimal results so far (NCT02017717, NCT02617589 and NCT02667587) (19,40,41), ICIs still hold promise for treatment of gliomas, such as in combination with other immune and non-immune treatments as well as use after optimization of patient selection or neoadjuvant administration as described in this section (42–45).

In regard to optimal patient selection, ICI treatment for temozolomide (TMZ)-induced hypermutated GBM has been the subject of debate for years (24,46). Frequent use of TMZ, an alkylating agent used in standard-of-care, often leads to a significant accumulation of single-nucleotide variant (missense) mutations in recurrent tumor compared to non-hypermutated counterparts (median mutation burden: 50.8 vs. 2.6 mutation per Mb) (47). The incidence of hypermutation is estimated to be 10–20% of post-TMZ recurrent tumors (47,48). The strong correlation between efficacy and the tumor mutational burden observed in other cancers (49) as well as several case reports on inherent mismatch repair-deficient patients with hypermutated GBM, who exhibited objective responses to ICI treatment (50,51), have culminated into the above-mentioned hypothesis. However, recent studies have provided opposing data regarding the efficacy of ICI on TMZ-induced hypermutated GBM (47,52). The isocitrate dehydrogenase (IDH) mutation-induced immunosuppressive TME, as well as the sparsity of insertion/deletion/frameshift-type mutations, may preclude ICI efficacy in these tumors (53–55). Currently, in two separate clinical trials, pembrolizumab (anti-PD-1 mAb) and avelumab (anti-PD-L1 mAb) are being prospectively investigated for patients with recurrent hypermutated gliomas/GBMs (NCT02658279, NCT02968940) (56). The studies are expected to bring more robust insight on ICI efficacy in hypermutated GBM.

Another interesting strategy involves neoadjuvant (presurgical) administration of ICIs (57). The rationale is to proactively enhance systemic immunity against tumor antigens, eliminating micrometastatic/disseminated tumor cells that would otherwise be the source of future relapse. Another possible advantage includes preventing early exhaustion of tumor-infiltrating leukocytes (TILs) interacting with higher levels of endogenous tumor antigen prior to resection, and thereby enhancing T-cell priming. Cloughesy et al. (43) recently reported the results of a randomized, open-label pilot study of neoadjuvant versus adjuvant pembrolizumab treatment in patients with recurrent GBM. Although the sample size was limited (16 patients each), the study showed a significant improvement in overall and progression-free survival (OS and PFS) in the neoadjuvant group. Of note, the resected tumor specimens in the neoadjuvant group were characterized by enhanced interferon gamma (IFNγ)-related gene signatures and PD-L1 expression. In addition, T-cell receptor (TCR) repertoire sequencing highlighted the gradual overlapping expansion of the T-cell clones between tumor and blood, indicating a coordinated local and systemic T-cell response elicited by neoadjuvant ICI. In the other paper published back-to-back with the above-mentioned literature, Schalper et al. (44) reported a phase-II single-arm trial in which patients with newly diagnosed and recurrent, resectable GBMs were treated with neoadjuvant plus adjuvant nivolumab. Neoadjuvant therapy resulted in (i) enhanced expression of chemokines, (ii) enhanced immune cell infiltration and (iii) augmented TCR clonal diversity among tumor-infiltrating T lymphocytes, indicating a local immunomodulatory effect of treatment. Intriguingly, a variable degree of PD-1 occupancy was observed in the infiltrating T-cells within the resected specimens, providing evidence of their interaction with nivolumab. However, care should be taken in the interpretation of this data. First, both of the above-mentioned studies are early phase exploratory studies with limited cohort size and breadth of patient subtype. Second,

while multiple early phase studies on ICI monotherapy had suggested the efficacy in glioma in the past, none of them finally succeeded. Further validation is needed through carefully designed prospective, controlled clinical trials (58).

Vaccines for gliomas

Unlike those for infectious diseases, tumor vaccines are expected to induce a therapeutic adaptive immune response against a specific antigen rather than a prophylactic response. Among the various forms of tumor vaccines investigated (59) (Table 1 and 2), we specifically focus on peptide vaccines in this review. They can be further classified based on their target antigen type, such as (i) single peptide vaccines targeting shared neoantigens, (ii) multipeptide vaccines employing non-mutant shared antigens and (iii) multipeptide vaccines incorporating private neoantigens.

Vaccines targeting shared neoantigens originating from common mutations in gliomas, such as *IDH1*-R132H, H3.3-K27M and *EGFR*vIII, have been widely studied (24). One notable example, *EGFR*vIII, showed promise in preclinical and early phase trials (60); however, advanced phase (II/III) studies with the EGFRvIII vaccine, rindopepimut (CDX-110), have failed to show survival benefit for patients with newly diagnosed (ACT IV, NCT01480479) (20) and relapsed GBM (ReACT, NCT01498328) (61). Antigen loss was indeed observed in post-treatment specimens in the study, indicating cancer immunoediting as a result of effective treatment (62,63). On the other hand, *IDH1*-R132 and H3.3-K27M are truncal mutations, thus all tumor cells typically retain the mutant peptides (64), which is ideal in terms of immunotherapy, with rare exceptions of transient loss of IDH-mutations (65). Schumacher et al. (66) developed a peptide vaccine encompassing the IDH1-R132H mutation capable of inducing both epitope-specific CD4+ T-helper-cell responses (T_H1) as well as humoral responses. Two separate first-in-human phase I trials with IDH1-R132H vaccines, NOA-16 (NCT02454634) and RESIST (NCT02193347), are currently ongoing (67). NOA-16 has so far shown optimal results in terms of safety as well as induction of cellular and humoral immunity (67). In pediatric DMGs/DIPGs, the H3.3-K27M mutation-derived epitope bound to HLA-A*02:01 was concurrently identified by two research groups (68,69). A first-in-human pilot clinical trial of H3.3-K27M peptide vaccine in patients with newly diagnosed DMGs (PNOC-007 trial, NCT02960230) was recently completed. Preliminary results showed the safety, the successful induction of antigen-specific T-cells in peripheral blood mononuclear cells (PBMCs), and a correlation between elicited adaptive immune response and survival benefit (70,71).

Multipeptide vaccines targeting non-mutant antigens is another strategy that holds several advantages over single antigen and neoantigen vaccines, including preconfirmed immunogenicity and the clinical feasibility of the off-the-shelf approach. One recent example is IMA950, composed of 11 tumor-associated antigens, of which robust immunogenicity had been previously demonstrated in the context of HLA-A2 (72). A single-arm, phase I/II study, demonstrated not only feasible tolerability, but also an improved survival benefit in the IMA950/poly-ICLC protocol compared with IMA950/granulocyte macrophage colony-stimulating factor (GM-CSF) used in the previous study (NCT01920191) (73,74). Interestingly, the researchers amended the vaccine administration route from intradermal (i.d.) to intramuscular (i.m.) or subcutaneous (s.c.) injections in the middle of the study, significantly improving the induction of CD8+ and CD4+ T-cell responses. The vaccine is currently being investigated in combination with varlilumab (anti-CD27-mAb) (NCT02924038) and with pembrolizumab (NCT03665545). Narita et al. (75) recently reported another study of multiple peptide vaccines conducted in Japan. In this randomized, double-blinded, phase-III trial, 58 HLA-A24+ patients were administered 4 out of 12 warehouse peptides (ITK-1) carefully chosen based on their baseline peptide-specific IgG responses (76). Although the study failed to show survival benefits, interestingly, the selection of one warehouse peptide (SART2–93) significantly correlated with shorter survival and poorer cellular immunity compared with the others.

Another recent advancement involves a more personalized approach, through the targeting of private neoantigens—tumor-specific protein-coding mutations—on an individual basis. In 2019, Hilf et al. (77) reported the results of a phase I GAPVAC-101 trial (NCT02149225), in which 15 HLA-A*02:01 or 24:02 patients with newly diagnosed GBM were treated with the 'personalized' vaccine cocktails. This study employed two separate steps of antigen selection for the actively personalized vaccine 1 (APVAC1) and APVAC2. For APVAC1, 6–7 antigens were carefully selected from a warehouse of pre-manufactured, non-mutant HLA class-I peptides and administered together with GM-CSF and poly-ICLC (78). For APVAC2, the neoantigens were preferentially selected based on transcriptome, HLA class I peptidome and pre-vaccine T-cell reactivity tests on an individual basis while the ligandome-based approach failed in neoantigen detection in this study cohort (79). The overall results from the GAPVAC-101 trial were encouraging in terms of safety as well as inducement of robust immunogenicity. In particular, an induction of APVAC1-antigen-reactive CD8+ T-cells was observed in PBMCs from most patients, accompanied by a shift to a memory phenotype. On the other hand, neoantigen vaccines in APVAC2 preferentially induced a CD4+ T-cell response. In another paper published back-to-back with the above-mentioned literature, Keskin et al. (80) also reported encouraging results from their phase I/Ib study of a personalized neoantigen vaccine (NeoVax study, NCT02287428). Using entirely *in silico* neoantigen prediction, 8 patients with newly diagnosed GBM were treated with a median of 12 synthetic peptides. Of note, two patients, not requiring dexamethasone during the vaccine-priming period, exhibited robust *de novo* T-cell responses against multiple neoantigens. CD8+ and CD4+ T-cells were both induced and enriched in an antigen-experienced memory phenotype with poly-functionality. In addition, post-vaccine tumor specimens from these two patients showed significant increases in tumor-infiltrating T-cells. TCR repertoire analyses revealed that a fraction of neoantigen-reactive T-cell clonotypes was shared between the post-vaccine tumor and blood samples, with increased frequency in the tumor, indicating the successful trafficking of vaccine-induced neoantigen-reactive T-cells to the tumor site.

Cellular immunotherapy for gliomas

Compared to ICIs and vaccines, cellular immunotherapy is a more straightforward approach, as preactivated T-cells will be directed to the tumor bed after infusion. Although TIL therapy has shown high-objective response rates in metastatic melanoma (81), its applications in primary brain tumors are limited (82,83), possibly owing to the sparsity of TILs and tumor-specific antigens. Instead, several modes of genetically engineered cellular immunotherapies have been actively investigated in gliomas, including CAR-T targeting cell-surface antigens, such as IL13Rα2 (84), HER2 (85), EphA2 (86) and

Table 1. Recently completed clinical trials of vaccine for glioma

Clinical trial ID	Active treatment	Adjuvant	Study design	Study subject	N	Primary endpoint	Main findings
Phase III							
NCT01480479 (ACT-IV) (20)	EGFRvIII vaccine (Rindopepimut)	GM-CSF	Multi-center, randomized, double-blind	Newly diagnosed EGFRvIII+ GBM	745	OS	Median-OS: 20.1 vs 20.0 m (HR 1.01, 95% CI 0.79–1.30; $P = 0.93$)
Phase II							
NCT01498328 (ReACT) (61)	EGFRvIII vaccine (Rindopepimut) + beva-cizumab	GM-CSF	Multi-center, randomized, double-blind	Relapsed EGFRvIII+ GBM	73	6 m-PFS	6 m-PFS: 28% for rindopepimut vs 16% for control ($P = 0.12$, one-sided)
NCT01280552 (167)	DCs pulsed with 6 synthetic GAA peptides (ICT-107)	-	Multi-center, randomized, double-blind, placebo-controlled	HLA-A1 or A2+ newly diagnosed GBM	124	OS	Median-OS: 17.0 vs 15.0 m (HR 0.87, $P = 0.58$) HLA-A2+ patients showed higher cellular immune response
Phase I							
NCT02454634 (NOA-16) (67)	IDH1-R132H peptide vaccine	Montanide + imiquimod	Single-arm, open-label	Grade III-IV glioma with IDH1 R132H mutation	39	Safety	Neither RLT nor severe AE were observed. Mutation-specific T-cell or humoral immune response was induced in 80% and 87%, respectively
NCT02960230 (PNOC-007) (70,71)	H3.3-K27M peptide vaccine	Montanide + TT + poly-ICLC	2-arm, open-label	H3.3K27M+ pediatric DMG/DIPG	49	Safety, 12 m-OS	Seven Grade 3 and zero Grade 4 treatment related AE. 12 m-OS: 40% for DIPG and 39% for other DMG. CyTOF analyses of PBMCs revealed the expansion of mutation-specific CD8+ T-cells
NCT01250470 (168)	Survivin peptide vaccine (SurVaxM)	Montanide + GM-CSF	single-arm, open-label	Survivin+ relapsed malignant glioma	9	Safety	No grade 3–4 vaccine-related AE. Both cellular and humoral immune responses were induced in 75% (6 of 8)
NCT01920191 (74)	Multi-GAA peptide vaccine (IMA950)	Poly-ICLC	Single-arm, open-label	HLA-A2+ newly diagnosed GBM	19	Safety	Four patients developed cerebral edema with rapid recovery. CD8 T-cell responses to a single or multiple peptides were observed in 63.2% and 36.8%, respectively. Protocol modification significantly improved vaccine efficacy
NCT02149225 (GAPVAC-101) (77)	Personalized multi-GAA and neoantigen vaccine (APVAC1/2)	Poly-ICLC + GM-CSF	Multi-center, open-label	HLA-A*02:01+ or 24:02+ newly diagnosed GBM	16	Safety, cellular immune responses	Favorable safety and robust immunogenicity were confirmed. APVAC1 and 2 antigens preferentially induced sustained CD8+ Tcm and CD4+ Th1, respectively

GM-CSF, granulocyte macrophage colony-stimulating factor; GBM, glioblastoma; OS, overall survival; PFS, progression-free survival; DC, dendritic cell; GAA, glioma-associated antigen; RLT, regime-limiting toxicity; TT, tetanus toxoid peptide; Tcm, central memory T; Th1, helper 1 type T.

EGFRvIII (87) as well as TCR-T-cell therapy targeting intracellular antigens like H3.3-K27M (69). Brown et al. (88) conducted a phase I trial of IL13Rα2-directed CAR-T with various administration routes for patients with recurrent malignant gliomas, in which a representative case exhibited a dramatic and sustained clinical response after intraventricular infusion (NCT02208362). Recently, the researchers initiated another phase I trial to test the therapy in combination with ICIs (NCT04003649).

Table 2. Ongoing clinical trials of vaccines for glioma

Clinical trial ID	Active treatment	Adjuvant	Study design	Study subject	N	Primary endpoint	Status
Phase III							
NCT02546102 (STING)	DCs pulsed with six synthetic GAA peptides (ICT-107)	-	Multi-center, randomized, double-blind, placebo-controlled	HLA-A2+ newly diagnosed GBM	414	OS	Suspended
NCT00045968 (DCVax-L) (169)	DCs pulsed with autologous tumor lysate	-	Multi-center, randomized, double-blind, placebo-controlled	Newly diagnosed GBM	348	PFS	Unknown
Phase II							
NCT02455557	Survivin peptide vaccine (SurVaxM)	Montanide + GM-CSF	Multi-center, open-label	Survivin+ newly diagnosed GBM	64	6 m-PFS	Active
NCT04013672	Survivin peptide vaccine (SurVaxM) + pembrolizumab	Montanide + GM-CSF	Single-arm, open-label	Relapsed GBM	51	6 m-PFS	Recruiting
NCT01204684	DCs pulsed with autologous tumor lysate	Resiquimod or poly-ICLC	Randomized, open-label	Newly diagnosed grade III-IV glioma	60	Most effective combination	Active
Phase I							
NCT02193347 (RESIST)	IDH1 R132H peptide vaccine (PEPIDH1M)	Td	Single-arm, open-label	Grade II, relapsed glioma with IDH1 R132H mutation	24	Safety	Active
NCT02924038	Multi-GAA peptide vaccine (IMA950) + neoadjuvant varlilumab	Poly-ICLC	Randomized, open-label	HLA-A2+ grade II glioma	30	Safety, cellular immune responce	Recruiting
NCT03665545 (IMA950–106)	Multi-GAA peptide vaccine (IMA950) + pembrolizumab	Poly-ICLC	Randomized, open-label	HLA-A*02:01+ relapsed GBM	24	Safety	Recruiting
NCT02287428 (NeoVax) (80)	Personalized neoantigen vaccine + pembrolizumab (later added)	-	4-Arm, randomized, open-label	Newly diagnosed GBM	56	Safety	Active

Td, tetanus-diphtheria toxoid.

An emerging cell-surface target for CAR-T is disialoganglioside (GD2) (89). Its expression is primarily restricted to the CNS after birth whereas its aberrant expression is observed in some neuroectodermal-origin tumors, such as melanoma (90) and neuroblastoma (91). Owing to its high tumor specificity, immunotherapy targeting GD2 has been investigated since the 1980s mainly for neuroblastoma (92). Rossig et al. (93) first described a GD2-targeting CAR-T. Although GD2 is expressed on neurons at low levels, neurotoxicity has not been observed clinically (94–96). Encouraged by these studies, Mount et al. (97) found uniformly high expression of GD2 in patient-derived H3-K27M-mutant DIPG cultures, which was even higher than neuroblastoma and sarcoma cell lines. By contrast, the expression was far lower in H3 wild-type DIPG cultures. The researchers also developed a GD2-CAR-T for DIPG and tested its cytotoxicity in xenograft preclinical models. The CAR-T eradicated H3 K27M-mutant tumors in a GD2-dependent manner both *in vitro* and *in vivo*. In May 2020, a phase I clinical trial started enrolling patients for the treatment of DIPGs and spinal DMGs with the GD2-CAR-T (NCT04196413). Other such representative, ongoing clinical trials are summarized in Table 3.

Viral immunotherapy for gliomas

Oncolytic viruses (OV) not only have cytolytic effects on cancer cells but also trigger various pro-inflammatory signals, which recruit cytotoxic T-cells to the TME (98). Therefore, OV therapies are designed with the expectation of inciting direct tumor-targeted oncolysis as well as acting as an *in situ* tumor vaccination. Among a variety of viral immunotherapy currently investigated in gliomas (99,100), we briefly touch on the following: adenovirus (DNX-2401/-2440), herpes simplex virus-1 (HSV-1) (G207 and G47Δ), and retroviral replicating vector (RRV) (Toca-511/5-FC), all of which have demonstrated the most remarkable advances in both preclinical and clinical investigation.

Table 3. Ongoing clinical trials of genetically-engineered cellular immunotherapy for glioma

Clinical trial ID	Active treatment	Administration route	Study subject	N	Primary endpoint	Status
Phase I						
NCT04196413 (97)	GD2 CAR	N/A	H3K27M-DMG/DIPG	54	Feasibility, MTD, safety	Recruiting
NCT02208362 (88)	IL13Rα2 CAR	Intratumoral or intracavitary or intraventricular	Recurrent grade III-IV glioma	92	Safety	Recruiting
NCT04185038	B7H3 CAR	Intracavitary or intraventricular	Recurrent CNS tumor or DMG/DIPG	70	Safety and feasibility	Recruiting
NCT03500991	HER2 CAR	Intracavitary (arm A) or intraventricular (arm B)	HER2+ recurrent pediatric CNS tumor	48	Safety and feasibility	Recruiting
NCT03638167	EGFR806-specific CAR	Intracavitary (arm A) or intraventricular (arm B)	Recurrent EGFR+ CNS tumors	36	Safety and feasibility	Recruiting
NCT04077866	B7H3 CAR	Intratumoral or intraventricular	Recurrent GBM	40	OS	Recruiting
NCT04003649	IL13Rα2 CAR + nivolumab +/− ipilimumab	Intracavitary or intraventricular	Recurrent GBM	60	Safety, feasibility, and 9 m-survival	Recruiting
NCT04045847	CD147 CAR	Intracavitary	Recurrent GBM	31	Safety	Recruiting
NCT04214392 (170)	Chrolotoxin (CLTX) CAR	Intracranial (dual delivery)	MMP2+ recurrent GBM	36	Safety	Recruiting
NCT03726515	EGFRvIII CAR + pembrolizumab	Intravenous	Newly diagnosed, EGFRvIII+, MGMT-unmethylated GBM	7	Safety	Recruiting
NCT02442297	HER2 CAR	Intracranial	HER2+ recurrent CNS tumors, pediatric and adult	28	Safety	Recruiting
NCT03412877	Mutated neoantigen TCR (virally engineered) +/− pembrolizumab	intravenous	GBM and other types of cancer	270	Response rate	Recruiting
NCT04102436	Mutated neoantigen TCR (non-virally engineered)	intravenous	GBM and other types of cancer	210	Response rate	Recruiting

CAR, chimeric antigen receptor; DMG, diffuse midline glioma; DIPG, diffuse intrinsic pontine glioma; MTD, maximum tolerated dose; CNS, central nervous system; MMP2, metalloproteinase; MGMT, O-6-methylguanine-DNA methyltransferase; TCR, T-cell receptor.

Adenovirus (DNX-2401). DNX-2401 (originally delta-24-RGD) is a second generation conditionally replicative oncolytic adenovirus (CRAd) designed to selectively replicate in tumor cells with Rb pathway dysregulation (101). In a phase I trial (NCT00805376), DNX-2401 was administered to patients with recurrent, high-grade gliomas via intratumoral injection alone (group A) or followed 2 weeks later by *en bloc* tumor resection (group B). A sufficient safety profile as well as clinical efficacy with a 3-year survival rate of 20% was demonstrated in group A. In group B, successful spread and replication of the virus and induction of an antitumor immune response were confirmed in 55% of the post-treatment tumor specimens (102). A variety of phase I-II clinical trials have been subsequently conducted, in combination with dose-dense TMZ (NCT01956734), pembrolizumab (NCT02798406) (103), IFNγ (NCT02197169) (102) and mesenchymal or neural stem cell delivery (NCT03896568, NCT03072134) (104,105). In addition, preclinical models of pediatric high-grade glioma or DIPG have shown that DNX-2401 enhanced radio-sensitivity and increased both CD4+ and CD8+ T-cell infiltration, providing a rationale for combination therapy with radiotherapy (RT) (106,107). Based on this evidence, a phase I trial combining these treatments was conducted for a small cohort of pediatric DIPG patients and completed in April 2020 with results yet to be reported (NCT03178032). Moreover, a third generation, DNX-2440 (or Delta-24-RGDOX) was recently developed, and is now being tested in a phase I trial (NCT03714334) (108).

HSV-1 (G207 and G47Δ). A second-generation HSV-1, G207, in which the viral ribonucleotide reductase (RR) is inactivated so that replication of G207 is limited to dividing cells, has been tested for more than two decades (109). Subsequent to a series of phase I/Ib

trials (NCT00028158) (110,111), Ring et al. (112) reported that a variety of aggressive pediatric brain tumor cells were more sensitive to G207 (by 22-fold) than adult GBM due to significantly higher levels of the virus-entry receptor, CD111 (nectin-1). Encouraged by this finding, G207 is currently being tested in two phase I clinical trials with or without RT in pediatric patients with recurrent/progressive malignant brain tumors (NCT02457845 and NCT03911388) (113).

A third-generation HSV-1, G47Δ, was developed by Todo et al. (114). A modification of G207 through a deletion in the alpha47 gene resulted in enhanced viral replication, immediate oncolytic activity and improved stimulation of TILs. Following a phase I/IIa trial (UMIN000002661) confirming safety, a phase II trial was conducted for patients with residual or recurrent GBM (UMIN000015995), in which G47Δ was stereotactically and repeatedly injected into the tumor ∼6 times. A 1-year survival rate of 92.3% was shown in the interim analysis, leading to early termination of the trial as it fulfilled predetermined criteria (115).

RRV (Toca 511/Toca FC). Toca 511 (vocimagene amiretrorepvec)/Toca FC is the most advanced viral immunotherapy for treatment of glioma in terms of development and clinical trial phase. Toca 511 has an RRV backbone containing a yeast-derived cytosine deaminase (CD) transgene (116). This treatment strategy is described as prodrug-activator gene therapy since the major role of the Toca 511 RRV is to convert the infected cells into stable, vector-producing cells through integration of the CD gene into the tumor cell genome (117). The expressed CD enzyme intracellularly converts the antifungal pro-drug, 5-fluorocytosine (5-FC, administered as Toca FC) into the anticancer drug, 5-fluorouracil (5-FU). Preclinical studies provide evidence of a significant improvement of survival as well as favorable changes in antitumor immunity, such as a reduction of immunosuppressive myeloid cells, polarization away from T_H2 and T_H17 in CD4+ T-cells and an increase of IFNγ-expressing CD8+ T-cells (116,118,119). Three phase I clinical trials showed a tolerable safety profile and improved survival benefit as compared with external controls, while overall viral loads were controlled systematically (NCT01156584, NCT01470794 and NCT01985256) (120–122). Recently, a phase 2/3 trial was conducted, in which Toca 511 was directly injected into the tumor resection cavity and Toca FC was initiated at 6 weeks post-surgery (NCT02414165). This study, however, was terminated in 2019 after failing to meet both primary and secondary endpoints, the improvement of OS and objective responses in the treatment group as compared with the standard-of-care group (median OS: 11.1 vs 12.2 months), while final results are yet to be published as of June 2020 (123). Although another phase II/III clinical trial involving Toca 511 (NRG-BN006) had been prepared for patients with newly diagnosed GBMs, it was later withdrawn, possibly owing to the failure of the aforementioned study.

Recent advances for brain metastases

In this section, we overview the current status of ICIs and cellular immunotherapies for BMs. In the past, clinical studies with chemotherapeutic agents as well as immunotherapies had generally excluded patients with BMs from participation. However, this notion has been eventually revised as described in following sections.

ICIs for brain metastases

In contrast to gliomas, the efficacy of ICIs is nowadays well-acknowledged for BMs. A retrospective analysis of a phase III trial of ipilimumab (anti-CTLA-4 mAb) for advanced melanomas, unexpectedly found that the treatment efficacy was comparable between patients with and without BMs (16). This observation was further supported by a series of prospective and retrospective studies (124–127). A subsequent phase II, prospective study with pembrolizumab was conducted for patients with melanoma or non–small-cell lung cancer (NSCLC) BMs, and showed durable objective responses of 22% and 33%, respectively (NCT02085070) (128). Long-term follow-up data for the 34 patients in NSCLC cohort reported a CNS response rate of 29.4% and a 2-year OS rate of 31% (17). CheckMate-204, a phase II study of ipilimumab and nivolumab for patients with BMs of melanoma (NCT02320058), showed an intracranial response rate of 57%, including a complete response rate of 26% (18). Of note, intra- and extra-cranial responses were highly concordant. Similar findings were reported in another phase II study on BMs of melanoma conducted in Australia (ABC trial, NCT02374242) (129). To further validate these findings, a randomized phase III study testing (i) fotemustine (a nitrosourea alkylating agent) alone vs. (ii) fotemustine and ipilimumab or (iii) ipilimumab and nivolumab is currently ongoing for patients with melanoma BMs (NIBIT-M2 trial, NCT02460068).

Cellular immunotherapy for brain metastases

Progress has also been made using cellular immunotherapies for treatment of BMs. For example, Priceman et al. (130) investigated their HER2-CAR-T for treatment of breast cancer BMs in preclinical models. This treatment strategy is motivated by the observation that BMs occur in ∼50% of HER2+ breast cancer patients. It was hypothesized that a cellular immunotherapy approach could cross the BBB, overcoming the challenge observed in HER2-targeted mAbs. As previous clinical studies have demonstrated safety and efficacy of systemic administration of HER2-CAR-T in patients with sarcoma and recurrent GBM (131,132), the authors sought to optimize the administration routes, the CAR constructs, preconditioning and the dose. In their xenograft mice models, trastuzumab-based HER2-BBζ CAR-T successfully eradicated HER2+ breast cancer BMs after local intratumoral or regional intraventricular delivery while systemic administration was significantly less effective. Interestingly, intraventricular delivery showed robust antitumor activity even against multifocal disease and leptomeningeal spread, which is a relatively common issue in metastatic breast cancer. Based on the therapeutic efficacy in this preclinical study, a phase I clinical trial was recently launched in which HER2-positive breast cancer patients with BMs and/or leptomeningeal carcinomatosis are administered local or regional delivery of HER2-CAR-T (NCT03696030).

Immunotherapy for primary and secondary brain tumors: the contrariety in efficacy

As described in previous section, the development of successful immunotherapies for glioma is far behind many other types of tumors. On the other hand, the efficacy of ICIs and cellular therapies has been recognized to be comparable between intra- and extra-cranial lesions for the treatment of melanoma or lung cancer BMs. Why is there such a disparity in efficacy with similar therapeutics in tumors that both occur in the CNS? An explanation could be contrived from commonly acknowledged major obstacles to therapy for gliomas including (i) the paucity of and spatial, temporal and intertumoral heterogeneity of antigens (48,62,133); (ii) the glioma-induced immunosuppressive TME characterized by enrichment

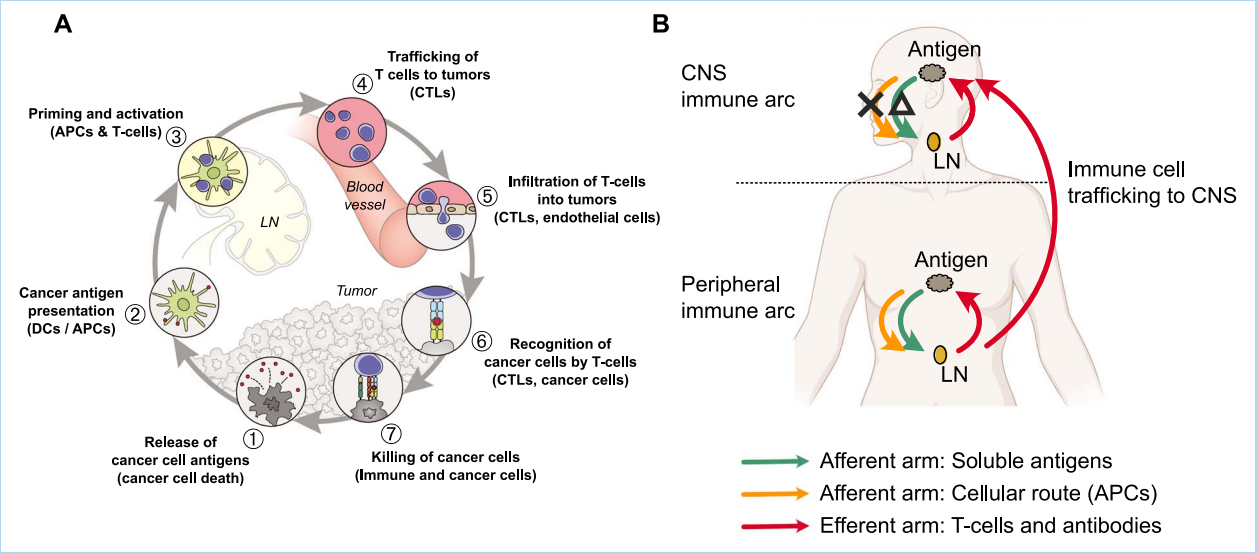

Figure 1. Cancer-immunity cycle and immune surveillance of CNS and non-CNS tumors. A. Cancer-Immunity Cycle. Source: *Reprinted with permission from Elsevier. Chen DS and Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. Immunity 2013.* (142) B. Difference in afferent and efferent arm of immune-surveillance between CNS and non-CNS tumors, Source: *Adapted from Galea et al.* (138). LN, lymph node; APCs, antigen-presenting cells.

of microglia/macrophages and upregulated TGF-ß, IL-10, STAT3 and IDO/kynurenine (25,134–137); (iii) the anatomically and functionally restricted antigen presentation due to downregulated MHC expression, the presence of the BBB and the absence of intracranial regional lymph nodes (LNs) in the CNS (14,138,139); and (iv) a highly infiltrative growth pattern that makes it harder for therapeutic agents to traffic to (140,141). Some features are shared with BMs but others are not. Among them, it is our particular interest to shed more light on the issue of restricted antigen presentation in the CNS, and the difference of the TME between primary and metastatic brain tumors.

A restricted afferent arm of immune surveillance in the CNS

When unique features of CNS immunity are discussed, the presence of the BBB often takes precedence as a restrictive entity. However, this is not always the case for T-cell migration across the BBB. To better understand the trafficking of T-cells throughout the CNS, we will use the cancer-immunity cycle (142) composed of seven key elements pertinent to the function and connectivity of the CNS (Fig. 1A). It is known that deep cervical LNs function as the primary draining LNs for intracranial lesions (relevant to element 3) (143–145). It is also appreciated that once 'educated', activated effector T-cells can traffic to the CNS and travel through the BBB (element 4 and 5) (146,147). There is accumulated evidence demonstrating antigen-specific T-cell migration and resultant tumor immunoediting in response (elements 6 and 7) (80,148).

On the other hand, with respect to elements 1 and 2, the CNS parenchyma has highly isolated afferent communication with lymphatic systems owing to functional and anatomical restrictions (Fig. 1B). To overcome element 2 in the cancer-immune cycle, tumor antigens must be taken up by antigen-presenting cells (APCs), such as dendritic cells (DCs), macrophages and B cells, either inside or outside the tumor bed (149). For example, conventional DCs have been identified in the choroid plexus and meninges in animal models (139). However, it is uncertain whether beneficial antigen presentation occurs inside the glioma TME, even if a small number of DCs are present, because of the enriched population of tumor-associated, immunosuppressive, pro-tumorigenic microglia/macrophages (TAMs) as described later.

When considering the modes of antigen presentation outside the tumor bed, it is crucial to understand the drainage routes of extracellular fluid in the CNS—interstitial fluid (ISF) and cerebrospinal fluid (CSF) (145). They drain into regional LNs via different routes, as reviewed in detail in the following references (14,147,150-152) (Fig. 2A). ISF and solute antigens drain from the parenchyma to cervical LNs along 100–150 nm wide arterial intramural perivascular spaces (Fig. 2B), as experimentally demonstrated both in animals and humans (153,154). Since this pathway is too narrow for APCs to migrate (154), they must rely on the CSF drainage route. Perivascular space surrounding postcapillary venules is connected with the CSF space consisting of the subarachnoid and intraventricular spaces (Fig. 2C). CSF can drain to the cervical LNs, mainly passing through the cribriform plate of the ethmoid bone to the nasal mucosa, or through the recently discovered peri-venous sinus dural lymphatics (Fig. 2A). However, there remain critical unanswered questions: which type of APCs play a leading role in antigen capture, where does this occur, and by what mechanism(s)? One possible explanation involves the intraventricular and subarachnoid spaces, where a small number of DCs are present. Nevertheless, the communication between ISF and CSF is strictly restricted by the 'glymphatic system', a perivascular channel system formed by astrocytic endfeet and basement membrane; only a tiny fraction of ISF (15%) is secreted into the CSF (14), and the role of the glymphatic system in this process still remains controversial (147,155). Another scenario focuses on macrophages localized in the perivascular space of the postcapillary venules (Fig. 2B). But their role in this context is still undetermined (156).

Consequently, the afferent arm of immune surveillance, or element 2 in the cancer-immunity cycle, is highly restricted in the CNS; the adaptive immune response is hardly triggered spontaneously to the antigens of primary CNS tumors. On the other hand, such a conclusion is not applicable to the case of BMs since a more efficient

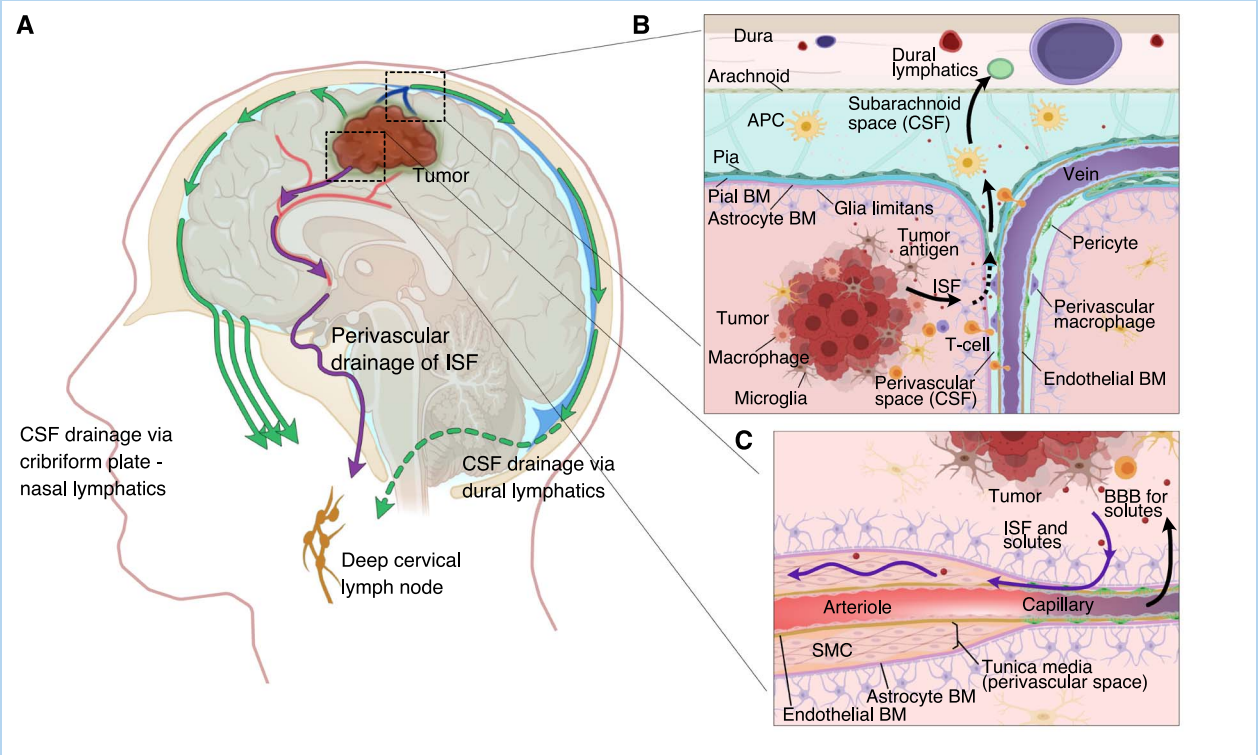

Figure 2. Neuroanatomical restriction of antigen presentation. A. Three main potential routes out of the intracranial diseases for antigen presentation at the deep cervical LN. ISF containing antigens can migrate through arterial perivascular space into the LN (shown in purple), but this space is too narrow for immune cells. In contrast, immune cells that are capable of capturing the antigens may reach to the LN using the CSF routes, either cribriform plate-nasal lymphatics, or peri-sinus dural lymphatics (shown in green). B. Perivascular space of postcapillary venules directly connected to the subarachnoid space. Extravasation of immune cells can take place at neither arteriole nor capillary but at postcapillary venule levels. Although ISF-CSF exchange is restricted by glia-limitans, it is thought that APCs in the CSF spaces, such as dendritic cells may play a key role in the tumor antigen capture. C. On the other hand, ISF containing the soluble antigens can drain at capillary levels and migrate within the arteriole perivascular space (tunica media) toward the LN, retrograde to blood flow. BBB, blood–brain barrier; BM, basement membrane; CSF, cerebrospinal fruid; ISF, interstitial fluid; SMC, smooth muscle cell. Source: *Adapted from Engelhardt et al.* (14) and *Ratnam et al.* (152).

antigen presentation is expected at the extracranial primary disease site (142,150,157). Hence, the contrasting clinical efficacy of ICIs for gliomas and BMs can be explained by the differences in magnitude and range of antigen presentation in either case. Currently, many of the immunotherapies in development, such as vaccines and immune cell therapies, are designed to bypass the issue of poor antigen presentation in a more targeted fashion.

Difference in TME

The TME is comprised of cancer cells as well as many different noncancerous cell types, such as endothelial cells, fibroblasts and immune cells. In addition, tissue-resident cell types in the CNS, such as microglia, astrocytes and neurons contribute to the formulation of an immunosuppressive environment (36,158). It is well acknowledged that ~30–50% of cellular components in the TME of GBM are comprised of myeloid cells, such as microglia, macrophages and myeloid-derived suppressor cells (MDSCs) (25). In particular, immunosuppressive 'M2' TAMs are recruited by CSF-1 secreted by glioma cells (159), and lack the costimulatory molecules CD80, CD86 and CD40 essential for T-cell activation (160). In addition, secretion of IL-10 and TGF-ß by these M2-TAMs not only downregulates the expression of MHC class-II on glioma and myeloid cells, but also suppresses DC maturation (161,162), provoking defects in the antigen-presenting machinery (25,134). Moreover, beyond the organ specificity, disease-specific genetic alterations, such as IDH1 mutation (53,54,163), NF1 loss (46) and N-Myc amplification (164), can all contribute to local immunosuppression.

Furthermore, two separate studies recently dissected the disease-specific features of the TME with high-resolution analyses (165,166). Friebel et al. (165) conducted a mass cytometry-based single-cell analysis of surgically resected brain tumors and non-tumor controls from 38 patients. Concordantly with the other study, they found TAMs dominated the TME in gliomas (~80% of leukocytes) whereas lymphocytes dominated in BMs (~50%). Of note, glioma predominantly harbored TAMs of microglial origin, whereas BMs were highly invaded by a higher number of monocyte-derived macrophages (MDMs). Interestingly, macrophage composition was very similar between IDH-mutant gliomas and non-tumor brain tissue. By analyzing the developmental trajectory of monocyte-to-MDM transition, the researchers clarified that even the composition of MDMs was distinct among diseases. In addition, regarding T-cells, the glioma TME was characterized by lower expression of activation markers whereas the metastatic TME was composed of activated/exhausted T-cells.

Klemm et al. (166) also investigated the difference of the TME among non-tumor brain tissue, IDH-mutant and wild-type gliomas, as well as several types of BMs using flow-cytometry,

immunofluorescence (IF), RNA-sequencing (RNAseq) and spatial tissue characterization. Within CD45+ cells in clinical tumor specimens, a significant enrichment in myeloid cells was observed in gliomas regardless of IDH status, while the abundance of lymphoid lineage cells was significantly higher in IDH-wild-type gliomas and BMs. RNA-seq analyses demonstrated that a substantially higher proportion of lymphocytes with the most diverse landscape was observed in BMs, especially those originating from melanoma. Interestingly, the lymphocyte composition also varied among BMs of different origins; for example, T cells were dominant in melanoma BMs, whereas breast cancer BMs were characterized by the highest neutrophil infiltration. In addition, the analysis on the spatial relationship of TILs using IF-phenotyped tissue sections revealed that both TAM populations resided close to T cells more frequently in BMs, suggesting their interaction. By contrast, in IDH-wild-type gliomas, tumor-associated microglia and MDMs lacked T cells in their close vicinity.

In summary, both studies consistently demonstrated that immune cell composition and their respective phenotypic and functional features are dependent on the disease type, instead of the CNS tissue environment itself. It is reasonable that the difference of ICI efficacy may be, at least in part, explained by such a difference of the TME. In addition, although encouraging data have been reported for treatment of melanoma and lung cancer BMs with ICIs so far, their efficacy may not be uniform among BMs originating from other cancer types with different TME compositions.

Concluding remarks

As discussed, although no established, effective immunotherapy has been developed for glioma thus far, several promising approaches have been proposed in the last few years, such as neoadjuvant ICIs, personalized multipeptide vaccines, CAR-T therapies and virus-based immunotherapies. In addition, accumulated data has demonstrated that ICIs can be an effective treatment option for some types of BMs. Although the CNS can no longer be considered completely immune-privileged, this does not lighten the remaining challenges immunotherapeutic modalities must overcome, especially in encountering restricted antigen presentation and the immunosuppressive TME. This has been partially reflected in the differences in TIL composition and ICI efficacy between primary and secondary brain tumors. Better understanding of these unique immunological characteristics of the CNS as well as thoughtful design of combinatorial therapeutic modalities will be required for future success in immunotherapy for both primary and secondary tumors.

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Conflict of interest statement

Hideho Okada is an inventor of the following US utility patent applications; 'H3.3 CTL peptides and uses thereof' (Case Number, SF2015–163), which has been exclusively licensed to Tmunity, Inc., 'Anti-EGFRvIII chimeric antigen receptor (Case Number , U Penn 02980), which has been exclusively licensed to Novartis Pharma, Inc. and 'Identification of an IL-13 Receptor Alpha2 Peptide Analogue Capable of Enhancing Stimulation of Glioma-Specific CTL Response', which has been exclusively licensed to Stemline, Inc.

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