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Aptamers in neuro-oncology: An emerging therapeutic modality

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Abstract

Despite recent advances in the understanding of brain tumor pathophysiology, challenges associated with tumor location and characteristics have prevented significant improvement in neuro-oncology therapies. Aptamers are short, single-stranded DNA or RNA oligonucleotides that fold into sequence-specific, 3-dimensional shapes that, like protein antibodies, interact with targeted ligands with high affinity and specificity. Aptamer technology has recently been applied to neuro-oncology as a potential approach to innovative therapy. Preclinical research has demonstrated the ability of aptamers to overcome some obstacles that have traditionally rendered neuro-oncology therapies ineffective. Potential aptamer advantages include their small size, ability in some cases to penetrate the blood-brain barrier, inherent lack of immunogenicity, and applicability for discovering novel biomarkers. Herein, we review recent reports of aptamer applications in neuro-oncology including aptamers found by cell- and in vivo-Systematic Evolution of Ligands by Exponential Enrichment approaches, aptamer-targeted therapeutic delivery modalities, and aptamers in diagnostics and imaging. We further identify crucial future directions for the field that will be important to advance aptamer-based drugs or tools to clinical application in neuro-oncology.

Key Points

- Nucleic acid aptamers are emerging as diagnostic tools and targeting moieties for novel therapeutics. Promising target specificity, blood-brain barrier penetrance, non-immunogenicity, and stability characteristics make aptamers intriguing therapeutic modalities for neuro-oncology.
- The Systematic Evolution of Ligands by Exponential Enrichment method of developing aptamers is analogous to a drug screen of a hundred trillion molecular shapes and can be used to identify novel biomarkers and tumor cell surface targets, selective reagents, and can be implemented in vivo to optimize delivery.
- In some preclinical studies, aptamers have demonstrated superiority at targeting orthotopic brain tumor models and delivering therapeutics through a diversity of modalities. Aptamer target specificity and small size show promise in diagnostic applications, imaging, and surgery. While aptamers are promising in neuro-oncology, much work will be required to realize clinical utility.

Despite the last decade's surge in innovations for multimodal brain tumor management, translation into clinical efficacy for adult and pediatric patients remains minimal.[1] In high-grade gliomas, such as glioblastoma (GBM), median survival is 12 months with less than 10% of patients surviving 2 years from diagnosis.[2] In pediatric diffuse midline glioma harboring H3K27M mutation, prognosis is worse with a median survival of only 9 months.[3,4] The need for clinical advances is therefore urgent and ongoing.

Therapeutic efficacy in neuro-oncology is hindered by many obstacles, including the complexity of the blood-brain barrier (BBB), cerebral spinal fluid (CSF), and the tumor itself, which

together oppose adequate drug delivery and accumulation (Figure 1).[5–7] Transport across the BBB favors diffusion of small molecules (<500 Da) and even "successful" drugs initially internalized are rapidly effluxed via endothelial multi-drug resistant transporters.[8] Tumor delivery is further complicated by the rapid circulation of CSF, which replenishes every 4–5 hours, contributing to an estimated 10-fold decrease in drug concentration for every mm of distance penetrated by a water-soluble drug into the brain.[9,10] Furthermore, tumors display extensive inter-/intra-tumor heterogeneity and an immunosuppressive microenvironment.[5–7] High-grade gliomas such as GB are a composition of cancer stem cells and various clonal and subclonal differentiated tumor cells, which seed plasticity and resistance.[11,12] These characteristics drive both tumor aggressiveness and resistance, increasing the challenges of management.[5–7]

Identification of novel therapeutic targets over the last decade has facilitated the search for targeting modalities that improve therapeutic accumulation in tumor tissues. Neuro-oncology requires BBB-penetrating and cell-targeting tools. Physicians currently lack these targeted tools and therefore often rely on long-term systemic delivery of chemotherapy and radiation. This approach is associated with co-morbidities in brain tumor survivors.[13] Thus, an urgent need exists for development of novel therapeutics that facilitate adequate drug delivery across the BBB, rapid tumor homing to avoid clearance via the CSF, and targeting diversity to address tumor heterogeneity.

The use of folded oligonucleotide "aptamers" has emerged as a new paradigm in therapeutics. Aptamers often have molecular weights in the 6–30 kDa range, making them significantly smaller than antibodies. They are non-immunogenic and, in some cases, capable of crossing the BBB. Aptamers tightly engage targets through interactions based on favorable electrostatic, hydrogen-bonding, and van der Waals interactions driven by their complementary 3-dimensional shapes. Like antibodies, aptamer interactions with specific targets are often highly specific and characterized by nanomolar-range equilibrium dissociation constants. Increasingly, aptamers are being developed for precision drug delivery to cells or whole tissues as antitumor agents. In this review, we will discuss recent developments in the application of aptamers in neuro-oncology, including the selection of tumor- and tissue-specific aptamers, post-selection conjugation of aptamers to drugs for targeted delivery, their use in imaging and diagnosis of tumors, and current challenges for their translational use in clinical settings.

Unique Aptamer Characteristics

Promising advantages of nucleic acid-based pharmaceuticals have placed oligonucleotide therapies, including siRNAs, miRNAs, gene therapies, antisense oligonucleotides, aptamers, and nucleic acid vaccines, in the spotlight of recent medical advancements.[14–21] In contrast to other oligonucleotide therapies whose primary function is regulating transcription or translation of a specific gene, aptamers typically function by binding to, and sometimes altering the activity of, many kinds of molecular targets. Thus, the 3-dimensional shape, not the coding sequence, determines an aptamers functionality.

Aptamers are short, single-stranded RNA or DNA molecules with tens of nucleotides in length generated enzymatically or by solid-phase chemical synthesis. Given their composition as polymers, these single-strands fold into various 3-dimensional shapes exclusive to nucleic acids including hairpins, internal loops, crosses, pseudoknots, and G-quadruplexes.[22,23] These features serve as the scaffolding for highly specific binding interactions that, like antigen-antibody complexes, can occur with nanomolar-range equilibrium dissociation constants.[23] Thus, given preparation by scalable chemical synthesis and their ability to be "trained" to recognize a diverse range of molecular targets, aptamers are often referred to as chemical antibodies.[22]

Aptamers have distinct qualities potentially advantageous for the treatment of brain tumors as compared to other targeting moieties. These characteristics include their relatively small size, non-immunogenic character, and the potential for high target specificity (Figure 2).[22,24–28] Typical aptamers are ~5–10-fold smaller than typical IgG antibodies (~12–30 kDa vs. 150–170 kDa, respectively), potentially enabling more effective penetration of the highly selective BBB. Based on mathematical modeling, the predicted efficiency of delivery of a 34-nucleotide aptamer across the BBB is ~12% of injected dose, an estimated 100 times more than an antibody.[29,30] From a therapeutic perspective, aptamers are inherently non-immunogenic and nontoxic, even at doses 1000-fold higher than typical antibody therapeutics, making them potentially advantageous for human use.[31] While preclinical and clinical trials demonstrate that oligonucleotides do not elicit development of neutralizing antibodies in trial subjects, it is important to note that aptamer conjugation to other substances such as poly(ethylene glycol) (PEG) may trigger an immunogenic response that inhibits aptamer function.[32–35] Though not aptamers, intensely chemically modified antisense oligonucleotides have been attributed in one report to cause severe hepatotoxicity in animals even after a single dose.[36] As with therapeutic antibodies, aptamer targets must also be selected carefully as their high-affinity interactions may occur with targets found in both tumor and non-tumor cells.[37] CpG oligonucleotides have been explored as immunostimulatory vaccine adjuvants, but this effect is motif-specific and is therefore not shared by all aptamers.[38] Despite the inherent non-immunogenicity of nucleic acids, aptamers must therefore be carefully targeted and chemically modified to prevent side effects.

Aptamer production avoids the need for animals or cell cultures and can be significantly faster than manufacture of antibodies, making the selection process more cost-effective and potentially enabling the rapid development of patient-specific aptamers. Finally, aptamers have the added potential benefit that the target does not necessarily need to be identified, as aptamers can be trained using heterogeneous or undefined targets (protein, lipid, glycoprotein, etc.) present on different cells or tissues.

Modified RNA and DNA oligonucleotides can typically be stored for extended periods of time at low temperatures or lyophilized, and are readily reconstituted and can withstand repeated freeze-thaw cycles, unlike antibody-based

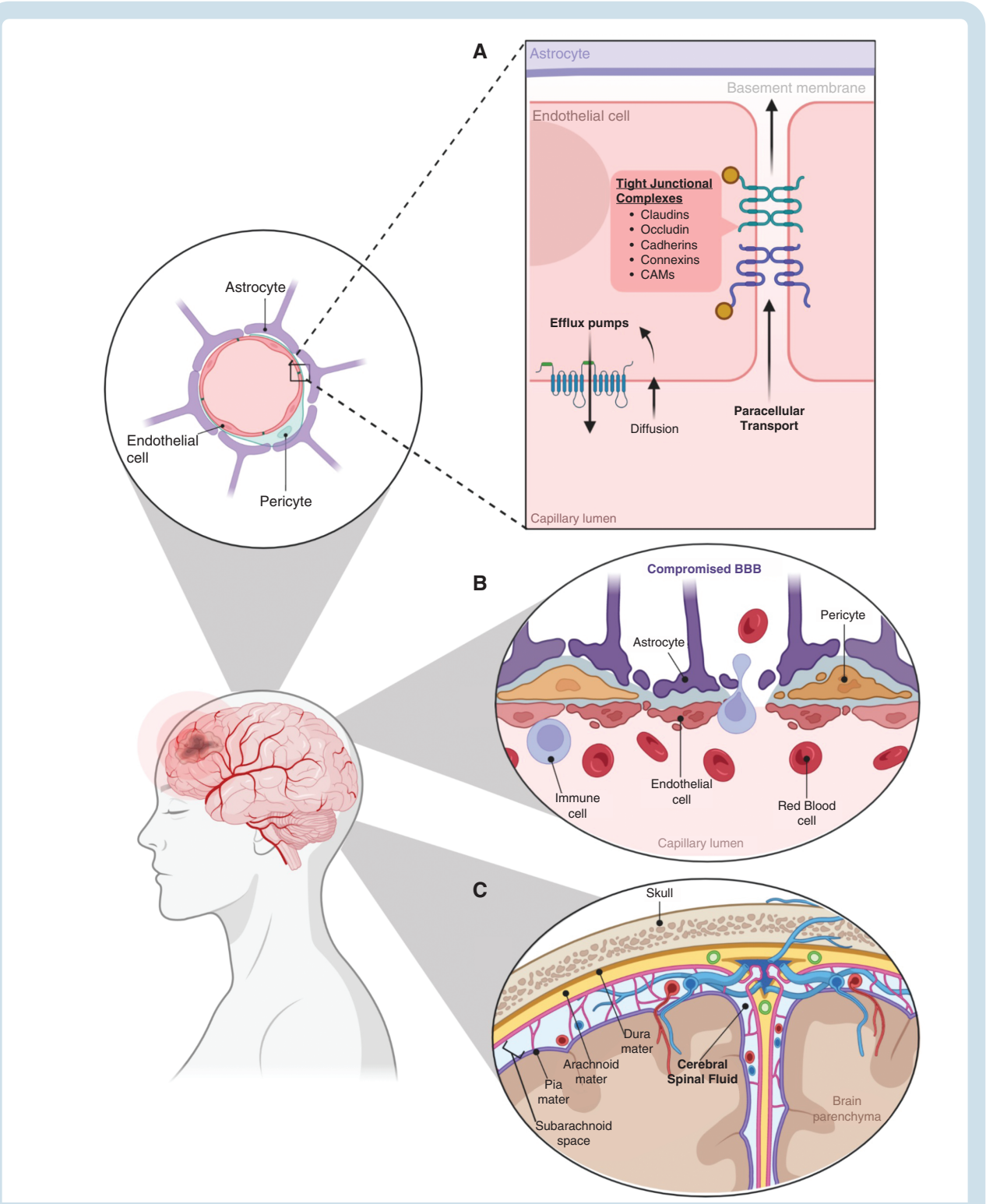

Figure 1. Obstacles to brain tumor therapy. (A) Multi-drug resistant ABC transporters and other efflux pumps remove lipophilic small molecule drugs. Paracellular transport of large hydrophilic molecules (eg, antibodies) limited by tight junctions. (B) The blood-brain tumor barrier can result in a compromised blood-brain barrier, causing the blood-brain barrier to become heterogenously "leakier." (C) Diffusion through the cerebral spinal fluid (CSF) is poor with an estimated 10-fold decrease in water-soluble drug concentration for every mm of distance into the brain. The CSF volume is turned over once every 4–5 hours per day, making it difficult to achieve steady-state in the brain. Figure was created with BioRender.com.

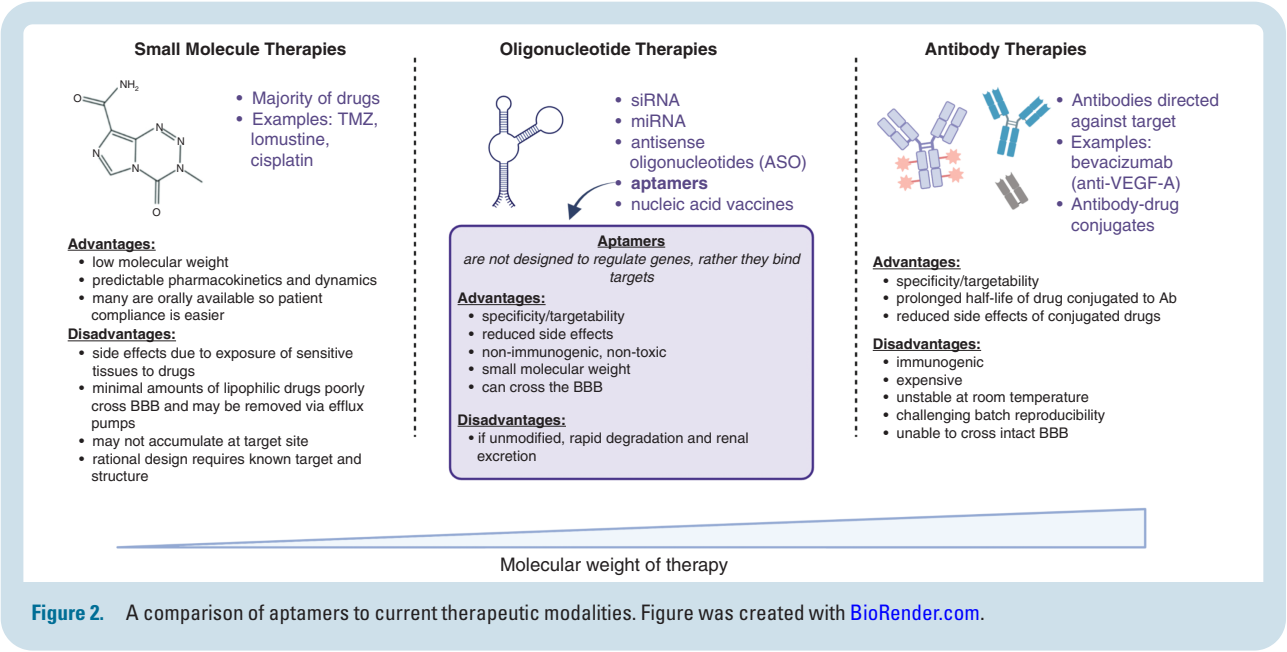

Figure 2. A comparison of aptamers to current therapeutic modalities. Figure was created with BioRender.com.

therapeutics.[39] Importantly, as synthetic polymers produced by standardized and repeatable methods, aptamers can be manufactured with a high degree of batch-to-batch consistency.

Thus, the potential for high target specificity, BBB-penetrance, non-immunogenicity, and stability in addition to relatively low manufacturing cost and a high degree of batch-to-batch reproducibility make aptamers attractive therapeutic modalities for application in neuro-oncology.

Systematic Evolution of Ligands by Exponential Enrichment and Aptamer Development

The activity of aptamers arises from their development: the principles of natural selection are used to "train" molecules to selectively identify a target through a process called Systematic Evolution of Ligands by Exponential Enrichment (SELEX; Figure 3). A typical SELEX process begins with a library of tens to hundreds of trillions of oligonucleotides containing randomly synthesized regions typically 20–60 nucleotides in length, flanked by two terminal primer binding regions enabling simultaneous PCR amplification of all sequences in the library. SELEX is characterized by the 3 defining steps that create a single round of selection: (1) incubation of a random oligonucleotide library with the target of interest, (2) removal of unbound oligonucleotides and capture of oligonucleotides bound to the target, and (3) rewarding target-bound molecules with reproduction by PCR amplification. The latter step regenerates a narrowed pool of candidate aptamers to be used as the starting material for the following round. This process is repeated until library enrichment is observed.

While early SELEX approaches identified aptamers against purified proteins or small molecules, selection now occurs against increasingly complex targets including living mammalian cells (cell-SELEX), whole tissues, or tumors animals (in vivo SELEX), among others.[40–45] Advanced technology-driven SELEX strategies including fully automated robotic selections and molecular docking simulation-based in silico aptamer selection have also become available over the last decade, but these technologies produce aptamers limited in therapeutic application.[46–49] Toggle-SELEX involves introducing the library to both positive and negative targets to reduce unintended binding in cases where aptamers are inclined to recognize common features of a SELEX design.[50–53]

Consequently, SELEX uniquely primes aptamers to address the issue of inter-/intra-tumor heterogeneity. Rather than having a specific molecular target identified, SELEX trains aptamers to distinguish differences.[54] Selection conditions can be designed so that aptamers identify novel tumor cell surface biomarkers. In theory, cocktails of aptamers targeting different molecules can be conjugated to therapeutics, broadening the cellular targeting capacity, and thus improving treatment efficacy by addressing tumor heterogeneity.

Chemical Modifications

Two Approaches to Chemical Modifications

Aptamers intended for use in cell culture, animal, or human applications are often modified for increased stability to nucleolytic degradation. Both RNA and DNA aptamers are vulnerable to nuclease degradation in human serum, and RNA aptamers are susceptible to hydrolysis at physiological pH.[55] The half-life of unmodified aptamers in serum can be as low as minutes if no steps are taken to protect from nucleases.[56] Chemical modification may increase half-life on the order of several days in circulation.[56] The most prominent extracellular DNAses are DNAse1 family members, which nonspecifically cleave single- and double-stranded DNA phosphodiester bonds at

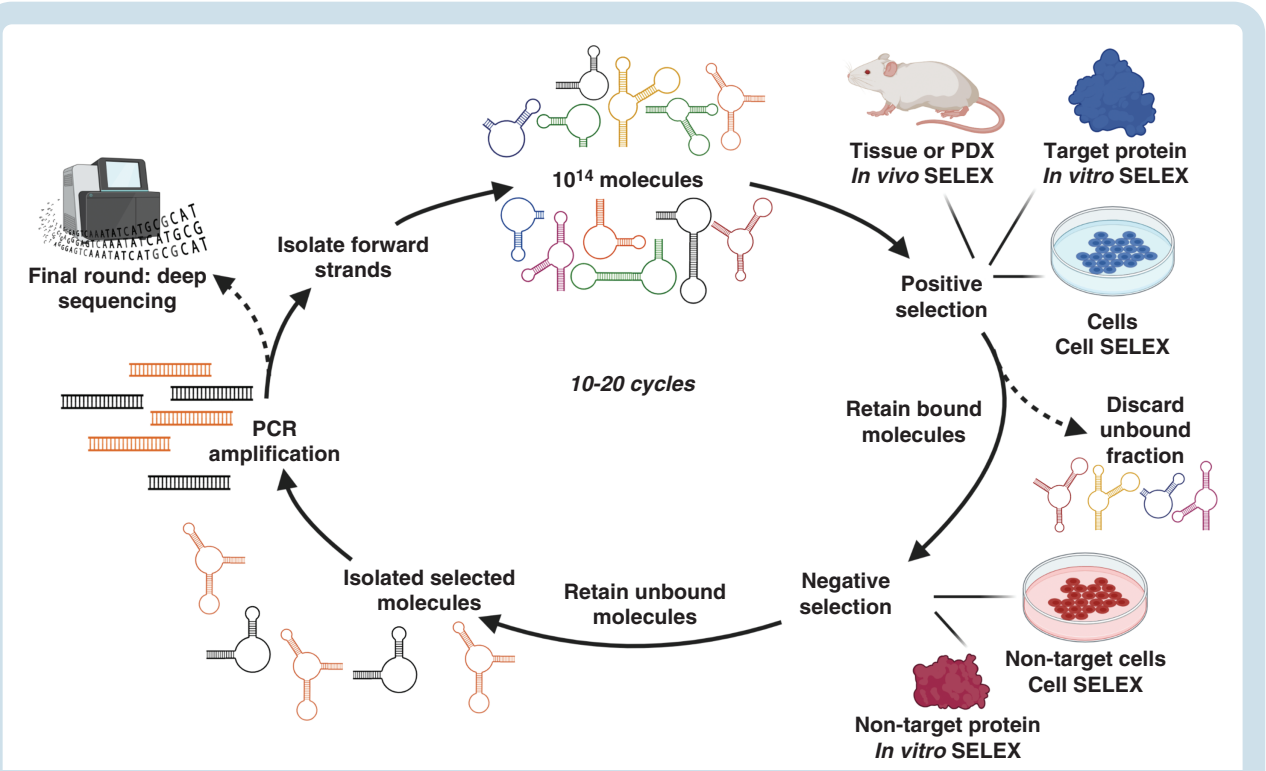

Figure 3. A graphical representation of the conventional SELEX method. Figure was created with BioRender.com.

pyrimidine residues.[57] Several RNAses are also present in serum and CSF.[58] Aptamer efficacy is therefore limited by stability in vivo, which has necessitated the development of approaches to alter and protect aptamers.

Two chemical modification strategies have emerged: (1) select an unmodified aptamer for its behavior before chemical modification, or (2) build the modifications into the selection process by using chemically modified libraries.

In the first approach, aptamers are selected against a target, and then undergo post-SELEX iterations and modifications to enhance stability and binding in vivo. One well-characterized example of multiple classes of modifications is the anti-VEGF RNA aptamer Pegaptanib, which is currently used as anti-angiogenic treatment for macular degeneration.[59]This and several other RNA aptamers were first identified without modifications in a report describing their ability to bind and inhibit vascular endothelial growth factor (VEGF) in vitro.[60] In subsequent phases of development, selection was performed on libraries seeded with these anti-VEGF sequences including various iterations of 2′-NH_2-pyrimidine and 2′-OMe purine substitutions to develop a nuclease resistant version of the aptamer.[59,61] Subsequent post-SELEX modifications led to the addition of phosphorothioate-linked poly(deoxythymidine) caps at both the 5′- and 3′-termini, 2′-F pyrimidines, 5′ terminal 40 kDa PEG addition, and a 3′ inverted dT cap. This medicinal chemistry approach resulted an aptamer half-life of 90–100 hours following intravitreal administration with detectable activity up to 28 days post-injection.[26,62]

Recently, a second approach has been used in which aptamers are selected in physiological conditions and/or bearing the cargo that they are intended to carry as a therapeutic. Consequently, survivors of these selections not only successfully bind targets, but also are optimized pharmacaologically.[44,45,63–65] For example, in an orthotopic xenograft mouse model for hormone-refractory prostate cancer, aptamer D3P-21 was selected by in vivo-SELEX.[63] Not only did the aptamer demonstrate remarkable tumor-homing capacity, but it also maintained in vivo efficacy with minimal modifications as it consistently survived systemic circulation and reached the target during selection rounds.[63] A notable challenge is that, unless creative alternatives are found, chemical modifications of aptamer libraries must be compatible with the enzymatic polymerization steps required for PCR.[66–68] To date, no aptamers have been reported using this method to select molecules targeting brain tumors. As this approach has evolved in the last few years, the clinical success of aptamers selected in this method has yet to be determined.

Chemical Modifications Examples

Chemical modifications can be made to the phosphodiester backbone, the deoxyribose/ribose sugars, or the nitrogenous bases (Figure 4). Alterations to the phosphodiester backbone or the sugars have been reported to increase aptamer resistance to nuclease degradation.[69–72] Base substitutions with modified nucleotides can alter the affinity of aptamers for their targets.[73] One hypothesis for the increased systemic stability and delivery of aptamers carrying hydrophobic bases is enhanced association with serum albumin as a protective bloodstream carrier with presumed aptamer dissociation when in contact with tumor cells.[74]

Along with stability to nuclease degradation, avoiding rapid renal clearance is crucial in translating aptamers to clinical application. 5′- or 3′-terminal modifications have also been introduced with the goal slowing clearance or improving cellular uptake. Many aptamers (~26 kDa for an 80-nucleotide single-stranded DNA) are below the size required to resist human glomerular filtration (30–50 kDa).[75] Terminal PEG modification is commonly used to address this and can increase aptamer retention in circulation from minutes to days.[76] Terminal N-acetylgalactosamine (GalNAc) has also been added to improve cellular uptake. Figure was created with BioRender.com.

Conjugates

As opposed to terminal and internal modifications which improve stability, aptamer conjugations are applied post-establishment of aptamer specificity and stability with the goal of delivering cargo to targeted cells or tissues. Like terminal modifications, conjugation can also protect from rapid renal excretion and degradation in vivo. Commonly considered payloads include chemotherapeutics, siRNAs, nanoparticles, radionuclides, and proteins.[24] Bifunctional aptamers, in which two oligonucleotides are conjugated or combined, have also been created.[26,77]

Other strategies for aptamer-cargo conjugation include terminal cholesterol modification for lipid nanoparticle incorporation, covalent attachment to radionuclides and metal nanoparticles, or non-covalent but highly stable biotin-streptavidin interactions.[78–84]

Aptamers in Preclinical Neuro-Oncology

Many aptamer applications in neuro-oncology have been accomplished in the last 15 years. Most new aptamers were selected against GBM models via cell-SELEX. However, the primary paradigm in neuro-oncology has been to apply

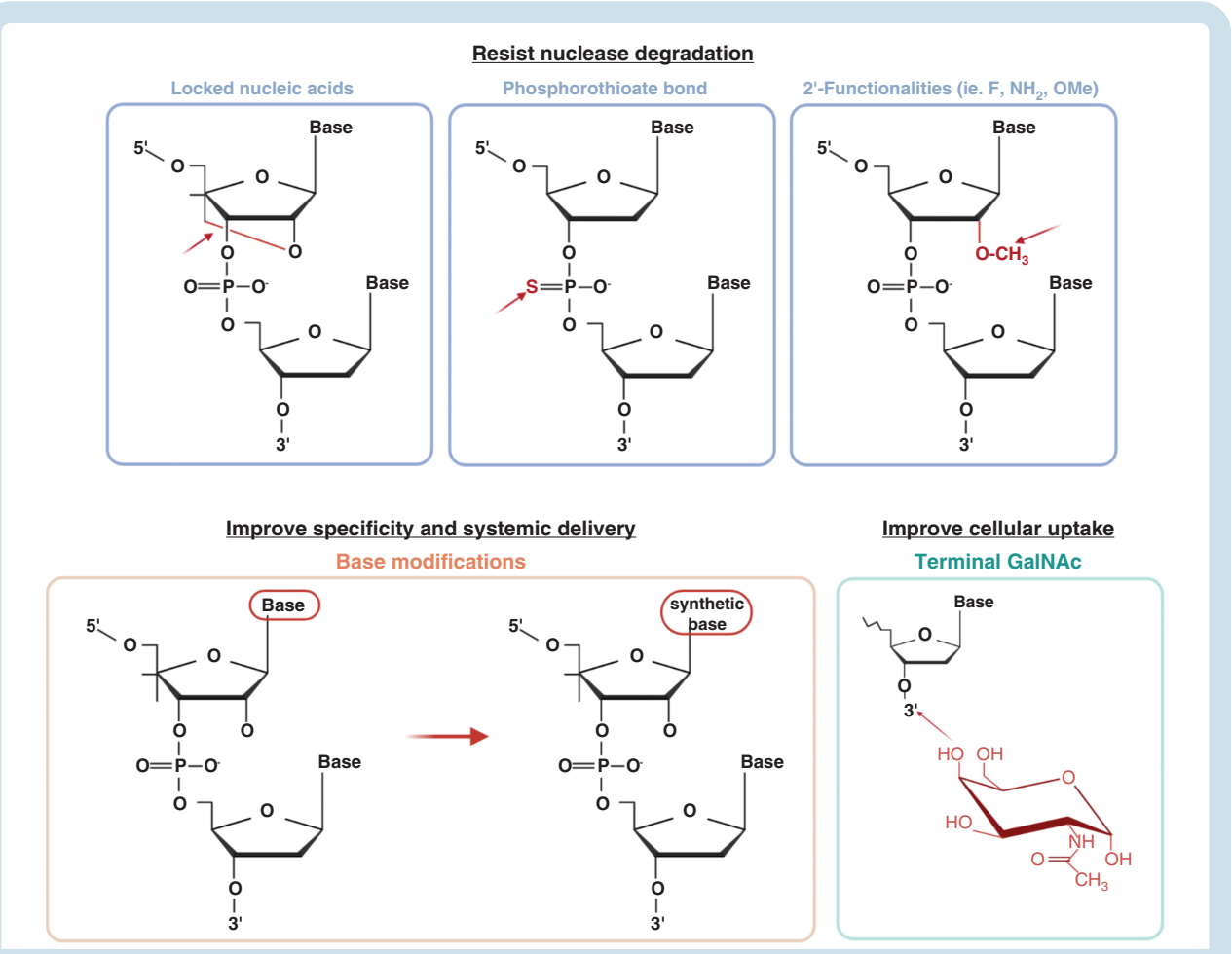

Figure 4. Examples of chemical modifications and their effect on aptamers. Altering the phosphodiester bond of the backbone with either phosphorothioate or phosphorodiothioate analogs has been shown to increase aptamer resistance to nuclease degradation.[69,70] Sugar modifications such as locked nucleic acids (LNAs) reportedly increase nuclease stability of the aptamer and certain base pairing interactions.[71,72] Base substitutions with modified nucleotides can alter the affinity of aptamers for their targets.[73] In one example, the addition of a hydrophobic 3,5-bis(trifluoromethyl)benzoyl analog at two terminal and three internal positions reportedly improved tumor specificity, systemic delivery, and stability in vivo.[74] One hypothesis for the increased systemic stability and delivery of aptamers carrying hydrophobic bases is enhanced association with serum albumin as a protective bloodstream carrier with presumed aptamer dissociation when in contact with tumor cells.[74]

previously selected aptamers to neuro-oncologic therapies as targeting moieties, rather than seeking novel aptamer targets. It is important to note that, to date, no aptamers have entered clinical trials for neuro-oncology. Thus, it is imperative to understand the status of preclinical research in this field and obstacles to application in the clinic.

Aptamers Identified by Cell-SELEX

Cell-SELEX has been used to develop several preclinical aptamers relevant to neuro-oncology (Table 1). Aptamers developed from this method report stringent target specificity and, in some cases, receptor inhibition similar to small molecule pharmaceuticals. However, they commonly require modifications to optimize for therapeutic use. Cell-SELEX can be a tool for either specific targeting or biomarker identification as the target ligand is typically unknown.

By using a human GBM line overexpressing EGFRvIII (U87-EGFRvIII) as the positive selection and the parental GBM line as the negative selection, three different anti-EGFRvIII aptamers have been developed. Wu et al. used this approach to identify anti-EGFRvIII aptamer U2 and reported that ^{188}Re radiolabeled U2 accumulates in mouse GBM xenografts with significant anti-tumoral effects.[79,88] Further testing suggested that aptamer binding to EGFR inhibited downstream tyrosine kinase signaling, inhibiting DNA repair after radiation.[88] It was reported that U2 induced apoptosis in the target cells (U87-EGFRvIII), but not the parental cells, and inhibited cell migration and invasion.[88] Aptamers 32 and CL4 are two more DNA oligonucleotides developed by similar approaches and reportedly bind to EGFRvIII.[86,89,93] Like U2, CL4 has been reported to bind EGFRvIII in a manner that inhibits tyrosine kinase autophosphorylation, resulting in inhibited cell migration, invasion, and growth.[89]

Target-blind cell-SELEX against GBM cells has identified aptamers that interact with PDGFRβ and tenascin-C. Camorani et al. selected against a parental human GBM line (U87MG) and developed aptamer Gint4.T, which appears to binds the human PDGFRβ ectodomain and is reported to strongly inhibit receptor-dependent activation and downstream signaling in GBM cells.[87] Testing of Gint4.T in vitro suggested that Gin4.T aptamer treatment inhibits cell migration and proliferation. In vivo, Gint4.T was reported to induce GBM cell differentiation and slow tumor growth.[87] GBI-10 is an aptamer selected against human GBM line U251 and is believed to target malignancy-related tenascin-C.[85]

GBM128 and GBM131 are additional GBM-specific aptamers with unknown targets. Both reportedly bind multiple GBM cell lines and weakly interact with non-GBM tissues.[50] Taken together, cell-SELEX has produced a number of aptamers with potential therapeutic relevance to neuro-oncology.

Two additional cell-SELEX aptamers of neuro-oncologic interest target human glioma cells and human glioma stem cells (GSC), rather than GBM. DNA aptamer S6-1b was selected against human glioma cell line SHG44 and negatively selected against human astrocyte cell line SVGp12.[91] S6-1b is reported to be specific for its target cells in vitro, demonstrating binding to targeted SHG44 cells but ignorance to human colon carcinoma cells, hepatoma cells, gliosarcoma, colon carcinoma, lung adenocarcinoma, or triple negative breast cancer cells.[91] In mouse models, S6-1b reportedly demonstrated homing to subcutaneous glioma tumors for up to 4 hours following intravenous administration.[91] Preliminary evidence suggested that S6-1b binds fibronectin, though this has not been validated.[91] Recognizing the challenge presented by stem cells in brain tumor heterogeneity, Wu et al. used freshly prepared GSC to develop aptamer W5-7.[90] GSCs (CD133+) were used as the positive selection target, while U87 GBM cells were used for negative selection. The authors reported that W5-7 strongly binds GSCs, but did not bind two human GBM cell lines, hepatocellular carcinoma, cervical cancer, triple negative breast cancer, colon cancer, or gliosarcoma cells, suggesting selectivity even among different brain tumor cell models.[90] W5-7 was modified post-selection with four 2′-OMe nucleotides at both the 5′ and 3′ termini, reportedly conferring aptamer stability in CSF for over 36 hrs.[90] This GSC-specific aptamer was hypothesized by the authors to bind CD133, but this awaits experimental verification.

Aptamers Identified by In Vivo SELEX

In vivo SELEX is a relatively new approach that has yet to produce preclinical neuro-oncology aptamers (Table 1). Currently, the most relevant published preclinical aptamers identified by in vivo SELEX have been against normal mouse brain parenchyma, human pediatric neuroblastoma, human hormone-refractory prostate cancer, human non-small cell lung carcinoma, and human colorectal carcinoma.[63–65,92] In vivo aptamer selection has many theoretical advantages. Thus, lessons learned from targeting other cancers using this method help illuminate benefits and limitations of in vivo selection for development of aptamers in neuro-oncology.

In vivo selection involves injection of the aptamer library into a living organism, with subsequent recovery of aptamers from the desired tissue and/or sub-cellular location after a desired time of circulation. If scrupulous perfusion is used to remove blood-born aptamers prior to tumor harvest, in vivo SELEX has the potential to select aptamers that can cross the BBB and access target tissues within the brain. The reportedly brain-specific aptamer A15 was selected by injecting a library of 2′-F-pyrimidine modified aptamers into the mouse tail vein and allowing circulation for 1 hour (rounds 1-8) or 3 hours (rounds 9-22) with thorough perfusion post-mortem before the brain was extracted and brain-specific aptamers amplified for the next selection round.[65] A resulting aptamer, A15, reportedly bound brain capillary endothelia and penetrated into healthy mouse brain parenchyma, unlike a scrambled negative control sequence, as determined by using a capillary depletion protocol with aptamer detection via qPCR.[65] This result was supported histologically using an in vitro hybridization experiment, identifying the presence of aptamers in capillaries versus parenchyma.[65] The A15 target was not identified, but this work provided foundational evidence that aptamers can be selected to cross the BBB.

Table 1. Preclinical aptamers developed or tested in neuro-oncology via cell-SELEX or in vivo-SELEX.

Preclinical Cell-SELEX Selected Aptamers								
Aptamer Name	Year	SELEX Method	Nucleic Acid	Cells		Molecular Target	Base Modifications	Citation
GBI-10	2003	Cell SELEX	DNA	U251 cells	Glioblastoma	tenascin-C	None	85
GBM128	2012	Cell SELEX	DNA	U118-MG cells	Glioblastoma	unknown	None	50
Aptamer 32	2013	Cell SELEX	DNA	U87Δ cells (overexpress EGFRvIII)	Glioblastoma	EGFRvIII	None	86
Gint4.T	2014	Cell SELEX	RNA	U87-MG cells	Glioblastoma	PDGFRβ ectodomain	2′F-Pyrimidines	87
U2	2014	Cell SELEX	DNA	U87-EGFRvIII cells	Glioblastoma	EGFRvIII	None	79
	2019						None	88
CL4	2015	Cell SELEX	RNA	A549 cells	Lung adeno-carcinoma (applied to GBM)	wild-type EGFR	2′F-Pyrimidines	89
W5-7	2019	Cell SELEX	DNA	Glioma stem cells		Suggest CD133, but not confirmed	First 4 bases in 5′ and 3′ termini with 2′-O-Me	90
S6-1b	2021	Cell SELEX	DNA	SHG44 cells	Human glioma	unknown	None	91
Preclinical in vivo-SELEX selected aptamers								
A15	2013	In vivo SELEX	RNA	Healthy brain	Neurology	unknown	2′F-Pyrimidines	65
D3P-21	2019	In vivo SELEX	DNA	Orthotopic prostate cancer model	Oncology	unknown	PEG	63
RA16	2018	In vivo SELEX	RNA	Non-small cell lung cancer xenograft	Oncology	unknown	2′F-Pyrimidines, PEG	64
S-1	2016	In vivo SELEX	RNA	Colorectal cancer PDX	Oncology	DHX9 (helicase)	2′F-Pyrimidines	45
RNA 14-16	2010	In vivo SELEX	RNA	Intrahepatic colorectal cancer metastases	Oncology	p68 (helicase)	2′F-Pyrimidines	44
DB99	2021	In vivo SELEX	DNA	Neuroblastoma xenograft	Neuroendocrine oncology	GD2	5′ mono thiophosphate	92

In principle, in vivo SELEX *applies selective pressure to optimize pharmacological parameters such as bioavailability and pharmacodynamics. In one example,* in vivo SELEX *developed aptamer D3P-21, which reportedly homes to hormone-refractory prostate tumors.*[63] Interestingly, the authors reported that D3P-21 induced 10-fold less TNFα secretion than an untrained random library in an in vitro model of immortalized murine embryonic stem cell-derived macrophages.[63] This result suggests that the original library contained structural motifs capable of TLR innate immunity recognition, but that this property was not retained in D3P-21 after whole-animal selection.[63] Similarly, Zhang et al. reported the selection of an anti-glycolipid GD2 aptamer using pediatric neuroblastoma tumors.[92] This selection involved a hybrid of in vivo and in vitro SELEX to produce an aptamer that reportedly survives systemic circulation and homes to neuroblastoma tumors without inducing an observable immune response in a mouse model of the disease.[92] The authors reported that therapeutic doses of free doxycycline resulted in greater whole-animal tissue damage than the same dose of doxycycline intercalated between base pairs of the folded anti-GD2 aptamer. The free doxycycline dose required for tumor reduction reportedly resulted in bleeding, inflammation, or cell damage in the heart, lung, kidney, liver, and spleen of treated animals, whereas GD2-doxcycline was not reported to induce damage. This result suggests potential for aptamer-conjugated therapeutics in neuro-oncology to target tumors with high specificity and fewer side effects than traditional systemic delivery. Whole-animal GD2-doxycycline delivery demonstrates the intriguing potential to exploit in vivo SELEX *to select for aptamers that specifically perform well in systemic circulation.*[92]

In vivo *SELEX* has also identified aptamers that reportedly inhibit cancer cell proliferation without the addition of cytotoxic agents. RA16 is a PEGylated 2′F-pyrimidine RNA aptamer selected against NCI-H460 non-small cell lung cancer cells in a mouse underarm tumor model.[64] Not only was this aptamer reported to accumulate in tumors in vivo, but it also reportedly inhibited growth of NCI-H460 cells in vitro and in vivo.[64] When RA16 was conjugated with epirubicin, it was reported to demonstrate higher toxicity

to these same cells both in vivo and in vitro.[64] This suggests aptamers have the potential to act simultaneously as targeting moieties and inhibitors, a principle that might be similarly explored in neuro-oncology.

Finally, *in vivo* selections against tumors can identify novel pathways and targets. In two *in vivo* selections, aptamers were reported to selectively bind either colorectal carcinoma xenografts or intrahepatic colon carcinoma metastases.[44,45] Further analysis suggested that these aptamers bind two different RNA helicases, DHX9 and p68, believed to be upregulated in the respective tumor models.[44,45] This work supported previous experiments suggesting that these upregulated helicases shuttle between the nucleus and cytoplasm, perhaps transiently appearing at the cell surface as has been reported for nucleolin, thus elucidating a potentially new therapeutic target and novel pathway to be further studied in cancer biology.[44,45] This work exemplifies the potential value of *in vivo* SELEX *in the neuro-oncology field to identify novel disease biomarkers.*

Aptamers as a Platform for Targeted Delivery

Much of the preclinical work applying aptamers in neuro-oncology has focused on using aptamers as targeting moieties by direct conjugation to therapeutics, intercalation of the therapeutic between base pairs, or decoration of nano-delivery systems (**Table 2**). Novel aptamers were not selected in most of these conjugation studies. Instead, previously described aptamers were added to a delivery system or therapeutic, and delivery was tested in relevant disease models. Although there remains a need for selection of new aptamers in neuro-oncology models, the themes observed in these preclinical studies using known aptamers as targeting moieties hint at their potential power and utility.

Several classes of aptamer-drug conjugates (ApDC) have been applied in cancer treatment strategies targeting both primary tumor sites and metastatic lesions. In a study of breast cancer metastases in brain, a bifunctional aptamer with a pH-sensitive linker separating domains targeting the transferrin receptor (TfR) and epithelial cell adhesion molecule (EpCAM) was tested with doxorubicin believed to be non-covalently intercalated at about 6 sites per aptamer.[77] The authors reported that even with the addition of doxorubicin, the aptamer maintained specificity, selectivity, and internalization. Interestingly, when brain-metastatic breast cancer cells were treated with the ApDC, doxorubicin retention was reportedly greater than for free drug, suggesting that the aptamer not only contributes to cellular uptake but also to retention of the drug.[77] This ApDC reportedly demonstrated transcytosis of the BBB in an *in vivo* model and specifically targeted tumor cells in the brain *in vivo*.[77]

Aptamers have frequently been reported to successfully deliver siRNAs after hybridization to form stable duplexes formed by complementary regions between the aptamer and siRNA. Using AS1411 (targeting nucleolin) and a "CpG" oligodeoxynucleotide (targeting TLR9), bi-specific aptamers were applied with the goal of delivering siRNA against osteopontin (OPN) mRNA, which is highly expressed in GBM and is thought to contribute to the malignant biology of GBM.[26,100] In another study, Gint4.T (targeting PDGFRβ) was conjugated to a STAT3 siRNA to elucidate tumor delivery in a subcutaneous GBM tumor-bearing mouse model.[94] Aptamer conjugation was reported to enhance knockdown relative to the free siRNA in cell culture, and the combination aptamer-siRNA therapy reportedly led to reduced expression of STAT3 and STAT3 target genes *in vivo*.[94] Aptamer-mediated STAT3 siRNA delivery reportedly reduced tumor cell proliferation and neovascularization relative to the siRNA hybridized to a nonfunctional negative control oligonucleotide.[94] The most promising example of siRNA delivery involved anti-GD2 aptamer DB99, which was formulated into a self-assembled complex of the synthetic aptamer, a neuroblastoma-specific MYCN siRNA, and self-loading doxorubicin.[92] The conjugated aptamer retained selectivity for $GD2^+$ neuroblastoma tumors.[92] DB99 conjugates were reported to simultaneously release doxorubicin and induce MYCN silencing in tumors, resulting in reduced tumor growth *in vivo*. Importantly, the complexes were reported to exhibit a nontoxic profile even as the amount of the chemotherapy reached twice the standard dose.[92] As this work suggests, ApDCs may be able to maintain aptamer specificity and function, while carrying conjugated therapeutics, potentially increasing efficacy of the agent.

Future aptamer-mediated siRNA delivery may also aim to regulate expression of fusion oncogenes.[101] Fusion genes are prevalent in neurologic tumors and in some cases indicate poor prognosis relative to standard outcomes.[102,103] Importantly, siRNAs can be designed against fusion oncogenes such that they bridge the junction site of two transcripts to reduce expression of the fusion protein without impairing expression of normal protein. This approach has been explored in vitro in glioblastoma[104] and other non-neurological cancers[105,106] with success, but has not yet been applied in animal models of neuro-oncology disease or with aptamer delivery. siRNA could be delivered by either loading into aptamer-conjugated nanovesicles or by direct duplex hybridization to terminal unstructured aptamer regions.

A second focus of current aptamer studies in neuro-oncology involve conjugation of well-characterized aptamers to nano-delivery systems. Aptamer AS1411 (targeting nucleolin), has been the most commonly used aptamer in neuro-oncology.[81,84,95,98] Luo et al. added AS1411 to a (L-γ-glutamyl-glutamine)-paclitaxel (PGG-PTX) nanoconjugate drug delivery system, creating AS1411-PGG-PTX.[95] AS1411 conjugation reportedly resulted in increased cellular binding of the complex and subsequent internalization.[95] Conjugation of AS1411 was reported to increase retention of the complex in tumors. Peak fluorescent intensity in a GBM animal model was shown to be 24 hours post-injection, and the therapy was detectable in systemic circulation for 48 hours.[95] Though this aptamer was conjugated to a large cargo, AS1411-PGG-PTX reportedly penetrated *in vitro* tumor spheroids more effectively than the untargeted delivery system.[95] Brain penetration and bloodstream half-life of the targeted nanoconjugate were reportedly slightly better than the unconjugated

Table 2. Preclinical aptamers in neuro-oncology that have been used as a targeting moiety.

Aptamer Name	Year	Nucleic Acid	Aptamer Target	Aptamer Modifications	Conjugated Drug	Model Tested		Citation
Aptamer-drug conjugates (ApDC)								
TEPP	2020	DNA	TfR, and EpCAM	None	Doxorubicin	Orthotopic human brain-metastatic breast cancer xenograft		77
Aptamer-nucleic acid therapies								
Gint4.T	2018	RNA	PDGFRβ	2′F	siRNA to STAT3	Subcutaneous GBM xenograft mouse model		94
A1411 and a CpG ODN	2022	DNA	Nucleolin and TLR9	Phosphothioate on CpG aptamer	siRNA to osteopontin	Orthotopic mouse glioma model		26
Aptamer as targeting moieties on nanocomplex								
AS1411	2012	DNA	Nucleolin	None	PEG-PCL nanoparticles (AsTNP)	Docetaxel	Orthotopic glioma model	81
AS1411 or GBI-10	2013	DNA	Nucleolin or Tenascin-C	Biotinylated	adenovirus	--	Human GBM cell culture	84
AS1411	2017	DNA	Nucleolin	None	poly (l-γ-glutamyl-glutamine)-paclitaxel (PGG-PTX) nanoconjugates drug delivery system	Paclitaxel	Orthotopic GBM xenograft	95
Gint4.T	2017	RNA	PDGFRβ	2′F	Polymeric nanoparticle [poly(lactic-co-glycolic)-block-poly ethylene glycol (PLGA-b-PEG) copolymer]	PI3K-mTOR inhibitor	Orthotopic GBM xenograft	96
DB99	2021	DNA	GD2 glycolipid	5′ monothiophosphate	3 ssDNA sequences	Doxorubicin	Neuroblastoma flank tumors	92
GMT8	2022	DNA	U87 cells	None	PEGylated Ag@Au core-shell nanoparticles	--	Orthotopic GBM xenograft	97
AS1411	2022	DNA	Nucleolin	cholesterol conjugated AS1411 for embedding in exosomes	Aptamer coated macrophage exosomes, encapsulating glutathione-responsive biodegradable silica nanoparticles (CAT@SiO2)	Catalase	Orthotopic GBM xenograft	98
CD133 RNA Aptamer	2022	RNA	CD133	2′F	Exosome	TMZ	Orthotopic GBM xenograft	99
TfR aptamer	2022	DNA	Transferrin	Cholesterol conjugated for embedding in exosomes	biomimetic nanocomplexes (TMPsM)	PEP-TPE, siRNA to Transglutaminase 2	Neuro-oncology	27

PGG-PTX control, and median survival of tumor-bearing mice was increased relative to controls.[95]

In addition to delivering chemotherapy, AS1411 has been tested for driving larger payloads to brain tumors. Wu et al. encapsulated catalase into silica nanoparticles "CAT@SiO_2," loaded with a sensitizer, and transfected the nanoparticles with modified macrophage exosomes decorated with AS1411.[98] The strategy was intended to prime tissues for sonotherapy (ultrasound-induced creation of local tumoricidal reactive oxygen species), by co-delivering sensitizer and oxygen generator to tumor cells.[98] If nanoparticles were endocytosed by tumor cells, catalase release was intended to convert hydrogen peroxide to O_2, potentially reducing local tumor hypoxia and providing an oxygen source for reactive oxygen species generation by sonotherapy.[98] Tumor targeting is critical for the efficacy of this concept. Aptamer addition reportedly resulted in marked tumor cell-targeting *in vitro* and *vivo*.[98] Similar to the PGG-PTX nanoconjugate, this platform appeared to increase aptamer in circulation as the peak measurements in the orthotopic xenograft brain tumor were reported 12 hours post-injection.[98] The majority of

the conjugate was reportedly eliminated through the liver and spleen over 7 days.[98]

AS1411 has also been applied as a conjugate to increase viral uptake. Aptamers AS1411 (targeting nucleolin) or GBI-10 (targeting Tenascin-C) were biotinylated and conjugated to Ad5 adenovirus with coat protein expressed as a fusion with a biotin acceptor peptide.[84] U251 human GBM cells, which overexpress nucleolin and Tenascin-C, were transduced by aptamer-coated adenovirus delivering a luciferase expression vector as a readout of transduction efficiency. Aptamer-coated viruses were reported to transduce GBM cells 4 to 5-fold more efficiently than HUVEC cells, which express normal levels of nucleolin and Tenascin-C.[84] Approaches of this nature remain relatively unexplored to date, but may be useful for driving targeted oncolytic virus uptake in tumors.

Gint4.T, an aptamer believed to target PDGFRβ (commonly overexpressed in GBM), was used to decorate the surface of a copolymer nanocomplex of poly(lactic-co-glycolic)-block-polyethylene glycol (PLGA-*b*-PEG). This complex contained the potent dual PI3K-mTOR inhibitor Dactolisib (NVP-BEZ235).[96] Aptamer-conjugated nanocomplexes with Dactolisib ("@PNPs-Gint4.T") were reportedly internalized by PDGFRβ-overexpressing cells *in vitro* and the authors reported the drug to be at least 1000-fold more toxic when delivered by nanovector than as a free addition to media. Additionally, the complex was 4-fold more toxic than the nanocarrier with scrambled negative control aptamer acting as the targeting moiety.[96] Given the rapid turnover of the CSF, it is crucial that drug is quickly up taken into target cells. In this preclinical study, the authors reported that Dactolisib accumulation in targeted cells was 6500-fold improved in @PNPs-Gint4.T versus free drug and 65-fold higher than in conjugates with a negative control oligonucleotide after only 40 minutes of incubation. Aptamer conjugation in this study therefore apparently drove rapid and efficient uptake of drug into target cells, resulting in and more efficient GBM toxicity.[96] As expected, significant uptake in brain and accumulation in the tumor was seen *in vivo*.[96]

Aptamers have also been suggested as radiotherapy enhancers. GMT8, an aptamer intended to target U87 cells, was added to the surface of PEG-coated Ag@Au core-shell nanoparticles (GSGNPs) that had previously been reported to enhance the efficacy of radiotherapy.[97] Mice with orthotopic GBM xenografts receiving radiation therapy alone had a mean survival time of 36.5 days, whereas mice receiving GSGNPs reportedly had a mean survival time of 58.5 days.[97] When the tumors were analyzed ex vivo 24 hours post-injection, it was reported that the GSGNP-treated mice demonstrated a ~9-fold increase in the presence of the nanoparticles in the tumor compared to the nanoparticles lacking the aptamer.[97]

Similar targeting has been reported for other aptamers. Exosomes loaded with Temozolomide (TMZ) and O^6-benzylguanine and decorated with anti-angiopep-2 peptide and anti-CD133 RNA aptamer (EXO-An2-Apt) were reported to increase survival by ~44% in orthotopic GBM xenograft mice by compared treatment with free TMZ and O^6-benzylguanine.[99] Histological analysis reportedly revealed limited systemic toxicity from the targeted exosome payloads, though TMZ and O^6-benzylguanine-treated mice demonstrated alveolar congestion and disordered arrangement of cardiomyocytes and hepatocytes. In contrast, organs of the EXO-An2-Apt were reported to share appearance with tissues of PBS-injected mice.[99]

Diagnostic Aptamers

Given their perceived character as "chemical antibodies," aptamers are promising diagnostic tools for staining solid tissue biopsy samples, identifying novel CSF biomarkers, and developing liquid biopsy diagnostic techniques. The relative ease of aptamer selection, their antibody-like specificity, and the potential to identify novel biomarkers using the SELEX process all suggest potential opportunities for aptamers in cancer diagnostics.

An example aptamer relevant to neuro-oncology solid tissue biopsy diagnostics is H02 (**Table 3**).[107] This aptamer is believed to target integrin α5β1, which is implicated in GBM tumor angiogenesis and aggressiveness.[107] As emphasized previously and demonstrated with H02, highly stringent SELEX is required for defined target specificity. Using a hybrid of protein- and cell-SELEX methods that progressively increased stringency, a report describes selection of a nuclease-resistant RNA aptamer thought to be specific for the α5 subunit of α5β1.[107] The authors characterized the ability of the H02 aptamer to distinguish between 10 human GBM cell lines expressing different levels of the α5 subunit, resulting in a reported 0.78 correlation coefficient between traditional antibody immunofluorescence and aptafluorescence.[107] Histochemical analysis was performed on two patient-derived GBM xenografts in which mRNA integrin α5β1 expression levels had been found by other methods to differ by 7.6-fold by qPCR. Antibody immunofluorescence reportedly detected a 2-fold difference while aptafluorescence suggested a 4-fold difference in the same tumor samples.[107] The authors hypothesize that the more accurate quantitation was due to better fixed tissue penetration by the smaller aptamers.[107] Not only do these study results demonstrate the limitations of current methods, but they potential advantages of aptamers for fixed biopsy staining. While aptamer H02 is among only a few studied for use as a diagnostic for solid tissue biopsies in neuro-oncology, in principle most aptamers selected via stringent cell-SELEX are potential candidates for further development as diagnostic staining tools (**Table 1**).

The liquid biopsy has emerged as a noninvasive method for establishing the prognosis of various tumors. Diagnostic aptamers are increasingly considered for several cancers but have yet to be applied to liquid biopsies in neuro-oncology.[111,112] A number of aptamer probes ("aptasensors") have been developed to detect and quantify tumor-derived extracellular vesicles, including circulating serum exosomes in breast, lung, ovarian, cervical, and hepatocellular carcinoma patients.[113–116] Additional work has been done toward the goal of identifying novel biomarkers via aptamer selection ("aptamarkers") when SELEX is performed using extracts of patient serum or urine.[117,118] An advanced commercial aptamer-based proteomics technology ("SOMAscan") enables a single sample to be screened for the presence of over 7,000 different

proteins using unique aptamers for each. This approach has recently been used to identify a potential biomarker in CSF of patients with mental illness.[119] Though none of these preclinical studies have yet been applied in the context of neuro-oncology, they suggest that applications of aptamers in liquid biopsies may increase sensitivity and specificity, improvements that would be especially useful.

Aptamers in Imaging

Aptamers can readily be conjugated to fluorophores and radionuclides. This allows researchers to attempt targeting of specific tissues with high specificity and signal retention. Preclinical results suggest promising applications of aptamers for imaging in neuro-oncology (**Table 3**). TTA1, an aptamer believed to target extracellular matrix protein tenascin-C, was prepared as fluorescent (rhodamine Red-X-labeled) or radiolabeled with ^{99m}Tc via conjugation to a chelator, MAG_2.[108] The two compositions were compared to an inert form lacking five nucleotides thought to be critical for target binding.[108] Analysis of GBM xenograft tumors harvested at various times after i.v. injection of the fluorescent aptamer reportedly demonstrated perivascular fluorescence within 10 minutes of delivery and diffuse fluorescent infiltration into the tumor parenchyma after 3 hours. The same fluorescent signal was completely absent after treatment with the control, non-binding aptamer.[108] Biodistribution was characterized for the radiolabeled aptamer, which reportedly displayed rapid tumor penetration between 10 and 60 minutes, with specific tumor labeling reportedly persisting for 18 hours.[108] The authors reported rapid clearance from blood along with tumor retention yielding a tumor-to-blood ratio of 50 within 3 hours, stated to be favorable for imaging.[108] The authors analyzed labeling of multiple tumor types including colon, breast, GBM, rhabdomyosarcoma, and squamous cell carcinoma, with GBM reported as showing the strongest labeling among all tumors tested.[108]

Other aptamer imaging approaches have proposed to use the anti-nucleolin aptamer AS1411. In one study, AS1411 was conjugated with fluorophore Cy3 and BBB targeting peptide TGN with the intent of increasing intracranial mouse glioma tumor labeling versus TGN alone.[109] AS1411 has also been added to the surface of cobalt-ferrite nanoparticles containing fluorescent rhodamine within a silica shell matrix to deliver ^{65}Ga-citrate to tumors in glioma-bearing mice.[120] In a third study, multiple aptamers, including AS1411, RGD, and TTA1 were conjugated to MNP@SiO_2(RITC)-PEG/COOH/pro-N/NH_2 nanoparticles, creating the Simultaneously Multiple Aptamers and RGD Targeting cancer probe. Simultaneously Multiple Aptamers and RGD Targeting probing was claimed to targeted multiple cancer cell lines including prostate cancer metastases in brain.[121]

Aptamers in Surgery

For many brain tumors managed by surgery, the primary prognostic factor is extent of resection.[122] However, the balance between maximum safe resection and tumor infiltration can be difficult. Recently, fluorescent, tumor-targeted aptamers have been tested as an intraoperative tool to help define tumor borders.

Epidermal growth factor receptor variant (EGFRvIII) is overexpressed in many human gliomas.[110] In 120 specimens (110 glioma and 10 normal brain tissues), 42% of gliomas were positive for EGFRvIII whereas normal brain tissue was negative.[110] Aptamer A32 is intended to target EGFRvIII and was conjugated to a new generation

Table 3. Preclinical Aptamers in Neuro-Oncology That Have Been Used in Diagnostics, Imaging, and in Surgery.

Aptamer Name	Year	Nucleic Acid	Aptamer Target	Aptamer Modifications	Method	Model Tested	Comparison With Current Standard	Citation
Aptamers in diagnostics								
H02	2019	RNA	integrin α5β1	none	Aptafluorescence in solid tissue histology	GBM cells and PDX tissues	Demonstrated 2-fold increased sensitivity compared to antibody immunofluorescence in PDX samples	107
Aptamers in imaging								
TTA1	2006	RNA	tenascin-C	2′-O-Me purines, 2′-F pyrimidines, 3′-3′ inverted phosphodiester	Rhodamine Red-X-labeled or ^{99m}Tc	GBM xenograft tumors		108
AS1411	2014	DNA	nucleolin	none	Cy3	Glioma cells, tumor spheroids, and orthotopic glioma bearing mice		109
Aptamer as a surgical tool								
Aptamer A32	2017	DNA	EGFRvIII/ U87 cells	None	Fluorescent quantum dots (QD-aptamer)	Orthotopic glioma bearing mice		110

of brightly fluorescent quantum dots believed to have reduced toxicity, reticuloendothelial clearance, and adsorption by plasma proteins.[110] The QD-aptamer (QD-Apt) complex was reportedly nontoxic in vitro and in vivo, penetrated the BBB, selectively accumulated in mouse orthotopic gliomas, presumably by binding to EGFRvIII. This generated a strong fluorescent signal, making the macroscopic tumor margins clearly visible.[110] During in vivo experiments, the authors tested normal brain tissue, an EGFRvIII$^-$ tumor, and an EGFRvIII$^+$ tumor injecting QD with or without aptamer. They reported that QD-Apt positive signal was only observed for mice with EGFRvIII$^+$ tumors after systemic delivery of the agent. This suggests good specificity and potential utility during surgery.[110] Thus there are potential applications of aptamers perioperatively, as well as in diagnosis of biopsies and resected tumors.

Conclusions and Perspective

The field of neuro-oncology presents unique challenges to physicians. Treatment efficacy is severely limited by a lack of precise, BBB-penetrating tools capable of driving chemotherapeutic delivery, while providing a breadth of targeting moieties to address tumor heterogeneity. Unfortunately, physicians often must rely on systemic delivery of highly toxic drugs resulting in complications, even for rare cancer survivors. Current paradigms in tumor treatment continue to fall short of goals for patient outcomes despite recent improvements in the field.

A surge in innovation of SELEX methods has enabled researchers to identify nucleic acid aptamers with the promise of more rapid and specific homing to various tumors in animal models, including brain tumors. Several promising approaches in these models give hints of chemotherapeutic delivery to treat primary brain tumor or brain-metastatic sites with high efficacy and reduced side effects, potentially addressing the need for precision in neuro-oncology. Importantly, aptamers themselves are small and non-immunogenic, presumably allowing some to cross the BBB to target neurological tumors while evading immune response and minimizing damage to off-target tissues. In this regard, there is evidence for BBB-penetrant aptamers in both healthy and tumor-bearing mice.

Of the preclinical aptamers in neuro-oncology, AS1411 is likely the closest to clinical application. AS1411 was the first aptamer tested in cancer patients and is the only aptamer discussed here to have been through a Phase I and II clinical trial. In clinical trials, AS1411 demonstrated significant and durable anti-cancer effects with limited toxicity.[123–125] In the Phase I trial, 17 cancer patients with progressive metastatic disease received intravenous infusion of AS1411 at 1 mg/kg/day.[123,124] If no toxicity was observed at day 28, dosage was increased.[123,124] This study included patients with cancers including renal, colon, pancreatic, lung, prostate, and six others.[123,124] 50% of patients had stable disease two months post-treatment, which lasted for 2-9 months, and 1 patient had a near complete response for more than 6 months.[124]The Phase II trial was restricted to renal cell carcinoma patients who had failed kinase inhibitor treatment.[125] Of 35 patients, 1 had a drastic (84% reduction in tumor burden) and durable (2 years progression-free) response.[125] No other responses were seen and 34% of patients had a mild or moderate adverse event related to AS1411.[125] Other Phase II trials have used AS1411 in acute myeloid leukemia patients with unreported outcomes. The reported trials demonstrated that aptamers were nontoxic and have the potential to induce a dramatic and durable response, but more work is needed to identify predictive biomarkers of response to AS1411.

To date, there have been 31 clinical trials involving aptamers with 8 involving cancer. Ongoing clinical trials are using aptamers to discover biomarkers (NCT05745415, NCT02957370), as a targeting moiety in radiation therapy (NCT03385148), alongside chemotherapy (NCT01034410, NCT00512083), or as an individual targeted therapy (NCT00056199, NCT00881244, NCT00740441).

Translation of aptamers to clinical application has ongoing obstacles. Currently, *in vivo* challenges of serum stability and rapid renal clearance of unmodified aptamers are significant. RNA aptamers additionally face susceptibility to hydrolysis. The current paradigm is that aptamers must be chemically modified to enhance stability, conjugated with high molecular weight partners to prevent renal filtration, or both to survive in circulation. Pegaptanib successfully incorporates PEG conjugation and RNA base modifications, but other clinical trials have been stalled following PEG-specific immune responses. Pegaptanib delivery also utilizes intravitreal injection to circumnavigate the requirement for extended stability in circulation before reaching targets. A neuro-oncology specific challenge is the high rate of CSF turnover, but this can be combated by the development of aptamers with high-specificity interactions on target cells driving rapid delivery. Future efforts may identify elegant solutions enabling long-term stability in circulation to increase efficacy.

In addition to pharmacological challenges, there are trends in the field that appear to be slowing clinical application of aptamers. Most new aptamers for neuro-oncology application are selected on common cell culture lines. Another recurring strategy is to simply apply known aptamers to novel delivery systems. Thus, the full potential of aptamers is being underutilized in neuro-oncology. Aptamers are uniquely posed to target difficult problems in neuro-oncology such as H3K27 mutations, tumor heterogeneity, and passage of the BBB. The efficacy of an aptamer is in part governed by the applied selection approach, which must be designed thoughtfully and with clinical relevance in mind. As AS1411 demonstrated in clinical trials, aptamers are highly selective tools. It is key to identify tumor biomarkers that correspond with response.

Although much remains to be established and optimized, aptamer technology has the potential to bring significant advances in clinical neuro-oncology. To date, the field lacks reported efforts to merge leading neuro-oncology animal models with innovative in vivo SELEX methods. Future approaches are needed in which aptamers are selected for their ability to target orthotopic xenograft tumors in whole animals while delivering chemotherapeutics only to the targeted delivery site. Such approaches may eventually be extended to selection of patient-specific tumor-targeting

aptamers in PDX models for rapid clinical application. Future developments may include collections of brain tumor-specific, or even patient-specific, aptamer libraries that can be conjugated to therapeutics to address tumor heterogeneity, driving individualized medicine forward in neuro-oncology. As aptamers have been identified against histones, including H4,[126] H4-K16-acetylation,[127] histone H3,[128] and others, it is conceivable that aptamers specific to H3K27M may be identified. Eventually SELEX will be applied to identify aptamers that are sensitive and useful for both diagnostics and monitoring. Furthermore, aptamers offer promise for application in surgery and imaging.. Importantly, aptamers have taken steps into the field of adult neuro-oncology but applications in pediatrics are uncharted. While aptamer applications in neuro-oncology to date should be viewed with appropriate skepticism, there is legitimate reason for sustained research investment.

Keywords

drug delivery | diagnostics | imaging | nucleic acid therapy | targeted therapy

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