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MINIREVIEW

Rising to the challenge: recent aptamer-conjugate success in treating glioblastoma

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ABSTRACT

Glioblastoma reflects the most lethal and aggressive adult brain cancer with strong metastasising capabilities and molecular differences between various tumour types. These differences, among other factors, have contributed to the poor success rates of current therapeutics that often results in tumour recurrence. The highly invasive and toxic nature of current therapeutics leads to high mortality and low patient survivability rates, thereby demonstrating the importance of developing novel targeted therapies for treating glioblastoma. The main hurdle chemotherapeutics and drug delivery mechanisms face is the inability to cross the blood-brain barrier, however, aptamers have shown promise in overcoming this to effectively deliver drug payloads to cancerous cells. Aptamers are single-stranded oligonucleotide sequences that possess many desirable characteristics as a drug delivery vehicle over alternative targeted therapeutics, including a high binding specificity and sensitivity towards targets, minimal batch variability, lack of immunogenicity, and ease of modifications. While few researchers have been successful in generating aptamer-conjugates that can initiate therapeutic responses in glioblastoma tumours following blood-brain barrier crossing, many aptamer-conjugates show promise based on preliminary findings. Such conjugates include the addition of chemotherapeutic drugs, functionalised nanoparticle complexes, or small interfering RNA chimeras to aptamers. Herein, this review focuses on addressing why aptamers are ideal candidates for targeted therapeutic delivery, the difficulties chemotherapeutics and drug delivery mechanisms face, and an overall update of various aptamer-conjugates over the past 6 years that show promise in crossing the blood-brain barrier to treat glioblastoma.

KEYWORDS: Aptamer-conjugates, blood-brain barrier, functionalised nanoparticles, glioblastoma, siRNA chimeras, therapeutics

INTRODUCTION

Glioblastoma represents the most malignant and aggressive form of adult primary brain cancer with low patient survivability and high mortality rates. Regardless of patients receiving the gold standard treatment options of surgery, 6 weeks of concurrent radiation therapy with temozolomide, and 6 months of adjuvant temozolomide, median patient survival rates remain around 15 months post-treatment, while approximately 5% of patients survive 5 years (Fernandes et al, 2017). Recently, the World Health Organisation re-classified glioblastoma as the most serious grade IV brain tumour belonging to the adult-type diffuse glioma family with actively proliferating tumours capable of metastasising faster than other brain cancers (Louis et al, 2021). Additionally, glioblastoma is further categorised as an isocitrate dehydrogenase (IDH) wildtype with either epidermal growth factor receptor (EGFR) gene amplification, human telomerase reverse transcriptase gene promotor mutations, or a combination of +7/-10 changes to chromosome copy numbers (Louis et al, 2021). With a strong molecular heterogeneity, tumour immune system evasion techniques through local immunosuppression, and the blood-brain barrier (BBB) preventing entry of most chemotherapeutics, each factor contributes to the limited success of glioblastoma therapeutics today (Janjua et al, 2021). Currently,

dominating targeted therapeutic research to deliver a more personalised medical approach are immunotherapies as extensively reviewed by Chokshi et al (Chokshi et al, 2021). However, immunotherapies are costly, unstable in varying environmental conditions, and can induce immune responses in patients (Webster, 2022). Alternatively, aptamers have proven advantageous over the limitations of other targeted therapeutics, especially with the ease of modifications allowing aptamers to act as drug carriers, which was successfully proven to traverse the BBB, treating metastatic brain cancer *in vivo* (Macdonald et al, 2020).

Aptamers and aptamer selection for glioblastoma

Aptamers or chemical antibodies are single-stranded DNA or RNA oligonucleotide sequences that form unique 3D structures capable of binding to target biomarkers with high specificity and sensitivity (Hays et al, 2017). Therefore, aptamers are promising candidates for targeted therapeutics to cancer cell biomarkers, given additional characteristics such as low immunogenicity, minimal batch variability, and stability in various environmental conditions (Zhou and Rossi, 2017). Having first been described in 1990 through Systematic Evolution of Ligands via Exponential enrichment (SELEX), the SELEX process has been established to generate aptamers to a range of different targets including bacteria, cells, proteins, small molecules, or viruses (Ellington and Szostak, 1990; Tuerk and Gold, 1990; Zhou and Rossi, 2017). As such, there is no standardised SELEX protocol given the various targets, desired aptamer application, or library used. One such protocol, cell-SELEX as visualised in Figure 1, uses living cells to generate aptamers through iterative rounds that can bind to complex biomarkers on cancerous cells while being retained within natural environmental conditions (Shigdar et al, 2021). Completion

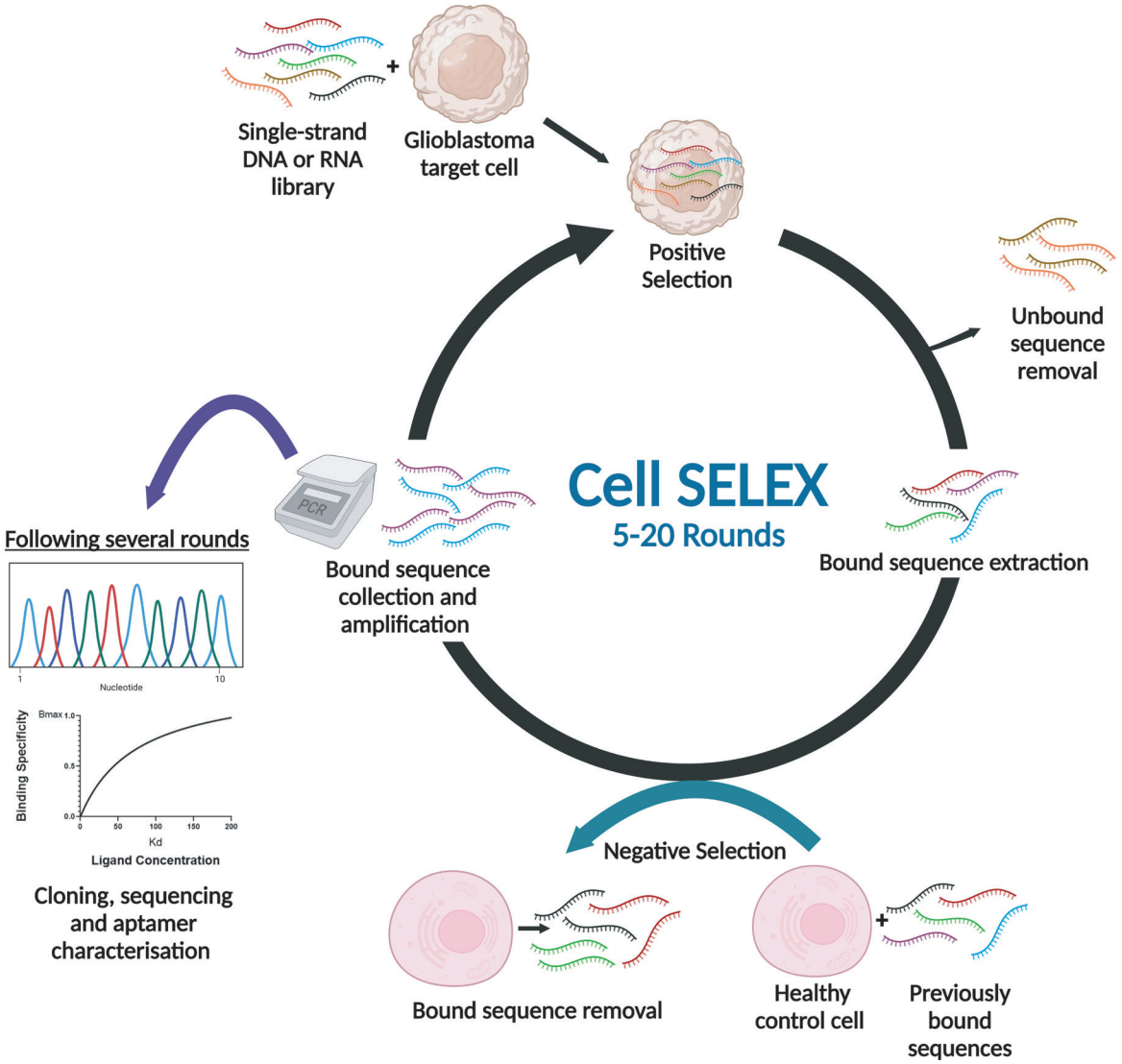

Figure 1. Representative diagram of the cell Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process using whole live cells to potentially select aptamers that can specifically bind to glioblastoma cells. An oligonucleotide library containing up to 10^{15} sequences of single-strand DNA or RNA that are flanked randomly by fixed primers on the 5' and 3' end undergoes iterative rounds of selection, partitioning, and amplification. Cell-SELEX predominately consists of positive selection rounds with a negative selection round using cells that share most biomarkers excluding the aptamer target. This potentially eliminates non-specific binding. Unbound sequences are partitioned while target sequences are amplified through polymerase chain reaction for DNA sequences or RNA transcription for RNA sequences. Following sufficient rounds, selected aptamers are sequenced, cloned, and the aptamer is characterised through various techniques. Adapted from (Hays et al, 2017). Created using BioRender (BioRender, 2022).

of cell-SELEX involves a series of high-throughput next-generation or Sanger sequencing techniques to determine the aptamer sequence, and subsequent determination of binding affinity through various techniques including flow cytometry and an enzyme-linked apta-sorbent assay (Nabavinia et al, 2018; Pleiko et al, 2019).

Aptamer conjugation for therapeutic purposes

The desirability of aptamers to be easily modified enhances the potential for aptamers to become therapeutic delivery vehicles by avoiding limitations of nuclease degradation within the circulatory system or rapid excretion through renal filtration (Heo et al, 2016; Lee et al, 2016). As a therapeutic, upon binding with the target receptor, aptamers can either act as agonists by activating target receptor functions, as an antagonist blocking target interactions, or as therapeutic agent carriers to delivery drug payloads to target cells or tissues (Zhou and Rossi, 2017). For example, chemotherapeutic drugs can be either conjugated to, or intercalated into a double-stranded guanine-cytosine rich helical strand of aptamers allowing them to act as a therapeutic delivery vehicle (Macdonald et al, 2018). Additionally, siRNA or functionalised nanoparticles containing various components such as therapeutic agents, microRNA (miRNA) or siRNA molecules can combine with aptamers for therapeutic effects following internalisation into cancerous cells as shown in Figure 2 (Zhang et al, 2014; Kruspe and Giangrande, 2017). Once an aptamer has bound with

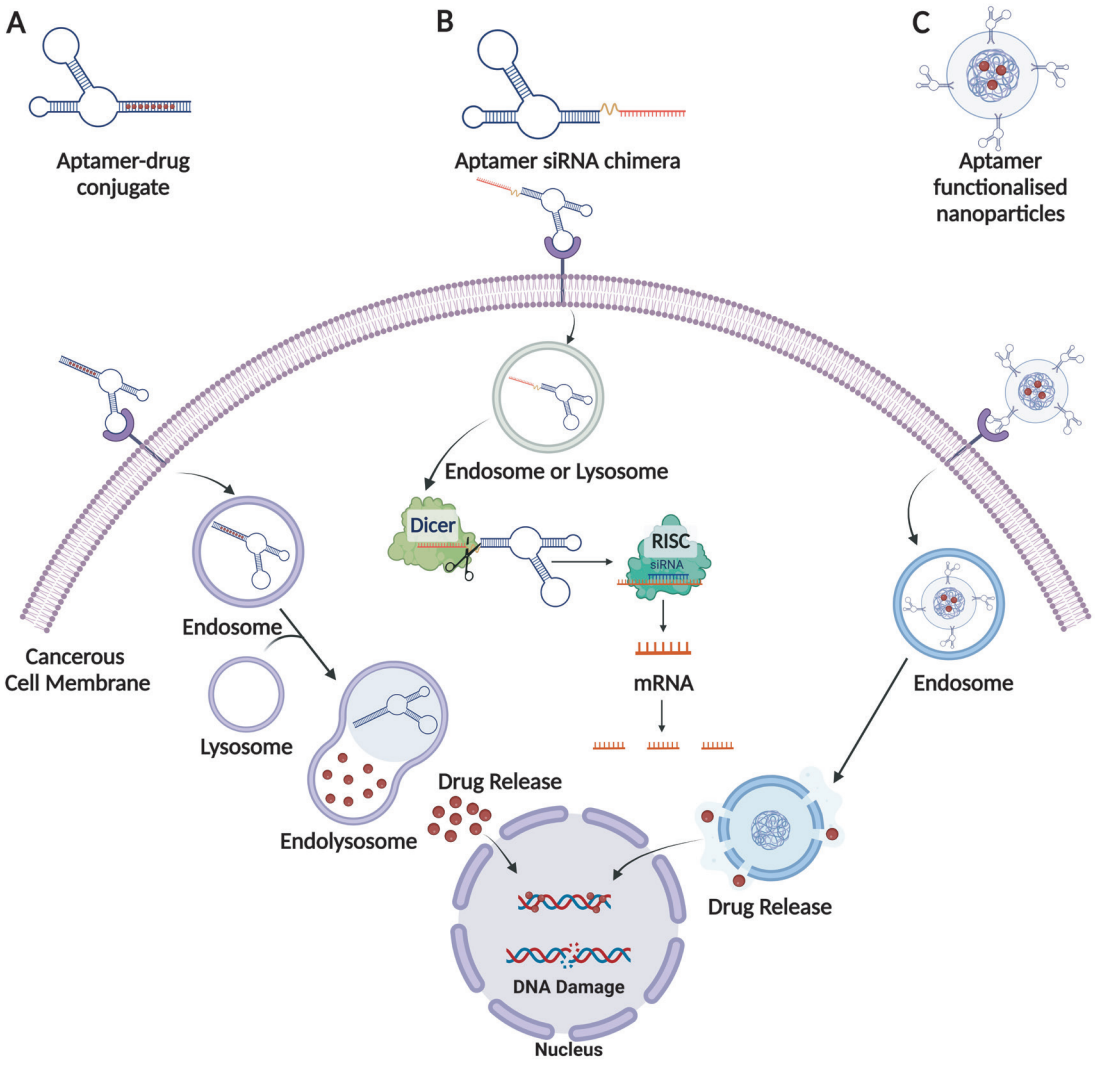

Figure 2. Different aptamer-conjugate mechanistic pathway actions following internalisation into a cancer cell by receptor-mediated endocytosis (RME). **A.** Aptamer-drug conjugate. Doxorubicin (DOX) conjugation is achieved through covalent linkages. Aptamer-DOX enters an endosome and is transformed into an endolysosome. The greater acidic conditions protonates DOX, releasing the drug into the cytoplasm before entering the nucleus of cancer cells, initiating cell death through DNA damage. Figure A adapted from (Macdonald et al, 2018). **B.** Aptamer-siRNA (AsiC) conjugate. Upon entry into the endosome or lysosome, AsiC escapes and is recognised by RNA interference machinery where the ribonuclease, Dicer, cleaves the aptamer allowing for siRNA loading into a RNA-induced silencing complex (RISC). Argonaute protein within the RISC complex unwinds double-stranded siRNA. Matching messenger RNA becomes cleaved with corresponding gene expression reduced within the cell. Figure B adapted from (Kruspe and Giangrande, 2017). **C.** Functionalised nanoparticle-aptamer conjugate. Following RME, the therapeutic agent dissociates from the functionalised complex, escaping the endosome and eliciting drug response within cancerous cells Figure C adapted from (Zhang et al, 2014). Created using BioRender (BioRender, 2022).

desired surface membrane receptors, many aptamers will internalise through receptor-mediated endocytosis (RME) by encapsulating the aptamer-conjugate within an endosome and allowing for specific delivery of payloads following the completion of RME (Kruspe and Giangrande, 2017).

Recent aptamer-conjugates with potential in treating glioblastoma

Over the past 6 years, many aptamer-conjugates have been described targeting various receptors expressed on glioblastoma cells showing great potential, however, BBB traversing capabilities are yet to be determined. Such receptors include prominin-1 or CD133+, EGFR variant III (EGFRvIII), platelet-derived growth factor receptor-β (PDGFR-β), or Axl (Table 1). In hopes of reducing cancer cell proliferation in stem cells and glioblastoma recurrence chance, Affinito et al (2019) used a previously generated 40L RNA aptamer and the respective truncated version A40s to assess internalisation within human patient-derived glioblastoma tissues. Both aptamers selectively bound to CD133+ glioblastoma stem cells (GSCs) compared with differentiated U251 and U87MG glioblastoma cells. However, after 30 minutes, while the A40s aptamer demonstrated almost complete internalisation, approximately 40% of the 40L aptamer was internalised into GSCs (Affinito et al, 2019). Thus, the miRNA inhibitor, anti-miR-10b, was combined with A40s to supress cell growth as visualised by a decrease in reported miR-10b expression levels, leading to the upregulation of B-cell lymphoma-like protein 11 (BIM) and inducing apoptosis (Affinito et al, 2019).

Focusing on targeting the EGFRvIII mutation found in 50% of glioblastoma patients, which correlates with chemoresistance and radiotherapy resistance, Zhang et al developed a U2 DNA aptamer to inhibit cell proliferation and improve radiosensitivity (Zhang et al, 2018; Hao and Guo, 2019). The U2 aptamer specifically bound to U87-EGFRvIII cells and displayed a time/dose-dependent inhibition, leading to significantly increased apoptotic rates and inhibition of cell proliferation with reduced migration capabilities *in vitro.* Additionally, following U87-EGFRvIII cell exposure to 2 gray (Gy) of radiation and treatment with the U2 aptamer, cells produced fewer cell colonies from a colony formation assay compared with the control scrambled sequence, suggesting that the U2 aptamer inhibits DNA damage repair mechanisms following radiation (Zhang et al, 2018). By combining U2 with the radioisotope rhenium-188, the ^{188}Re-U2 aptamer-conjugate saw significant antitumour effects in U87-EGFRvIII subcutaneously injected BALB/c mice with inhibition of tumour volume and weights detected compared to the ^{188}Re aptamer alone (Zhang et al, 2018). Therefore, this aptamer-conjugate displays potential as an inhibitor by interfering with tyrosine kinase receptor activation and downstream signalling pathways which helps to improve radiosensitivity (Zhang et al, 2018).

More recently, Esposito et al combined previously generated GL21.T-anti-miR-10b and Gint4.T-STAT3 aptamer-conjugates to determine if they could synergise, further resulting in antitumour effects upon binding to Axl+ and PDGFRβ+ GSCs (Esposito et al, 2020). By testing aptamer-conjugates in isolated human GSCs, only 20% of tumours greater than 50µm developed compared with approximately 50% for individual aptamer-conjugates. Additionally, similar percentages were seen with size reductions in tumours thereby reducing GSCs self-renewal potential (Esposito et al, 2020). Further investigation into the therapeutic effects *in vivo* for treating glioblastoma will be required for this bifunctional aptamer-conjugate.

BBB hurdle challenging glioblastoma therapeutic success

One major hurdle that hinders the success of brain cancer chemotherapeutics is the BBB. This semipermeable barrier is composed of a highly selective endothelial complex responsible for maintaining homeostasis through oxygen and nutrient supplementation to the brain, while protecting against neurotoxins and invading pathogens (García-Varela et al, 2021). With tight junctions between astrocytes, endothelial cells, and pericytes, the BBB restricts paracellular exchange between the central nervous system and systemic circulation. This ensures ions, molecules smaller than 500Da and essential nutrients can cross (Janjua et al, 2021). Consequently, 98% of small drug therapies cannot traverse the BBB thus, are ineffective for treating brain tumours (Clark et al, 2015). Furthermore, of those few lipophilic drugs that are actively transported across, efflux pumps such as P-glycoprotein limit drug accumulation

Table 1. Recent aptamer-conjugates demonstrate potential for treating glioblastoma.

Target	Aptamer	Conjugated with	Study form	Outcome	Reference
Axl	GL21.T	Anti-miR-10b	*In vitro*	Reduced tumour size and development from GSCs	(Esposito et al, 2020)
CD133	40L A40s	Anti-miR-10b	*In vitro*	Rapid internalisation into tissue causing apoptosis	(Affinito et al, 2019)
CD133	CD133	Telaglenastat (CB-839) GLS1 inhibitor and PEGylated gold nanoparticles	*In vitro*	Significantly decreased GSCs and glioblastoma cell viability in a dose-dependent manner	(Poonaki et al, 2022)
EGFRvIII	U2	Rhenium-188	*In vitro* *In vivo*	Enhanced radiosensitivity and significant antitumour effects in mice	(Zhang et al, 2018)
PDGFRβ	GMT8 Gint.4T	tFNA and paclitaxel	*In vitro*	Inhibits cell proliferation, migration, and invasion	(Shi et al, 2019)
	Gint4.T	STAT3, siRNA	*In vitro*	Reduced tumour size and development from GSCs	(Esposito et al, 2020)
TfR	TfR	RNV541	*In vitro*	miR21 inhibition	(Larcher et al, 2019)

within the BBB (García-Varela et al, 2021). Regardless of attempting BBB disruption techniques such as focused ultrasound or convection-enhanced delivery in hopes to enhance drug delivery, efflux pumps continue to reduce drug distribution despite membrane leakiness (de Gooijer et al, 2021). Instead, researchers have taken advantage of specific aptamer binding by exploiting receptor-mediated transcytosis (RMT) to deliver drug payloads across the BBB (Figure 3). Despite few researchers having attempted to cross the BBB to treat brain cancers, aptamers still have the potential to succeed by specifically targeting biomarkers expressed on both the BBB to traverse through RMT and target cancerous cells by internalising through the previously described RME (Figure 2).

For aptamers that have been unsuccessful in crossing the BBB, it is possible to combine any DNA aptamer with a transferrin receptor 1 (TfR1) aptamer to provide the BBB circumvention capabilities through RMT and allow for the therapeutic treatment of brain cancers, brain metastases, or neurodegenerative diseases as was previously demonstrated by Macdonald et al and Li et al (Li et al, 2020; Macdonald et al, 2020). Macdonald et al generated a bifunctional aptamer by fusing truncated and significantly modified versions of the GS24 and SYL3C aptamers, termed TEPP, which is capable of binding to TfR and epithelial cell adhesion molecules (EpCAM; Macdonald et al, 2017). Despite focusing on EpCAM positive brain metastases, TEPP could traverse an *in vitro* BBB model and

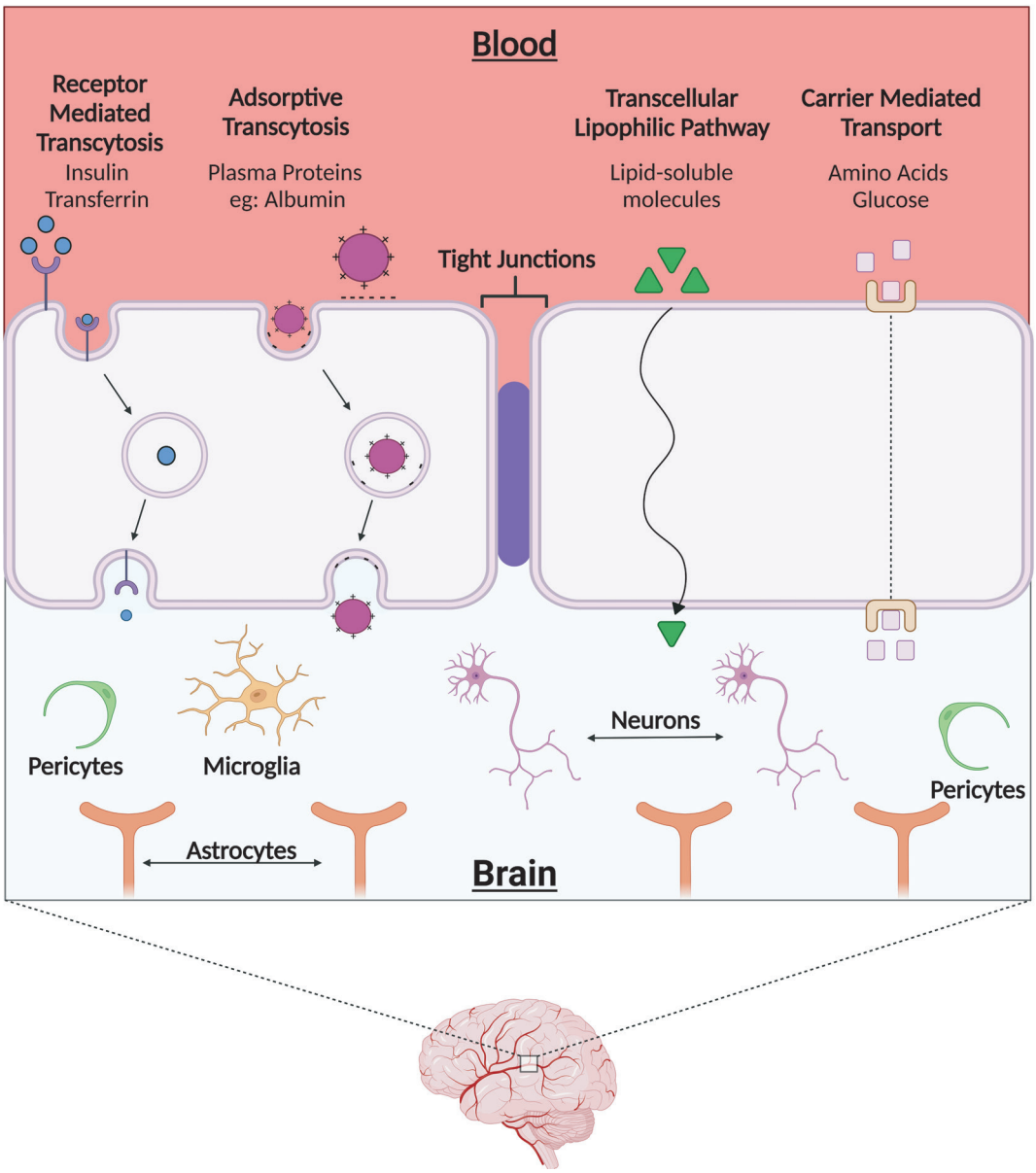

Figure 3. Schematic representation of the four primary traversing mechanisms across the BBB. Receptor-mediated transcytosis (RMT) requires specific receptor-ligand binding to surface receptors on the cell membrane, allowing for endosome formation and internalisation into the brain. Adsorptive transcytosis involves interactions between positively charged particles and the negatively charged cellular membrane for internalisation similar to RMT. The transcellular lipophilic pathway provides access for lipid-soluble molecules up to 500 daltons in size to cross the barrier. Lastly, carrier-mediated transport is for naturally occurring molecules. Adapted from (Chen and Liu, 2012). Created using BioRender (BioRender, 2022).

upon conjugation with doxorubicin (DOX), was shown to successfully reach cancerous cells within the brain following BBB circumvention in MDA-MB-231 metastatic mice models (Macdonald et al, 2020). This proof-of-concept that the TfR aptamer allows BBB penetration was further supported by Li et al combining with a Tau aptamer, the Tau-TfR bifunctional aptamer traversed the BBB in both *in vitro* and *in vivo* models with an improved stability in blood circulation and the disruption of tauopathy events that contribute to neurodegenerative diseases (Li et al, 2020). Thus, combining any DNA aptamer with the TfR aptamer will enhance the possibility of aptamer-conjugates to successfully cross the BBB and deliver payloads to glioblastoma cells.

Various aptamer-conjugates successfully cross the BBB treating glioblastoma

While so few aptamer-conjugates have assessed BBB traversing mechanisms and been successful in treating glioblastoma to date, it is important to acknowledge those studies that have, including those with the potential displayed in Table 1. Without being able to overcome the BBB hurdle, therapeutic efficacy for targeted therapeutics will remain minimal, however aptamers have opened the realm of possibilities. This can either be by targeting receptors heavily expressed on both the BBB and glioblastoma cells, or by combining two aptamers together using one that has previously shown crossing capabilities. Each successful aptamer-conjugate can be visualised within Figure 4 or is described in Table 2.

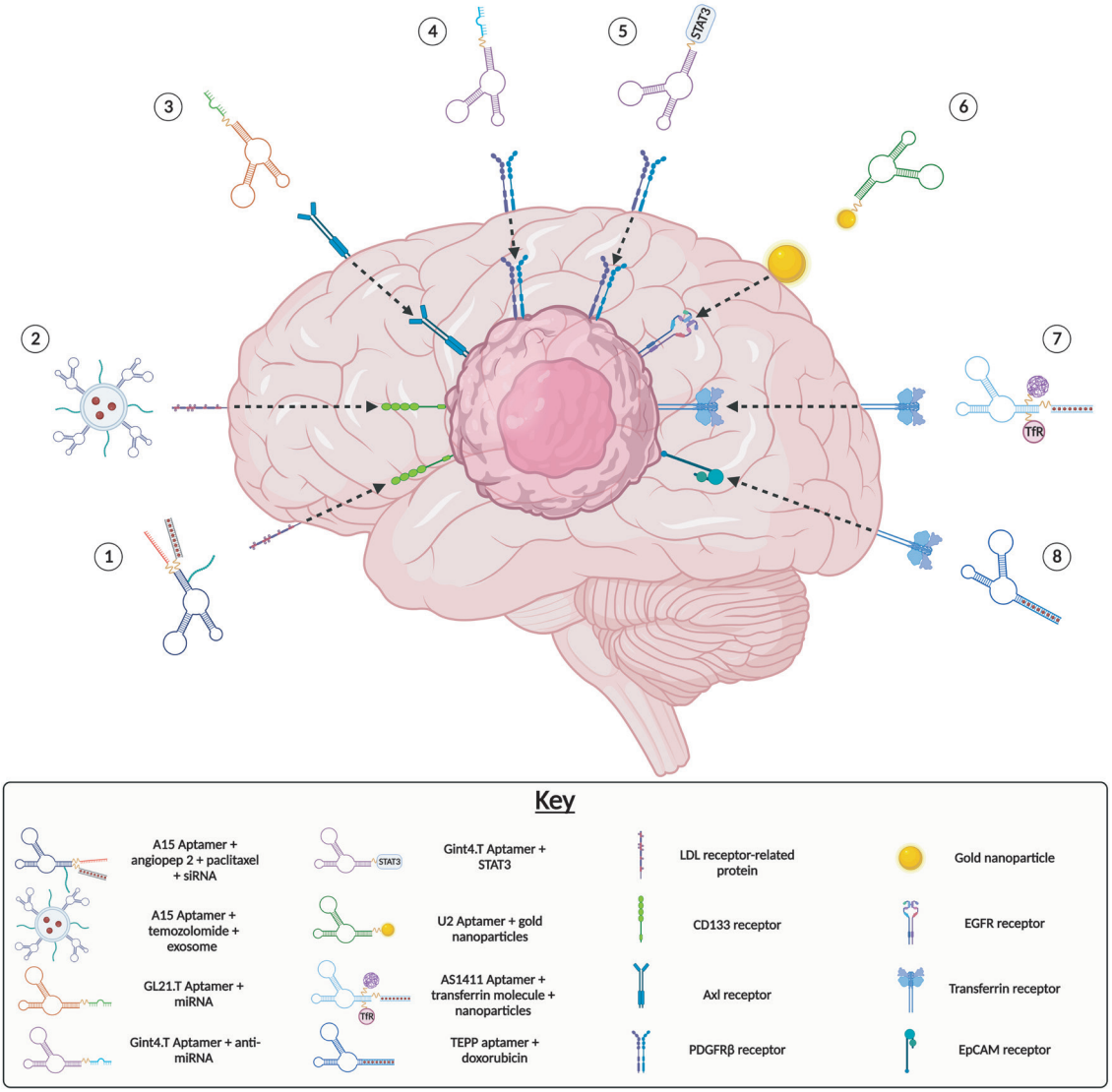

Figure 4. Diagrammatic representation of how successful aptamer-conjugates have been able to cross the BBB and treat glioblastoma or brain metastases over the last 6 years. The receptors on the outer brain are those expressed on the BBB which aptamers or other molecules bind to for RMT where the aptamer-conjugates internalise and begin to specifically target glioblastoma or brain metastases. The aptamers will bind with receptors on these cancer cells allowing for internalisation through RME where payloads can be delivered to initiate a therapeutic response. **1**) A15 aptamer with paclitaxel, survivin siRNA and angiopep 2 (Sun et al, 2018). **2**) A15 aptamer with temozolomide and an O^6-benzylguanine (BG) exosome (Liang et al, 2022). **3**). GL21.T aptamer with anti-miR-10b (Esposito et al, 2016). **4**) Gint4.T aptamer with miR-137 (Esposito et al, 2016). **5**) Gint4.T aptamer with STAT3 (Esposito et al, 2018). **6**) U2 aptamer with gold nanoparticles (Peng et al, 2020). **7**) AS1411 aptamer with transferrin molecule and mesoporous ruthenium nanoparticles (Zhu et al, 2018). **8**) TEPP bifunctional aptamer with DOX (Macdonald et al, 2020). Created using BioRender (BioRender 2022).

Table 2. Recent success of aptamer-conjugates crossing the BBB to deliver payloads to glioblastoma tumours, initiating therapeutic responses.

BBB/ Aptamer Targets	Aptamer	Conjugated with	Outcome	Reference
EphA2/ CD133	40L and A40s	2'-fluropyrimidine	Present in brain 1 hour post-injection in mice. Inhibits cell growth, stemness and migration of GSCs	(Affinito et al, 2020)
Macrophage exosome/ Nucleolin	AS1411	Catalase, silica nanoparticles, indocyanine green sonosensitiser	Present in brain 24 hours post-treatment in mice; biologically safe. Improved survival rate (>35 days) when combined with ultrasound irradiation	(Wu et al, 2022)
PDGFRβ	Gint4.T	Polymeric nanoparticles with PI3K-mTOR inhibitor	Strong drug bioavailability (EC_{50} = 38pM) inducing cytotoxicity. Significant presence in orthotopic mice 4 hours post-injection	(Monaco et al, 2017)
RNP / c-Met	SL1	DOX containing RNP on red blood cells	Prolonged survival time (23 vs 15.5 days)*. 2.17-fold greater accumulation in brain 48 hours post-injections in mice	(Lui et al, 2022)
TfR	GS24	Tetrahedral nucleic acid framework nanoparticle (tFNA), temozolomide	tFNA enhanced temozolomide cytotoxicity in sensitive and resistant glioblastoma cells. GS24-tFNA complex maintained in mouse brain for at least 1 hour	(Fu et al, 2019)
Not specified/ Nucleolin	AS1411	Poly(L-γ-glutamyl-glutamine) nanoparticles, paclitaxel	Strong accumulation in brain after 24 hours post-treatment in mice. Survival slightly increased (52 vs 47 days) *	(Luo et al, 2017)

*All outcome comparisons are made with the aptamer-conjugate vs without the presence of aptamers.

EphA2: Ephrin type-A receptor 2, RNP: red blood cell membrane-based nanoparticle drug delivery system.

Angiopep-2 delivers aptamer-conjugates for stem cell targeting

Sun et al developed a dual-modified cationic liposome complex consisting of the low-density lipoprotein angiopep-2, paclitaxel, survivin siRNA apoptotic inhibitor, and the A15 RNA aptamer originally generated by Shigdar et al specifically targeting CD133+ glioblastoma stem cells (Shigdar et al, 2013; Sun et al, 2018). Minimal cytotoxicity was reported within brain microvascular endothelial cells (BMEC) *in vitro* following liposome complex treatment while selectively inducing apoptosis in CD133+ GSCs (Sun et al, 2018). Additionally, temozolomide chemoresistance was ameliorated in U251 glioma cells due to an improved differentiation into non-stem cell lineages. This was evident based on decreased expression levels of survivin, nestin, a neuronal stem cell marker and various drug-resistant proteins, while the neuronal differentiation marker, glial fibrillary acidic protein (GFAP), experienced increased expression levels (Sun et al, 2018). Most importantly, angiopep-2 assisted delivery of liposome complex across the BBB of intracranial glioma tumour-bearing mice following tail vein injection at varying timepoints post tumour implantation (Sun et al, 2018). Bioilluminescent imaging revealed a stronger signal of the entire liposome complex within the brain 24 hours post-injection compared to without the A15 aptamer. Additionally, *ex vivo* evaluation of excised major organs revealed that the aptamers allowed for greater accumulation 24 hours post-injection within the brain compared to the complex without aptamers which failed to cross the brain and largely accumulated within the reticuloendothelial system (Sun et al, 2018). The success of this BBB crossing to deliver therapeutic payloads contributed to an improved median survival rate of 81 days in comparison to 45 days of the liposome complex without the enhanced specificity the A15 aptamer provided (Sun et al, 2018).

Liang et al, another research group targeting CD133+ GSCs trying to eliminate temozolomide-resistant glioblastoma, developed a dual receptor temozolomide and O^6-benzylguanine (BG) loaded exosome conjugated with angiopep-2 and the same A15 RNA aptamer from Shigdar et al (Shigdar et al, 2013; Liang et al, 2022). While exosomes are nanosized extracellular vesicles with natural BBB penetration abilities from the expression of transferrin and insulin receptors, they lack tumour targeting specificity. Thus, exosomes require conjugation with receptor-specific targeting moieties such as aptamers to accurately deliver drug payloads (Liang et al, 2022). With a strong accumulation of loaded exosome complexes in U87MG cells and GSCs, inhibition of cell proliferation was observed. This resulted from a 67.8 times greater drug uptake in cells with an improved temozolomide and BG efficacy in inducing apoptosis due to specific A15 aptamer binding compared to free temozolomide and BG alone (Liang et al, 2022). Next, U87MG-bearing mice were injected intravenously on the 7th, 10th, 13th, 16^{th,} and 19th day with temozolomide, BG, or the exosome complex following tumour implantation to determine survival rates. The median survival rates had increased by 43.75% to 46 days without adverse events following exosome complex treatment compared to 30 and 32 days for temozolomide and BG treatments, respectively (Liang et al, 2022). It was shown that angiopep-2 had assisted the exosome complex to penetrate the BBB through RMT and upon excising major organs including the brain post-treatment, the fluorescent intensity of the aptamer-exosome complex was almost 2x greater than the exosome complex alone. A favourable distribution to the tumour sites was observed

further supporting BBB penetration abilities (Liang et al, 2022).

PDGFRβ and Axl targeting provides aptamer-conjugate entry

Targeting both PDGFRβ and Axl tyrosine kinase receptors overexpressed on the BBB and glioblastoma cells identifies exemplary targets for glioblastoma therapeutics (Kim et al, 2012; Esposito et al, 2016). Esposito et al assessed the therapeutic effects of short miRNA antagonists including anti-miR-10b and the miR-137 oncosuppressor by combining with previously generated Gint4.T and GL21.T RNA aptamers respectively (Esposito et al, 2016). The strong synergistic effect from using combined inhibitory aptamers with gene regulatory function to bind to PDGFRβ (Gint4.T) and Axl (GL21.T) receptors saw a reduction in U87MG glioblastoma tumoursphere size due to the GSCs differentiating and losing propagation abilities (Esposito et al, 2016). Additionally, both aptamer-conjugates could traverse an in vitro tri-culture BBB model containing astrocytes, endothelial cells, and pericytes, as demonstrated by a decrease within the transendothelial electrical resistance that measured the BBB integrity, compared with the scrambled aptamer sequence alone. However, BBB permeability was enhanced for 6 hours following internalisation before resuming normal composition thus, potentially leaving the brain in a vulnerable state if assessed *in vivo* (Esposito et al, 2016). Alternatively, Esposito et al generated a novel aptamer-siRNA chimera targeting signal transducer and activator of transcription 3 (STAT3), termed Gint4.T-STAT3 to prevent GSC survival and propagation (Kim et al, 2012; Esposito et al, 2018). Alongside aptamer binding inhibitory effects and silencing of STAT3, Gint4.T-STAT3 assisted in reduced cell viability and migration within U87MG and T98G glioblastoma cells, and drastic tumour growth inhibition within subcutaneously injected U87MG xenograft mice models (Esposito et al, 2018). Additionally, reduced neovascularisation and tumour cell proliferation was detected *in vivo*, supporting Gint4.T-STAT3 as a glioblastoma therapeutic (Esposito et al, 2018). However, assessment into the biodistribution of aptamer-conjugate is recommended as this study assumes BBB traversing through RMT based on previous findings with the Gint4.T aptamer.

Gold nanoparticles allow entry of U2 aptamer across the BBB

Knowing that appropriately sized gold nanoparticles (AuNP) can cross the BBB, Peng et al modified their previously generated U2 DNA aptamer with a thiol group to allow for the conjugation of AuNPs (Peng et al, 2020). The U2-AuNP was shown to reduce phosphorylation levels of EGFRvIII 24 hours post-treatment with U2-AuNP in U87-EGFRvIII glioblastoma cells, thereby inhibiting cell proliferation and invasion (Peng et al, 2020). Aptamer signal could be detected 24 hours post-treatment in the brains of U87-EGFRvIII intracranial glioblastoma mouse models, while no signal was detected within the control group, reflecting successful BBB delivery of the aptamer-conjugate (Peng et al, 2020). To determine the therapeutic efficacy of the U2-AuNP aptamer-conjugate, every 3 days following U87-EGFRvIII tumour cell implantation, mice were injected with either U2-AuNP or NaCl as a control. This aptamer-conjugate was shown to improve survival rates to 30 days, compared with 24 days for the NaCl-treated mice, however additional studies are required to try and improve survival outcomes (Peng et al, 2020).

TfR allows BBB penetration and delivery of therapeutics that improve survivability

Zhu et al took advantage of the overexpression of TfR on the surface of the BBB and glioblastoma cells to assist delivery of a combined AS1411 aptamer with transferrin on the surface of mesoporous ruthenium nanoparticles to determine the therapeutic efficacy of photodynamic therapy (Zhu et al, 2018). Photodynamic therapy acts upon a photosensitiser generating reactive oxygen species following laser irradiation at particular wavelengths initiating apoptosis in tumour cells (Chen et al, 2017). Having previously identified the antitumour drug $[Ru(bpy)_2(tip)]^{2+}$ or RBT, Zhu et al included RBT within their nanosystem, termed RBT@MRN-SS-Tf/Apt (Chen et al, 2017; Zhu et al, 2018). This nanosystem demonstrated a significantly enhanced drug absorption within U87MG tumourspheres, penetrating deeply and inducing antitumour effects with significant delays in growth, which was further evident upon laser irradiation (Zhu et al, 2018). Using an *in vitro* BBB model constructed with human BMEC cells, following 3 hours of treatment with the RBT@MRN-SS-Tf/Apt complex, 42.3% of RBT was detected on the basolateral side of the BBB with corresponding decrease on the apical side demonstrating efficient migration of the aptamer-conjugate complex across the BBB through RMT. This concept was further supported within U87MG orthotopic mice models that were injected with U87MG cells using a stereotactic fixation device (Zhu et al, 2018). Twenty four hours following administration, the RBT@MRN-SS-Tf/Apt complex showed greater intensity in the brain supporting the BBB crossing through TfR and AS1411 binding to nucleolin, which following 48 hours was shown to achieve better drug retention than the complex without the AS1411 aptamer specificity. However, *ex vivo* analysis of excised major organs showed a strong distribution of the aptamer-conjugate within the liver and spleen, likely resulting from nanoparticle size which predominately accumulates within those organs. Regardless, a significantly prolonged median survival rate of 56 days with minimal weight loss in RBT@MRN-SS-Tf/Apt and laser treated U87MG orthotopic nude mice compared to 40 days without laser interference was observed (Zhu et al, 2018).

Aptamer success in glioblastoma clinical trials

Therapeutic based aptamers for treating cancers are slowly progressing into clinical trial stages with the first aptamer-based clinical trial for glioblastoma currently being evaluated. The phase 1/2 GLORIA trial assessed the safety and tolerability of the NOX-A12 RNA aptamer given concurrently with radiation therapy in 10 glioblastoma patients who experienced failed tumour biopsies or resections and expressed the unmethylated MGMT gene that renders temozolomide ineffective, while the control cohort received radiation therapy alone (ClinicalTrials.gov). The NOX-A12 aptamer binds to the CXCL12 chemokine disrupting the communication between tumour cells and the microenvironment known to promote tumour proliferation, vascularisation, and metastases (Pharma, 2022). Patients were given three different dosages of NOX-A12 (200mg, 400mg or 600mg per week) for 26 weeks and concurrently

received radiotherapy for the first 6 weeks at either 60Gy (2Gy over 30 sessions) or 40.05Gy (2.67 Gy over 15 sessions). This combination resulted in 90% of patients experiencing tumour size reduction compared with only 25% of patients in an identical cohort who received standard of care radiotherapy (ClinicalTrials.gov). Additionally, 40% of patients receiving NOX-A12 and radiotherapy achieved partial responses where a reduction of tumour size greater than 50% was reported and 30% of patients had one or more non-targeted lesions completely disappear compared with only 10% in the cohort receiving standard radiotherapy (ClinicalTrials.gov). Most importantly, this combination was safe and well tolerated in patients with no treatment-related deaths or dose-limiting toxicities reported, which is similar to what was seen in a phase 1 clinical trial using an ApTOLL DNA aptamer which targets the brain for stroke patients (ClinicalTrials.gov; Hernández-Jiménez et al, 2022). This, therefore, emphasises the importance of how successful and beneficial aptamers can be in the therapeutic sector with the absence of adverse events in human patients.

CONCLUSIONS

With such a poor disease prognosis and low five-year survivability of patients diagnosed with glioblastoma, it is important novel therapeutics are developed to overcome the limitations of current therapies. While the BBB presents a hurdle for most drug candidates, aptamer-conjugates have shown to be effective in delivering drug payloads in treating glioblastoma and brain metastases. Regardless of whether the aptamer conjugation was with chemotherapeutics, siRNA, or nanoparticle complexes, each study evaluated within this review that showed the ability to cross the BBB observed greater therapeutic potential in treating glioblastoma given the target sensitivity and specificity aptamers are known for. Additionally, the safety and tolerability of aptamers in treating brain-related morbidities reflects the importance of using aptamers as a targeted therapeutic. This article not only reflected on recent advancements within the last 6 years of aptamer conjugates as a therapeutic for glioblastoma, but highlighted a series of aptamers and aptamer targets that could be considered within future developmental studies.

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STATEMENT OF COMPETING INTERESTS

None declared.

LIST OF ABBREVIATIONS

AuNP: Gold nanoparticles
BBB: Blood-brain barrier
BG: O^6-benzylguanine
BIM: B-cell lymphoma-like protein 11
BMEC: Brain microvascular endothelial cells
DOX: Doxorubicin
EGFRvIII: Epidermal growth factor receptor variant III
EpCAM: Epithelial cell adhesion molecule
GFAP: Glial fibrillary acidic protein
GSCs: Glioblastoma stem cells
Gy: Gray
IDH: Isocitrate dehydrogenase
miRNA: microRNA
PDGFRβ: Platelet-derived growth factor β
RME: Receptor-mediated endocytosis
RMT: Receptor-mediated transcytosis
SELEX: Systematic Evolution of Ligands by EXponential enrichment
siRNA: small-interfering ribonucleic acid
STAT3: Signal transducer and activator of transcription 3
TfR: Transferrin receptor

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