Emerging nanomedical strategies for direct targeting of pediatric and adult diffuse gliomas

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INTRODUCTION

High-grade gliomas, in particularly diffuse midline glioma, H3K27-altered in children and glioblastoma in adults, are the most lethal brain tumour with a dismal prognosis. Developments in modern medicine are constantly being applied in the search for a cure, although finding the right strategy remains elusive. Circumventing the blood–brain barrier is one of the biggest challenges when it comes to treating brain tumours. The cat and mouse game of finding the Trojan horse to traverse this barrier and deliver therapeutics to the brain has been a long and hard-fought struggle. Research is ongoing to find new and feasible ways to reach specific targets in the brain, with a special focus on inoperable or recurring brain tumours. Many options and combinations of options have been tested to date and continue to be so in the search to find the most effective and least toxic treatment paradigm. Although improvements are often small and slow, some of these strategies have already shown promise, shining a light of hope that finding the cure is feasible. In this review, we discuss recent findings that elucidate promising but atypical strategies for targeting gliomas and the implications that this work has on developing new treatment regimens.

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only months. Other causes for therapeutic failure include the diffuse invasion of glioma cells into the surrounding brain, often sheltering them from both surgery and radiation, and the challenge of delivering sufficient doses of chemotherapy across the blood–brain barrier (BBB) [1].

Considering the overall poor outcome from current treatment options for both operable and inoperable glioma, the need for improved targeted therapies becomes evident in overcoming this highly lethal disease in children and in adults. In recent years, numerous review papers have been written outlining the conventional strategies in treating gliomas as well as those that have shown early in vitro or pre-clinical promise. Current strategies in overcoming the BBB for drug delivery, such as focused ultrasound and convection-enhanced delivery, as well as approaches involving nanoscale applications including liposomes, micelles, hydrogels and radiosensitisers, have previously been described in detail [9–11]. This review focuses on some of the more unconventional strategies that have had limited exposure but hold promise and potential in therapeutics targeting both pediatric and adult gliomas, from a directed perspective.

Although studies on both pediatric and adult gliomas are reviewed, most studies cited here are based on in vitro and pre-clinical adult glioma models, due to the lower availability of research material and a lack of literature in the pediatric field. In addition, enrolment of children in clinical trials is difficult, due to both low disease prevalence and parental reluctance, as well as lack of funding [12]. Nevertheless, registered clinical trials are steadily on the rise. Figure 2 outlines clinical trials registered on the ClinicalTrials.gov database between 22.07.2001 and 22.07.2021, based on condition or disease, of which just under 25% are pediatric (≤17 years).

### CELL-PENETRATING PEPTIDES

A major challenge in glioma therapy is the selective delivery of therapeutics to tumour cells behind the BBB, while limiting damage to other tissues. Although cell-penetrating peptides (CPPs) have been studied for nearly half a century, only the last decade or so has investigated their potential as a promising new strategy for targeted glioma treatment. CPPs are short peptides that can be coupled to various carriers for the selective delivery of drugs, imaging agents, nanoparticles, oligonucleotides and liposomes [13]. CPPs can target receptors which are overexpressed or limited to tumour cells as well as target abnormal signaling pathways. Through binding to their targets, these peptides can exert various effects, including the delivery of drugs. Although antibodies are often used in the clinic as tumour-targeting ligands, they nevertheless possess many limitations, including low stability in vivo, slow diffusion into the tumour tissue and high production costs. Compared to antibodies, tumour-targeting peptides have better BBB-penetrating capacities, traverse the cell membrane more readily and are easier to produce and modify [14]. Incorporating CPPs into drug delivery systems could therefore lead to more targeted and efficient glioma treatment.

Pre-clinical research has already shown promising results for CPP-mediated drug delivery in various cancer models, including glioma. A study in which the glioma homing CPP interleukin-13 peptide was anchored onto nanoparticles showed specific targeting and uptake by U87 MG cells, as well as enhanced penetration of particles into tumours both in vitro and in vivo [15]. When loaded with the chemotherapeutic docetaxel, these particles were also able to inhibit the growth of subcutaneous U87 MG tumours. In a similar study, conjugation of the glioma-targeting ligand transferrin with the CPP octa-arginine (R8) enhanced uptake and antiproliferative effects in U87 MG and GL261 glioma cell lines [16]. Another study showed a significant reduction of proliferation, migration and invasion of U87 MG cells in vitro following the uptake of a multifunctional construct consisting of the glioma homing peptide GL1, the tumour protein 53 (P53) C-terminal lysine-rich regulatory domain and the CPP polyarginine [17]. Similarly, it was shown that with small interfering RNAs a significant increase in gene-silencing specifically in U87...
MG cells and not in non-glioma cells can be achieved by combining the targeting peptide angiopep-2 and the PepFect peptide 14 as a delivery system [18].

Nanocarriers conjugating CPPs and polyethylene glycol (PEG)-lipid conjugates have also been designed to enhance the targetability of tumours through prolonged circulation time and exposure at the tumour site [19]. The high expression of matrix metalloprotease 2 (MMP2) in the tumour microenvironment was utilised to cleave the PEG chains specifically at the target location, exposing the CPP, in turn promoting the internalisation of the nanocarriers into the tumour cells. A gene delivery system based on the cleavage of PEG-lipid micelles within the MMP2-rich tumour microenvironment was recently used to effectively deliver the DNA repair inhibitor DNA strand break bait (Dbait) into glioma on the cleavage of PEG-lipid micelles within the MMP2-rich nanocarriers into the tumour cells. A gene delivery system based on the Dbait payload, which also acts as a radiosensitiser, specifically was conjugated to MMP2-responsive peptides, and used to deliver exposure at the tumour site [19]. The high expression of matrix lipid conjugates have also been designed to enhance the targeting of rat brain glioma [27]. In vitro assays showed that cytotoxicity of TMZ-SPIONs and TMZ-SPIONs-FA was 1.6 and 3.29-fold higher than free TMZ on rat C6 glioma cells, respectively. Analysis of MRI images before and after in vivo injections of TMZ-SPION-FA showed an increased influx of particles into the brain in the presence of an external magnetic field, indicating successful crossing of the BBB. To evaluate the biodistribution of the nanoparticles with and without FA, the heads of rats were placed in a magnetic field after injection with particles. The distribution of nanoparticles in the vital organs far from the field was significantly reduced, and a comparison of brain and tumour tissues showed that nanoparticles containing FA had a significantly higher distribution in tumour tissue. The therapeutic efficacy of this dual method was demonstrated by prolonged survival time and reduced tumour volume of C6 glioma-bearing rats.

Doxorubicin (DOX)-loaded SPIONs (DOX-SPIONs) have also been developed to study magnetic drug delivery to glioma cells both in vitro and in vivo [28, 29]. The uptake of DOX-SPIONs by C6 cells under a magnetic field was significantly increased compared to free DOX in vitro, leading to increased cytotoxicity. In glioma-bearing rats, ex vivo assays demonstrated that the DOX-SPIONs were delivered to the tumours by imposing external magnetic fields. Furthermore, this treatment led to the complete suppression of glioma growth after 28 days in vivo. The safety of these particles was verified through histological analysis of major organs. Similarly, a co-culture model was also used to examine the BBB permeability and anti-tumour activity of DOX-SPIONs compared to free DOX [29]. While DOX-SPIONs demonstrated enhanced permeability, cytotoxicity was similar in both studies. However, the combination of a cadherin binding peptide to temporarily open tight junctions with an external magnetic field significantly improved both DOX-SPION permeability and cytoxicity. As SPIONs clearly show potential, a follow-up study was performed in which a similar carrier loaded with salinomycin was established [30]. Consistently, results suggest that SPIONs combined with penetration enhancers and external magnetic fields could potentially lead to effective and specific targeting for glioma chemotherapy.

The potential of magnetic nanoparticles to target GBM cells that have spread within cerebrospinal fluid has also been explored [31]. Magnetic nanoparticles consisting of gold–iron cores bound to the chemotherapeutic agent etoposide, recognised for its intrathecal use, were established. These particles were shown to move at human size-distances in response to a rotating magnetic field. After remote targeting at 15 cm from the magnet, the etoposide particles significantly eradicated human U138 GBM cells compared to control particles in vitro. While further testing is necessary, intrathecal administration of magnetic drug carriers holds promise for the removal of glioma cells disseminated within the cerebrospinal fluid.

An alternative application of SPIONs is their utilisation as an imaging tool for delineation of resectable glioma in the operating room to guide surgeons. The use of fluorescent SPIONs for intraoperative imaging of GBM by targeting tumour-associated macrophages (TAMs) has already been investigated [32]. The fluorescent SPIONs selectively visualised TAM populations by in vitro live-cell imaging and in vivo fluorescence imaging. In orthotopic GBM xenograft models, the SPIONs could penetrate the BBB and successfully delineate the tumour. This study indicates the potential of SPIONs for improved intraoperative staging and more radical surgery.
Table 1. Examples of clinical trials incorporating the strategies being reviewed.

| ClinicalTrials.gov identifier | Year | Strategy                      | Phase | Sample size | Age (years) | Therapeutic applications                  | Endpoints                                      | Status                     |
|--------------------------------|------|-------------------------------|-------|-------------|-------------|-------------------------------------------|-----------------------------------------------|----------------------------|
| NCT01975116                    | 2013 | Cell-penetrating peptides     | I     | 18          | 3–21        | CNS tumours                               | Side-effects, dosage                          | Completed, no results       |
| NCT00914914                    | 2009 | Cell-penetrating peptides     | I     | 15          | ≥18         | Refractory solid tumour                    | Safety                                        | Completed, no results       |
| NCT00769093                    | 2008 | Iron oxide nanoparticles      | I     | 6           | ≥18         | HGG                                      | Observe microvascular changes                 | Terminated<sup>a</sup>     |
| NCT03179449                    | 2017 | Iron oxide nanoparticles      | I     | 10          | ≥2          | Malignant brain tumour                     | Macrophage characterisation                   | Recruiting                 |
| NCT03465618                    | 2018 | Carbon dots                   | I     | 10          | ≥18         | HGG                                      | Tumour localisation/distribution               | Recruiting                 |
| NCT01266096                    | 2010 | Carbon dots                   | N/A   | 23          | ≥18         | Malignant brain tumour                     | Characterisation                              | Active, not recruiting      |
| NCT04015700                    | 2019 | Neoantigens                   | I     | 12          | ≥18         | GBM                                      | Safety and feasibility                        | Recruiting                 |
| NCT03914768                    | 2019 | Neoantigens                   | I     | 10          | 1–75        | DIPG and GBM                              | Safety and potential benefit                  | Enrolling by invitation     |
| NCT04749641                    | 2021 | Neoantigens                   | I     | 30          | ≥5          | DIPG                                     | Safety and preliminary efficacy               | Recruiting                 |
| NCT04196413                    | 2019 | Neoantigens                   | I     | 54          | 2–30        | H3.3K27M<sup>+</sup> Glioma                | Feasibility, dosage                           | Recruiting                 |
| NCT05038150                    | 2021 | Genetically modified bacteria | I     | 50          | 18–75       | Refractory solid tumour                    | Safety and tolerability                       | Not yet recruiting          |
| NCT02718443                    | 2016 | Attenuated bacteria           | I     | 14          | ≥18         | rGBM                                     | Safety and tolerability                       | Completed                  |
| NCT03750071                    | 2018 | Attenuated bacteria           | I     | 30          | ≥18         | rGBM                                     | Efficacy and safety evaluation                | Recruiting                 |

Source: ClinicalTrials.gov.
CNS central nervous system, HGG high-grade glioma, GBM glioblastoma multiforme, DIPG diffuse intrinsic pontine glioma, rGBM recurrent GBM, N/A not applicable.

<sup>a</sup>Inadequate enrolment.
CARBON (NITRIDE QUANTUM) DOTS
Metal-free carbon nitride quantum dots (CDs) are nanoparticles with potential for biomedical applications as they harbour favourable properties such as good biocompatibility, photostability and tunable photoluminescence, as well as nontoxicity. Furthermore, being easy to produce and liquid dispersible makes them ideal candidates for application in bioimaging, drug delivery and theranostics [33]. The structural characteristics of CDs can also be modified for use in different biomedical applications. As CDs have a high loading capacity for chemotherapeutics, they are considered promising nanocarrier materials for cancer therapy. Non-selective interactions of some CDs with both tumour cells and normal cells have been previously reported and were seen as a disadvantage [34]. However, recent advances in CDs applications justify their investigation into glioma therapy strategies.

Using a simple one-step method, Ruan and colleagues prepared fluorescent carbon dots with demonstrated serum stability and low cytotoxicity, which could also be taken up by C6 glioma cells in vitro in a time- and concentration-dependent manner. In vivo, these carbon dots accumulated and distributed throughout the glioma in orthotopic C6 glioma-bearing mice, suggesting their potential in non-invasive glioma imaging applications [35]. Zheng and colleagues also prepared a fluorescent carbon dot variant, termed CD-Asp, based on D-glucose and L-aspartic acid, which exhibited excellent biocompatibility and a tunable emission spectrum. These carbon dots were seen to be highly selective for glioma cells in vitro, while in vivo analysis of C6 glioma-bearing mice showed that DC-Asp readily traversed the BBB and precisely targeted glioma tissue, with lower fluorescent signals being detected in normal brain tissue [36]. Both studies accentuate the potential of CDs in diagnostic, targeting and therapeutic functions, warranting further pre-clinical investigation.

Conjugates of CDs with the chemotherapeutic gemcitabine were investigated in their ability to selectively target and kill pediatric GBM cells in vitro [37]. Although gemcitabine has previously been shown to have potent anti-glioma effects in pre-clinical studies, human clinical trials have been largely disappointing [38], probably due to gemcitabine’s short plasma half-life, poor BBB penetration and dose-limiting toxicities. Conjugation of gemcitabine-CDs to transferrin showed the highly selective destruction of S/GMB2 glioma cells over non-cancerous HEK293 cells in vitro. BBB penetration capacity of these CDs was also demonstrated in vivo using a zebrashift model [37], highlighting the potential of CDs as nanocarriers for selective brain tumour drug delivery.

The synergistic effects of a triple conjugate targeting system, consisting of CDs conjugated to transferrin and the chemotherapeutic compounds epirubicin and TMZ, on pediatric and adult glioma cells, have also been investigated in vitro [39]. The efficacy of the triple system (two drugs and transferrin) was compared to dual systems (one drug and transferrin), non-transferrin CDs and free drugs. Results showed that the transferrin conjugated samples were more cytotoxic to the glioma cell lines compared to non-transferrin conjugates and that the triple systems were more cytotoxic than the dual systems. These results suggest that a triple conjugated CD system is a more capable therapeutic agent than single drug delivery systems.

Recently, a novel strategy to selectively image and deliver drugs to glioma using CDs structurally modified to mimic large amino acids that could bind to the large neutral amino acid transporter 1, which is expressed in most tumours, was examined [34]. As the BBB mostly limits drug delivery to brain tumours via traditional ligand-mediated methods, it was postulated that carrier transporters differentially upregulated in tumour cells might be a more effective approach. Results showed that intravenous injection of the CDs mimicking large amino acids selectively accumulated in an orthotopic mouse model of human glioma. Additionally, when loaded with the chemotherapeutic topotecan, these CDs enabled successful imaging of the tumours as well as significantly reduced tumour size without detectable toxicity.

A recent in vitro study combined the use of transferrin decorated magnetic nanoparticles with a different type of nontoxic quantum dots, namely InP/ZnS quantum dots, to investigate dual targeting and imaging of glioma [40]. The results demonstrated enhanced cellular uptake of these combined particles by glioma cells under an external magnetic field and showed both successful MRI imaging and improved fluorescence microscopy intensity. This is the first study to combine magnetic nanoparticles and quantum dots for anti-glioma therapy. However, further in vivo studies are needed to assess the safety and efficacy of this strategy in treating gliomas.

NEOANTIGENS AND TUMOUR-ASSOCIATED ANTIGENS
Neoantigens, or tumour-specific mutations, are more frequently being investigated as targets for glioma treatment utilising the immune response against the tumour. Advancements in cancer immunogenomics have allowed for the identification of targetable neoantigens, offering promise for the effectiveness of immunotherapy and priming it against tumours of the brain [41].

In comparison to adult brain cancer, pediatric brain cancers have less mutational load and corresponding neoantigens, and a tumour microenvironment with a smaller number of immune cell infiltrates. Consequently, immunotherapies developed for adult brain tumours are usually not as effective when applied to pediatric brain tumours, resulting in relatively fewer trials on immunotherapy for childhood glioma [42]. Fortunately, recent innovations have led to novel neoantigens and tumour-associated antigen strategies to be currently evaluated for adult as well as pediatric brain cancer.

A relatively recent neoantigen, lysine (K) to methionine (M) substitution at position 27 of histone 3 variant 3 (H3.3K27M), for glioma T cell therapy has been investigated rigorously since being identified [43]. The majority of pediatric diffuse midline gliomas (DMG) and more than 80% of DIPGs harbour this mutation, causing a global reduction of methylation on H3K27 and aberrant transcription of oncogenes. An HLA-A*02.01+ CD8+ T cell clone was established through stimulation with a synthetic peptide containing the H3.3K27M mutation. These T cells effectively killed HLA-A*02.01+ H3.3K27M+ glioma cells in an antigen- and HLA-specific manner in vitro. Transfer of these T cells also efficiently inhibited the progression of glioma xenografts in mice. Recently, the safety and efficacy of a H3.3K27M-targeted peptide vaccine were assessed in the Pediatric Neuro-Oncology Consortium [44]. Newly diagnosed patients, with HLA-A*02.01+ and H3.3K27M+ status, were enrolled in a DIPG (stratum A; 19 patients) or non-pontine DMG (stratum B; 10 patients) arm of the trial. The vaccine was administered together with an immunostimulant every 3 weeks for 8 cycles, followed by once every 6 weeks. Overall, the vaccine was well-tolerated and patients with H3.3K27M-specific CD8+ immunological responses showed prolonged overall survival compared to non-responders (16.1 vs. 9.8 months, p = 0.05). Although no significant differences in overall survival between the two strata were observed, these results warrant further studies targeting the H3.3K27M epitope.

The disialosanglioside GD2 is another tumour-associated antigen that has gained interest in cancer immunotherapeutics due to its limited expression in normal human tissues [45] and uniform, high expression in patient-derived H3.3K27M+ glioma cell cultures [46] and in glioblastoma [47]. A first-in-human trial was initiated in 2019 to determine the feasibility of manufacturing autologous GD2 expressing CAR-T cells, assess their safety and determine the maximum tolerated dose in children and young adults with H3.3K27M+ diffuse gliomas (ClinicalTrials.gov Identifier: NCT04196413). Results from the first four patients enrolled in the study were recently published, and show that the treatment is...
well-tolerated and that 3 of 4 patients derived clinical and radiographic benefits following intravenous administration of GD2-CAR-T cells. A second dose of GD2-CAR-T cells via intracerebroventricular administration provided additional radiographic/clinical benefits in three of three patients treated. Additionally, off-target, off-tumour toxicity was not observed in any of the patients. Although it is early in the trial, with only a few patients tested, the current results of this therapeutic approach are promising and encouraging for patients of glioma [48].

Tumour-associated antigens or neoantigens enable selective targeting (e.g. through immunotherapy) of the glioma microenvironment. However, such targets may be expressed on some tumour cells but not on others, which can result in some cells escaping immunotherapy. To limit tumour immune evasion, patients should receive immunotherapy that targets multiple neoantigens. For example, a recent study developed a novel multivalent vector protein, QUAD 3.0, which concurrently targets four GBM associated Eph receptors [49]. This approach allows the entire tumour microenvironment to be covered while minimising the chance of antigen loss and reducing therapeutic resistance. Additionally, QUAD 3.0 conjugated to DOX was shown to be cytotoxic to both established and patient-derived GBM cell lines in vitro, with IC50 values in the low nanomolar range. Given the positive results observed so far, multivalent vector proteins could be further used in pre-clinical models in the foreseeable future. Although immunotherapeutic intervention should be regarded with caution, the development of immunotherapies focusing on recruitment, activation and retention for pediatric glioma is warranted to harness the potential of this strategy in treating brain tumours in children.

TUMOUR-TARGETING BACTERIA
Recent developments in targeted therapy and immunotherapy have raised hopes for the treatment of glioma. However, some challenges concerning these approaches remain to be addressed, including toxicity to healthy tissues, the inability to treat deep tumour tissue and the possibility of inducing drug resistance in tumour cells [50, 51].

Although not a novel concept, live tumour-targeting bacteria offer a unique opportunity to meet these challenges. Compared to other strategies, tumour-targeting bacteria have multiple capabilities for suppressing cancer [50]. These bacteria preferably accumulate and proliferate within tumours, where they can trigger anticancer immune responses. For example, anaerobic bacteria specifically colonise the uniquely hypoxic tumour microenvironment and locally exert oncolytic effects. Bacteria can be modified via simple genetic manipulation or synthetic bioengineering to produce and deliver anti-tumour agents. Live tumour-targeting bacteria can be used either as a monotherapy or combined with other anticancer therapies to optimise clinical outcomes. The mechanisms of selective induction of tumour regression, different engineering methods and clinical trials involving tumour-targeting bacteria are reviewed elsewhere [51]. Here we describe recent advances in using live tumour-targeting bacteria for anti-glioma therapy.

It has previously been demonstrated that the attenuated tumour-targeting and highly mobile Salmonella typhimurium (S. typhimurium) A1-R strain can suppress and eliminate human glioma in an orthotopic mouse model [52]. S. typhimurium A1R is auxotrophic (leu/arg-dependent) but receives sufficient nutrition from tumour tissue and does not continuously infect normal tissue. In one study, glioma-bearing mice were treated with S. typhimurium by intravenous or intracranial injection once a week for three weeks and observed via intravitral microscopy. Intracranially administered bacteria were shown to inhibit brain tumour growth at 7.6-fold higher compared to untreated mice and significantly improved survival, with two of ten mice appearing to have their tumours eliminated. The same study showed that intravenous administration of bacteria was not effective. Another study used S. typhimurium A1-R to assess its anti-tumour efficacy through intravenous or intrathecal injection in orthotopic mouse models of spinal cord glioma [53]. Intrathecal treatment was seen to significantly improve survival and inhibited hind-limb paralysis compared to the intravenously treated and control groups.

Salmonella species pluralis are facultative anaerobes which can survive in oxygenated environments [51]. Through improved tumour targeting, reduced toxicity or increased efficacy of this bacteria could potentially be achieved. Since numerous cancer types, including glioma, overexpress the avb3 integrin, an S. Typhimurium strain displaying an integrin-binding Arg–Gly–Asp (RGD) peptide on its outer membrane to enhance specific targeting was designed [54]. This strain showed a >1000-fold enrichment in U87 MG xenografts in mice compared to control. In a similar study, a different type of enhanced tumour-targeting S. typhimurium bacteria was designed [55]. This strain had mutations in the msbB gene, blocking systemic toxicity, and in the purI gene, rendering it incapable of synthesising purines. Since purines are highly detectable in tumours but barely detectable in healthy tissues, these mutations generated a tumour seeking tendency that could be exploited. These bacterial carriers could induce apoptosis via a combination of the expression of tumour suppressor protein P53 and the pro-apoptotic drug Azurin. In a xenograft model of human U87 MG GBM in rats, intracranial injection of bacteria significantly prolonged survival. Histological and proteomic analyses showed no systemic toxicity and a restored neural environment in treated responders.

Clostridium novyi (C. novyi), an avirulent, highly mobile anaerobic bacteria that is extremely attracted to the hypoxic tumour microenvironment was attenuated and shown to induce an effective anti-tumour response in an orthotopic rat F98 glioma model [56]. The tumours were engineered to express luciferase, and a rapid fall of luciferase activity was observed within 48 h after intratumoral C. novyi spore injection, indicating a reduction in tumour volume. C. novyi specifically localised to the tumour, sparing surrounding normal cells and were observed to eliminate islands of microinvasive tumour cells that had diffused within healthy brain tissue. This bacterial treatment also resulted in a significant survival advantage. Abscess formation was not observed in this model with the adequate use of antibiotics. However, abscess formation in the brain remains a potential risk of C. novyi therapy in humans and would require excision and drainage. Given the poor prognosis of gliomas, the benefits of C. novyi therapy might compensate for these potential side-effects. This study also had encouraging results in a canine model, which ultimately led to treating a human patient. In a comparable study, intravenously injected spores of C. novyi led to tumour destruction and significantly prolonged survival in both rat F98 and human 060919 brainstem glioma xenograft models in rats [57]. As with the previous study, C. novyi germination was specific to the tumour, with sparing of the healthy brain parenchyma. However, oedema and increased intracranial pressure could have been fatal if not properly managed with hydration and antibiotics.

CONCLUSIONS
While this review is by no means exhaustive in describing the treatment strategies currently under investigation for treating high-grade gliomas, the aim is to bring to light some of the atypical recent advancements and stratagems that hold potential in advancing glioma treatments in children and adults. Gliomas present an unmet medical need and have done so since being described. The challenges that these tumours present can be seen in the radical strategies being attempted to improve prognosis, and eventually find a curative treatment for this disease. Although the ultimate goal is to defeat glioma, this victory must not be Pyrrhic, with considerations for quality of life intertwined with
treatment strategies. Delivery systems and combined modality treatment strategies need to be investigated thoroughly, with events such as neurotoxicity and additive effects of treatment elucidated in efficient experimental models so that a therapeutic index with a favourable benefit-risk ratio can be achieved. Table 1 shows a concise list of clinical trials incorporating the strategies being reviewed for brain neoplasms. Table 2 lists the strategies discussed above and their investigation over the last two decades, including studies and clinical trials that have been initiated and registered on the clinicaltrials.gov server [58], including those that are glioma specific.

### REFERENCES

1. Weller M, Wick W, Aldape K, Brada M, Berger M, Pfister SM, et al. Glioma. Nat Rev Dis Prim. 2015. https://doi.org/10.1038/nrdp.2015.17.
2. Fangusaro J. Pediatric high grade glioma: a review and update on tumor clinical characteristics and biology. Front Oncol. 2012;2:1–10. https://doi.org/10.3389/fonc.2012.00105.
3. Bassoe P, Aplebach CW. Glioma of the bulb and pons: a report of four cases. Arch Neurol Psychiatry. 1925;14:396–408. https://doi.org/10.1001/archneursys.1925.0200150109010.
4. Bailey P. Intracranial Tumors. Springfield, Ill: Thomas; 1933.
5. Bailey P, Cushing H. A classification of the tumours of the glioma group on a histogenetic basis, with a correlated study of prognosis. First edit. Philadelphia: JB Lipponcott; 1926.
6. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol. 2021;23:1231–51. https://doi.org/10.1093/neuonc/noab106.
7. Alentorn A, Duran-Peña A, Pingle SC, Piccioni DE, Idbaih A, Kesari S. Molecular characteristics and biology. Front Immunol. 2021;23:115011. https://doi.org/10.3389/fimmu.2021.655011.
8. Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C, et al. CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016. Neuro Oncol. 2019;21:v1–100. https://doi.org/10.1093/neuonc/noz150.
9. Haumann R, Videra JC, Kaspers GJL, van Vuurden DG, Hulmean E. Overview of current drug delivery methods across the blood-brain barrier for the treatment of primary brain tumors. CNS Drugs. 2020;34:1121–31. https://doi.org/10.1007/s40263-020-00766-w.
10. Liu Z, Ji X, He D, Zhang R, Liu Q, Xin T. Nanoscale drug delivery systems in glioblastoma. Nanoscale Res Lett. 2022;17:27 https://doi.org/10.1186/s11671-022-03668-6.
11. Raj D, Agraval P, Gaitsch H, Wicks E, Tyler B. Pharmacological strategies for improving the prognosis of glioblastoma. Expert Opin Pharmacother. 2021;22:29–31.
12. Martinez-Castaldi C, Silverstein M, Bauchner H. Child versus adult research: the gap in high-quality study design. Pediatrics. 2008;122:52–7. https://doi.org/10.1542/peds.2007-2849.
13. Xie J, Bi Y, Zhang H, Dong S, Teng L, Lee RJ, et al. Cell-penetrating peptides in diagnosis and treatment of human diseases: from preclinical research to clinical application. Front Pharmacol. 2020;11:697 https://doi.org/10.3389/fphar.2020.00697.
14. Raucher D. Tumor targeting peptides: novel therapeutic strategies in glioblastoma. Physiol Behav. 2016;176:100–6. https://doi.org/10.1016/j.physbeh.2019.01.006.
15. Gao H, Yang Z, Zhang S, Cao S, Pang Z, Yang X, et al. Glioma-homing peptide with a cell-penetrating effect for targeting delivery with enhanced glioma localisation, penetration and suppression of glioma growth. J Controlled Release. 2013;172:921–8. https://doi.org/10.1016/j.jconrel.2013.10.002.
16. Wang X, Zhao Y, Dong S, Lee RJ, Yang D, Zhang H, et al. Cell-penetrating peptide and transferrin co-modified liposomes for targeted therapy of glioma. Molecules. 2019;24:3540. https://doi.org/10.3390/molecules24193540.
17. Wang D, Guo M, Yu J, Wang X, Zhang G, Yang X, et al. Glioma targeting peptide in combination with the P53 C terminus inhibits glioma cell proliferation in vitro. Cytotherapy. 2018;20:353–61. https://doi.org/10.1016/j.jcyt.2018.01.011.
18. Srimanee A, Arvanitidou M, Kim K, Hallbrink M, Langel U. Cell-penetrating peptides for siRNA delivery to glioblastomas. Peptides (NY). 2018;104:62–9. https://doi.org/10.1016/j.peptides.2018.04.015.
19. Zhu L, Kate P, Torchilin VP. Matrix metalloprotease-2-responsive multifunctional liposomal nanocarrier for enhanced tumor targeting. ACS Nano. 2012;6:4911–8. https://doi.org/10.1021/nn300526f.
20. Xie J, Meng J, He M, Zhang CJ, Geng W, et al. Dual-targeting and microenvironment-responsive micelles as a gene delivery system to improve the sensitivity of glioma to radiotherapy. Acta Pharm Sin B. 2019;9:381–96. https://doi.org/10.1016/j.apsb.2018.12.001.
21. Yu J, Lefroy G, Bready D, Frenster J, Saldaña-Meyer R, Jin Y, et al. The H3K36me2 writer-reader dependency in H3K27M-DIPG. Sci Adv. 2021;7:eabg7444 https://doi.org/10.1126/sciadv.abg7444.
22. Cho CF, Wolfe JM, Fadzen CM, Calligaris D, Homburg K, Chiocca EA, et al. Blood-brain-barrier spheroids as in vitro screening platform for brain-penetrating agents. Nat Commun. 2017;8:1–14. https://doi.org/10.1038/ncomms15623.
23. Liu H, Zhang J, Chen X, Du XS, Zhang JL, Liu G, et al. Application of iron oxide nanoparticles in glioma imaging and therapy: from bench to bedside. Nanoscale. 2016;8:7808–26. https://doi.org/10.1039/c6nr00147e.
24. Marekova D, Turnovcova K, Sursal TH, Gandhi CD, Jendelova P, Janhwar-Uniyal M. Potential for treatment of glioblastoma: new aspects of superparamagnetic iron oxide nanoparticles. Anticancer Res. 2020;40:5989–94. https://doi.org/10.21873/ anticanceres.14619.
25. Wang X, Yang L, Zhang H, Tian B, Li R, Hou X, et al. Fluorescent magnetic PEI-PLGA nanoparticles loaded with paclitaxel for concurrent cell imaging, enhanced apoptosis and autophagy in human brain cancer. Colloids Surf B Biointerfaces. 2018;172:708–17. https://doi.org/10.1016/j.colsurfb.2018.09.033.
26. Gapenpinini L, Ucakar B, Joudiou N, Bianco J, Danhier F, Danhier P. Magnetic nanoparticles in glioma imaging and therapy: from bench to bedside. Nanoscale. 2021;13:4509–21. https://doi.org/10.1039/D1/NM015184.
27. Afzalipur R, Khoei S, Shervillouali S, Jamali Raoufi N, Motevalian M, et al. Dual-targeting temozolomide loaded in folate-conjugated magnetic triblock copolymer nanoparticles to improve the therapeutic efficiency of rat brain gliomas. ACS Biomater Sci Eng. 2019;5:6000–11. https://doi.org/10.1021/acsbiomaterials.9b00855.
28. Xu H-L, Mao K-L, Huang Y-P, Yang J-J, Xu J, Chen P-F, et al. Glioma-targeted superparamagnetic iron oxide nanoparticles as drug-carrying vehicles for thermoabatic effects. Nanoscale. 2016;8:14222–36. https://doi.org/10.1039/c6nr02448c.

### Table 2: Publications and clinical trials involving the five strategies discussed above in the last 20 years up to and including 22.07.2021.

| Strategy [2001–2021] | Published articles | Clinical trials |
|----------------------|-------------------|-----------------|
|                      | [22.07.2001–22.07.2021] |                |
| Cell-penetrating peptides | 4716 | 122 |
| Glioma specific | | 2 |
| Glioma specific | | 1 |
| SPIOs | 13,362 | 302 |
| Carbon (nitride quantum) dots | 180 | 3 |
| Neutrogenic (TAA) | 2400 (9848) | 58 (258) |
| Tumour-targeting bacteria | 21,400 | 502 |
| Glioma specific | | 68 |
| Glioma specific | | 6 |

TAA: tumour-associated antigens.
1200

Chheda ZS, Kohanbash G, Okada K, Jahan N, Sidney J, Pecoraro M, et al. Novel and etiologically bound magnetic nanoparticles designed for remote targeting of cancer cells disseminated within cerebrospinal fluid pathways. Front Neurol. 2020;11:1–14. https://doi.org/10.3389/fneur.2020.596632

Lee C, Kim GR, Yoon J, Kim SE, Yoo JS, Piao Y. In vivo delineation of glioblastoma by targeting tumor-associated macrophages with near-infrared fluorescent silica coated iron oxide nanoparticles in orthotopic xenografts for surgical guidance. Sci Rep. 2018;8:1–12. https://doi.org/10.1038/s41598-018-29424-4

Liu H, Wang X, Wang H, Nie R. Synthesis and biomedical applications of graphitic carbon nitride quantum dots. J Mater Chem B. 2019;7:5432–48. https://doi.org/10.1039/c9tb01410a

Li S, Su W, Wu H, Yuan T, Yuan C, Liu J, et al. Targeted tumour theranostics in mice via carbon quantum dots structurally mimicking large amino acids. Nat Biomed Eng. 2020;4:704–16. https://doi.org/10.1038/s41551-020-0540-9

Ruan S, Qian J, Shen S, Zhu J, Zhao H, et al. Self-targeting fluorescent carbon dots for diagnosis of brain cancer cells. ACS Nano. 2015;9:11455–61. https://doi.org/10.1021/acsnano.5b05575

Liyanage PY, Zhou Y, Al-Youbi AO, Bashamkhal AS, El-Shahawi MS, Vanni S, et al. Pediatric glioblastoma target-specific efficient delivery of gencitabine across the blood-brain barrier via carbon nitride dots. Nanoscale. 2020;12:7927–38. https://doi.org/10.1039/D0NR01647K

Veldhuizen van Zanten SEM, El-Khouly FE, Jansen MHA, Bakker DP, Sanchez Alliaga E, Haasbeek CJA, et al. A phase I/II study of gencitabine during radiotherapy in children with newly diagnosed diffuse intrinsic pontine glioma. J Neurooncol. 2017;135:307–15. https://doi.org/10.1007/s11060-017-2575-9

Hettiarachchi SD, Graham RM, Mintz KJ, Zhou Y, Vanni S, Peng Z, et al. Triple conjugated carbon dots as a nano-drug delivery model for glioblastoma brain tumors. Physiol Behav. 2017;176:139–48. https://doi.org/10.1016/j.physbeh.2017.01.018

Seleci DA, Maurer V, Barlas FB, Porsiel JC, Temel B, Ceylan E, et al. Transferrin-decorated niosomes with integrated irnp/zns quantum dots and magnetic iron oxide nanoparticles: Dual targeting and imaging of glioma. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22094556.

Schumacher TN, Schreiber RD. Neointegins in cancer immunotherapy. Science. 2015;348:669–74. https://doi.org/10.1126/science.aac4971. (1979)

Abedalhagh A, Mobark M, Al-Rashed M, AlHarbi M. Epigenomics and immune-therapeutic advances in pediatric brain tumors. npj Precis Oncol. 2021. https://doi.org/10.1038/s41598-021-00173-4.

Chheada ZS, Kohanbash G, Okada K, Jahan N, Sidney J, Pecoraro M, et al. Novel and shared neointegins derived from histone 3 variant H3K27M mutation for glioma cell therapy. J Exp Med. 2018;1215:141–57. https://doi.org/10.1084/jem.20171046

Moulins S, Taitt JM, Villanueva-Meyer JE, Bonner ER, Nepp T, Lulla RR, et al. Mass cytometry detects H3K27M-specific vaccine responses in diffuse midline glioma. J Clin Investig. 2020;130:6325–372. https://doi.org/10.1172/JCI114378

Nazha B, Inal C, Owoinikoko T. Disialoganglioside GD2 expression in solid tumors and role as a target for cancer therapy. Front Oncol. 2020. https://doi.org/10.3389/fonc.2020.01000.

Monte C, Mazzieri R, Sundaresh S, Arnold E, Kadapaakam M, Haile S, et al. Potential antitumor efficacy of anti-GD2 CAR T cells in H3K27M+ diffuse midline gliomas. Nat Med. 2018;24:527–9. https://doi.org/10.1038/s41591-018-0006-x

Golinelli G, Grisendi G, Prapa M, Bestagno M, Sponzo C, Rossignoli F, et al. Targeting GD2-positive glioblastoma by chimeric antigen receptor empowered mesenchymal progenitors. Cancer Gene Ther. 2020;27:358–70. https://doi.org/10.1038/s41417-018-0062-x

Majzer R, Ramakrishna S, Yeom K, Patel S, Chinnasamy H, Schultz L, et al. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. Nature. 2022;603:934–41. https://doi.org/10.1038/s41586-022-04489-4

Sharma P, Roberts C, Herpai D, Fokt ID, Pribe W, Debinski W. Drug conjugates for targeting EPH receptors in glioblastoma. Pharmaceuticals. 2020. https://doi.org/10.3390/ph13040077

Duong MTQ, Qin J, You SH, Min J. Bacteria-cancer interactions: bacteria-based cancer therapy. Exp Mol Med. 2019. https://doi.org/10.1038/s12276-019-0297-0.

Zhou S, Gravekamp C, Bermudes D, Liu K. Tumour-targeting bacteria engineered to fight cancer. Nat Rev Cancer. 2018;18:727–43. https://doi.org/10.1038/s41568-018-0070-z

Momiyama M, Zhao M, Kimura H, Tran B, Chishima T, Bouvet M, et al. Inhibition and eradication of human glioma with tumor-targeting Salmonella typhimurium in an orthotopic nude-mouse model. Cell Cycle. 2012;11:628–32. https://doi.org/10.1016/j.ccc.2011.13.19116

Murakami T, Hiroshima Y, Miyake K, Kiyuna T, Endo I, Zhao M, et al. Efficacy of tumor-targeting salmonella typhimurium A1-R against malignancies in patient-derived orthotopic xenograft (PDox) murine models. Cells. 2019. https://doi.org/10.3390/cells8060599.

Park SH, Zheng JH, Nguyen VH, Jiang SN, Kim DY, Szardeningis M, et al. RGD peptide cell-surface display enhances the targeting and therapeutic efficacy of attenuated salmonella-mediated cancer therapy. Theranostics. 2016;6:1672–82. https://doi.org/10.7150/thno.16135

Mehta N, Lyon JK, Patil K, Mokarram N, Kim C, Bellamkonda RV. Bacterial carriers for glioblastoma therapy. Mol Ther Oncol. 2017;4:1–17. https://doi.org/10.1016/j.mto.2016.12.003

Robert NJ, Zhang L, Janfu K, Collins A, Bai RY, Staatdke V, et al. Intratumoral injection of Clodirostium novyi-NT spores induces antitumor responses. Sci Transl Med. 2014. https://doi.org/10.1126/scitranslmed.3008962.

Staatdke V, Bai RY, Sun W, Huang J, Kibler K, Tyler BM, et al. Clodirostium novyi-NT can cause regression of orthotopically implanted glioblastomas in rats. Oncotarget. 2015;6:5536–46. https://doi.org/10.18632/oncotarget.3627.

US National Library of Medicine. ClinicalTrials.gov. 2021. Accessed 22 July 2021.

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