Burdenko's Journal of Neurosurgery
2025, No. 6, issue 2, pp. 44–49
https://doi.org/10.17116/neiro20258906244

Aptamer-based conjugated molecules in experimental and clinical approaches to treatment of glioblastoma

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ABSTRACT
Glioblastoma is the most common primary malignant tumor of nervous system and one of the most incurable human tumors. The median of overall survival is 16—17 months after comprehensive treatment. Among various difficulties for new therapeutic approaches, we should emphasize the blood-brain barrier complicating delivery of anti-cancer drugs to neoplasm and perifocal area. A promising approach is aptamer molecules as synthetic amino acid analogs capable of interacting and regulating activity of target proteins based on their spatial structural interactions. Aptamers possess high specificity and affinity to various receptors on cell surface and inside its structures if they penetrate cell membrane. In this review, we present various approaches to development of aptamer-based drugs against glioblastoma. Kopylov A.M. et al. synthesized a series of aptamers targeting EGFR and capable for delivery of toxic agents to tumor cells. Important clinical successes include the results of NOX-A12 drug and data on combined drugs based on trastuzumab in patients with metastatic breast cancer. NOX-A12 is an aptamer blocking *CXCL-12* gene and reducing resistance to irradiation of glioblastoma cells. Furthermore, very high control of breast cancer brain metastases in HER-2 positive cases was demonstrated for trastuzumab-emtansine and trastuzumab-deruxtecan. The last finding indicates the perspective for aptamer targeting glioblastoma tumor cells in conjugation with emtansine or deruxtecan.

Keywords: *aptamers, malignant tumors of nervous system, glioblastoma, neuro-oncology.*

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TO CITE THIS ARTICLE:
Kobyakov GL, Pavlova GV, Kopylov AM, Bezbabicheva TS, Usachev DYu. Aptamer-based conjugated molecules in experimental and clinical approaches to treatment of glioblastoma. *Burdenko's Journal of Neurosurgery.* 2025;89(6 vyp 2):44–49. (In Russ.).
https://doi.org/10.17116/neiro20258906244

Introduction

Standard treatment of glioblastoma implies resection followed by chemo- and radiotherapy with temozolomide and adjuvant temozolomide therapy. Second-line treatment includes bevacizumab (either alone or in combination with lomustine/irinotecan/carboplatin), redo resection and radiotherapy [1, 2]. Treatment outcomes remain disappointing: median overall survival ranges from 9.5 months in the US [3] to 15—17 months [1], and 5-year survival does not exceed 5% [3].

In recent decades, numerous preclinical and clinical studies have been conducted to identify new treatments for this tumor, but effective options have yet to be identified. This makes the problem particularly relevant. DNA aptamers conjugated to antitumor drug molecules (doxorubicin, paclitaxel, carboplatin, or monomethyl auristatin E) seem promising [4, 5].

Preclinical studies of aptamers in glioblastoma theranostics

Theranostics refers to substances and methods facilitating diagnosis and treatment of diseases.

Aptamers are synthetic single-stranded oligonucleotides capable of interacting and regulating activity of target proteins based on their spatial structural interactions. This is facilitated by their spatial structure resembling immunoglobulins. Aptamers demonstrate high specificity and affinity for specific receptors on cellular surface and inside cells, as well as may be conjugated to antitumor agents. These properties of aptamers make their discovery and use in theranostics perspective, including patients with glioblastoma [6—8].

One possible cause of ineffective therapeutic approaches to glioblastoma may be blood-brain barrier

(BBB) reducing availability of drugs to target cells. Unlike antibodies, aptamers are able to penetrate BBB. Moreover, aptamers, like antibodies, can act as blockers themselves and serve as vehicles for delivery of other agents. Low immunogenicity, high stability, easy tissue penetration and possible chemical modification make aptamers preferable instead of therapeutic antibodies [9].

Kopylov A.M. et al. developed bi(AID-1-T) aptamer specific for PARP1 enzyme, as well as GR20 and Gol1 specific for epidermal growth factor receptor (EGFR). The last ones are also characterized by good penetration through BBB and high specificity for glioblastoma [4—7]. Antiproliferative properties of bi(AID-1-T) suggest therapeutic potential of this aptamer. It is reported that GR20 may be used as a target molecule for delivery of known therapeutic agents in form of conjugates [6, 7].

Clinical trials of aptamers in the treatment of glioblastoma

When analyzing the PubMed and ClinicalTrials.gov databases, we found description of only one clinical trial devoted to efficacy of L-RNA aptamers in combination with radiotherapy for primary treatment of glioblastoma [10]. This report appeared in June 2024. Study design was a cohort phase I—II study with drug dose escalation. The study included patients who underwent surgery for newly diagnosed glioblastoma without mutations in the *IDH1* and *IDH2* genes, as well as with unmethylated *MGMT* gene (i.e., resistant to temozolomide). Briefly, the idea of therapeutic approach can be explained as follows. The *CXCL-12* gene encodes several isoforms of SDF-1 mRNA, which are capable of stimulating proliferation, survival, and invasion of tumor cells. The authors developed NOX-A12, i.e. aptamer blocking this gene and disrupting its function [10]. Patients were administered NOX-A12 aptamer at doses of 200, 400, and 600 mg weekly. Primary goals of this experiment were evaluation of safety and dose escalation. None patient discontinued treatment due to adverse events. The last patient enrolled in the study continued treatment beyond study duration (26 weeks) until clinical deterioration after 48 weeks. Treatment with radiotherapy and NOX-A12 was safe. No dose-limiting toxicity or treatment-related deaths were observed. Therefore, median dose was not reached, and 600 mg of NOX-A12 per week is the estimated dose selected for the ongoing study. Adverse events included only increase in liver enzymes (gamma-glutamyltransferase in 1 case and alanine aminotransferase and aspartate aminotransferase in 3 cases) that could be related to the drug. Treatment efficacy was assessed according to the modified RANO criteria. Among 10 patients, 8 ones demonstrated response to treatment according to at least one follow-up MRI. Partial response according to the mRANO criteria (>50% reduction in sum of transverse diameters in the largest MR scan) was recorded in 44% of cases. To assess antiangiogenic effects, the authors performed MRI with perfusion and diffusion. These additional parameters demonstrated improvement in 9 out of 10 patients (decrease of relative cerebral blood volume). ADC diffusion coefficient improved in 90% of patients with median best response 29.2%. The median of disease-free survival for all included patients was 174 days (range 58—260), 6-month disease-free survival — 40.0%, median OS — 389 days (range 144—562). Post-hoc exploratory analysis was conducted to identify potential biomarkers correlating with response to NOX-A12 treatment with particular focus on *CXCL12* (target for NOX-A12). Whole genome sequencing was performed for all tumor samples. Tumors with identical characteristics harvested from patients who received standard treatment (chemo- and radiotherapy with temozolomide) comprised the control group. Prevalence of *CXCL12*+cells was the highest in endothelial cells followed by pericytes, microglia, and glioma cells. Since *CXCL12* promotes radiation-induced neovasculogenesis and recurrence in preclinical models [10], the authors wondered whether *CXCL12* positivity might be predictive of response to NOX-A12 treatment. Increased *CXCL12* expression in endothelial cells and glioma cells was found to be significantly correlated with high disease-free survival. There was no correlation between disease-free survival and *CXCL12* in patients not received NOX-A12. Thus, the authors concluded that the drug was effective in glioblastoma cells with high levels of *CXCL12*.

Thus, the authors demonstrated safety of NOX-A12 across all dose escalation cohorts. Moreover, aptamer was successfully used for the first time in patients with glioblastoma without IDH mutations and *MGMT* gene methylation [10].

Aptamers conjugated to chemotherapeutic drugs

We will review some chemotherapeutic agents used in experimental studies for the treatment of glioblastoma. Some ones were translated into clinical trials, while others are still in preclinical stage. Doxorubicin, carboplatin and monomethyl auristatin E are potential agents for conjugation with aptamers for target delivery of chemotherapeutic agents to glioblastoma cells.

Doxorubicin in the treatment of glioblastoma

Available doxorubicin regimens, including its liposomal formulation, have failed to yield a significant clinical effect regarding 6-month disease-free and overall survival [11]. This is likely due to poor penetration of the drug through BBB. Therefore, there are ongoing researches of methods for delivering doxorubicin on glioblastoma cell lines and in animals. Ghaferi M. et al. [12] assessed antitumor efficacy of doxorubicin and carboplatin loaded into polyethylene glycol-conjugated liposomes (PEG-Lip) for glioblastoma in vitro and in vivo. PEG-Lip-DOX/CB complex with a size of 212±10 nm could release doxorubicin in a controlled

manner and significantly enhance cytotoxic effects of other drugs in a rat C6 glioblastoma cell line model. Oraiopoulou M.E. et al. [13] used a patient-derived glioblastoma spheroid model to evaluate efficacy of doxorubicin alone and in combination with temozolomide. Combination was more effective in vitro than monotherapy alone and suppressed tumor growth for up to 3 weeks after treatment. This combination may be a perspective therapeutic option when conjugated with aptamer enhancing target effect.

Platinum-based chemotherapeutic agents

Platinum-based chemotherapeutic agents (cisplatin, carboplatin and modern oxaliplatin) induce tumor cell death by inducing single- or double-strand breaks in DNA [14].

These drugs are polar macromolecules that hinders their penetration through BBB resulting in low efficacy against malignant gliomas and high risk of systemic toxicity (due to the need for high doses). Cisplatin therapy can cause nephrotoxicity and gastrointestinal toxicity, while carboplatin therapy can cause myelosuppression and ototoxicity. Nanoparticle-based and other carrier-based delivery methods are most often studied to overcome BBB. There are technologies for continuous intratumoral drug delivery (convection-enhanced delivery, CED) significantly increasing survival of rats with brain-implanted glioblastoma. Another method is intracarotid administration with temporary opening of BBB. Clinical efficacy of this invasive method is not confirmed. Aptamers may also be perspective in this case.

Monomethyluristatin E

Antibodies as a therapeutic agent (either alone or conjugated with toxic agent) have been the classic approach to targeted therapy for a long time. However, antibodies induce immune response and are unable to penetrate BBB.

Antibody-based drugs are being actively developed regarding penetration of BBB. Mao Y. et al. [15] developed homogeneous ADC 401-4 by conjugating monomethyluristatin E (MMAE) to humanized anti-B7-H3 mAb 401 via divinyl sulfonamide-mediated disulfide coupling. B7-H3 overexpression (immunoregulatory protein B7 homolog 3) is detected in cells of many malignancies including glioblastoma. Considering its minor expression in normal tissues, various researchers suggest this receptor protein as a perspective target for tumor therapy. In vitro studies of 401-4 demonstrated specific killing of various glioblastoma cell lines expressing B7-H3; 401-4 was additionally labeled with fluorescent substance Cy5.5 to produce fluorescent conjugate 401-4-Cy5.5. In vivo imaging studies showed that conjugate accumulated in tumor sites and could be used for targeted delivery.

Wu X. et al. [16] studied delivery of conjugate MMAE and antibody to human epidermal growth factor receptor 2 (HER2) linked via valine-citrulline linker to tumor cells in vitro and in vivo. In vitro and in vivo experiments confirmed selective delivery of RC48-ADC to HER2-positive tumor cells or tumor tissue, as well as MMAE release. This can reduce toxicity for healthy tissues and enhance antitumor efficacy.

The FDA has approved brentuzimab-vedotin, a conjugate containing MMAE, for the treatment of non-Hodgkin's lymphoma. This conjugate is a three-component molecule: 1) chimeric IgG1 antibody cAC10 specific for human CD30; 2) microtubule-disrupting agent, MMAE; 3) protease-cleavable linker covalently attaching MMAE to cAC10 [17].

Potential replacement of antibody in these conjugates with aptamer with specificity for similar targets appears perspective.

Paclitaxel and glioblastoma

Paclitaxel is a chemotherapeutic drug from taxane family. It is effective for lung, breast and colon cancers. Large molecule poorly penetrates BBB. Various models for delivering the drug to the brain are being studied (using nanoparticles or, in recent years, BBB opening techniques with targeted ultrasound). Available results demonstrate potentials of this method, and research is ongoing as phase II clinical trial (NCT04528680) [18]. Furthermore, potential delivery of paclitaxel to glioblastoma cells using aptamers appears worthwhile and potentially more clinically significant.

Discussion: what other antitumor "toxins" show promise for creation of aptamer conjugates?

In this review, we attempted to present modern and potential models for conjugating aptamers with anticancer chemotherapeutics for development of new effective drugs for the treatment of glioblastoma. Aptamers themselves are primarily used as a method for selective delivery to tumor cells. What other substances should be studied as potentially important for drug synthesis? Let's look at modern anticancer drugs and discover two targeted therapies for Her-2/neu mutation: trastuzumab-emtansine and trastuzumab-deruxtecan. Both drugs are used for breast cancer. Two toxic agents (emtansine and deruxtecan) are attached to the main trastuzumab molecule (tropic for Her-2/neu receptors). These molecules themselves are highly toxic and cannot selectively target tumors.

Updated results of the DESTINY-Breast03 study were presented at ASCO 2024 [19]. This trial evaluated the efficacy of trastuzumab and deruxtecan compared to trastuzumab and emtansine in patients with HER2-positive advanced breast cancer who had previously received taxanes and trastuzumab. The median of disease-free survival in the trastuzumab emtansine group was 7.2 months vs. 29.0 months in the trastuzumab deruxtecan group ($p<0.0001$). This reduced the risk of progression by 70%.

One of the areas in aptamer theranostics is target delivery of chemotherapeutic agents to tumors. Therefore, searching

for highly glioblastoma-targeted aptamer-toxin (deruxtecan or emtansine) conjugate seems important. This could theoretically lead to development of a new class of anticancer drugs for the treatment of glioblastoma. The goal of the experiment is to synthesize aptamers highly tropic to glioblastoma and attach a toxin molecule to these aptamers. Currently, a multiple-center team of scientists is working on selecting perspective aptamer molecules and their combination with antitumor drugs (paclitaxel, carboplatin and methyl-auristatin E). Another objective is analysis of their efficacy on native glioblastoma cell lines harvested from real tumors.

Author contribution:
Concept and design of the study — Kobyakov G.L.
Collection and analysis of data — Kobyakov G.L.
Writing — Kobyakov G.L., Bezbabicheva T.S.
Editing — Usachev D.Yu., Pavlova G.V., Kopylov A.M.

This study was supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement No. 075-15-2024-561 dated April 24, 2024).

The authors declare no conflicts of interest.

REFERENCES

1. Ulitin AYu, Zheludkova OG, Ivanov PI, Kobyakov GL, Matsko MV, Naskhletashvili DR, Protsenko SA, Ryzhova MF. Primary Tumors of the Central Nervous System. *Zlokachestvennye opukholi.* 2023;13(3s 2-1):120-147. (In Russ.). https://doi.org/10.18027/2224-5057-2023-13-3s2-1—120-147
2. Omuro A, Brandes AA, Carpentier AF, Idbaih A, Reardon DA, Cloughesy T, Sumrall A, Baehring J, van den Bent M, Bähr O, Lombardi G, Mulholland P, Tabatabai G, Lassen U, Sepulveda JM, Khasraw M, Vauleon E, Muragaki Y, Di Giacomo AM, Butowski N, Roth P, Qian X, Fu AZ, Liu Y, Potter V, Chalamandaris AG, Tatsuoka K, Lim M, Weller M. Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated MGMT promoter: An international randomized phase III trial. *Neuro-Oncology*. 2023; 25(1):123-134. https://doi.org/10.1093/neuonc/noac099
3. Price M, Ballard C, Benedetti J, Neff C, Cioffi G, Waite KA, Kruchko C, Barnholtz-Sloan JS, Ostrom QT. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2017-2021. *Neuro-Oncology*. 2024;26(Suppl 6): vi1-vi85. https://doi.org/10.1093/neuonc/noae145
4. Kopylov AM, Zavyalova EG, Pavlova GV, Pronin IN. Theranostics for glioblastoma with monoclonal antibodies to the epidermal growth factor receptor. Burdenko's Journal of Neurosurgery. 2020;84(3):113-118. (In Russ.). https://doi.org/10.17116/neiro202084031113
5. Kolesnikova V, Revishchin A, Fab L, Alekseeva A, Ryabova A, Pronin I, Usachev DY, Kopylov A, Pavlova G. GQIcombi application to subdue glioma via differentiation therapy. *Frontiers in Oncology*. 2024;14:1322795. https://doi.org/10.3389/fonc.2024.1322795
6. Golovin A, Dzarieva F, Rubetskaya K, Shamadykova D, Usachev D, Pavlova G, Kopylov A. In Silico Born Designed Anti-EGFR Aptamer Gol1 Has Anti-Proliferative Potential for Patient Glioblastoma Cells. *International Journal of Molecular Sciences*. 2025;26(3):1072. https://doi.org/10.3390/ijms26031072
7. Moiseenko VL, Antipova OM, Rybina AA, Mukhametova LI, Eremin SA, Pavlova GV, Kopylov AM. Post-Selection Design of Aptamers: Comparative Study of Affinity of the DNA Aptamers to Recombinant Extracellular Domain of Human Epidermal Growth Factor Receptors. *Biochemistry*. 2024;89(12):2183-2193. https://doi.org/10.1134/S0006297924120071
8. Brylev VA, Ryabukhina EV, Nazarova EV, Samoylenkova NS, Gulyak EL, Sapozhnikova KA, Dzarieva FM, Ustinov AV, Pronin IN, Usachev DY, Kopylov AM, Golovin AV, Pavlova GV, Ryazantsev DY, Korshun VA. Towards Aptamer-Targeted Drug Delivery to Brain Tumors: The Synthesis of Ramified Conjugates of an EGFR-Specific Aptamer with MMAE on a Cathepsin B-Cleavable Linker. *Pharmaceutics*. 2024; 16(11):1434. https://doi.org/10.3390/pharmaceutics16111434
9. Doherty CD, Wilbanks BA, Jain S, S Pearson K, Bakken KK, Burgenske DM, Lett NW, Sarkaria JN, Maher LJ 3rd. In vivo selection of anti-glioblastoma DNA aptamers in an orthotopic patient-derived xenograft model. *NAR Cancer*. 2025;7(1):zcaf005. https://doi.org/10.1093/narcan/zcaf005
10. Giordano FA, Layer JP, Leonardelli S, Friker LL, Turiello R, Corvino D, Zeyen T, Schaub C, Müller W, Sperk E, Schmeel LC, Sahm K, Oster C, Kebir S, Hambsch P, Pietsch T, Bisdas S, Platten M, Glas M, Seidel C, Herrlinger U, Hölzel M. L-RNA aptamer-based *CXCL12* inhibition combined with radiotherapy in newly-diagnosed glioblastoma: dose escalation of the phase I/II GLORIA trial. *Nature Communications*. 2024;15(1):4210. https://doi.org/10.1038/s41467-024-48416-9
11. Ananda S, Nowak AK, Cher L, Dowling A, Brown C, Simes J, Rosenthal MA; Cooperative Trials Group for Neuro-Oncology (COGNO). Phase 2 trial of temozolomide and pegylated liposomal doxorubicin in the treatment of patients with glioblastoma multiforme following concurrent radiotherapy and chemotherapy. *Journal of Clinical Neuroscience*. 2011;18(11):1444-1448. https://doi.org/10.1016/j.jocn.2011.02.026
12. Ghaferi M, Raza A, Koohi M, Zahra W, Akbarzadeh A, Ebrahimi Shahmabadi H, Alavi SE. Impact of PEGylated Liposomal Doxorubicin and Carboplatin Combination on Glioblastoma. *Pharmaceutics*. 2022;14(10): 2183. PMID: 36297618; PMCID: PMC9609487. https://doi.org/10.3390/pharmaceutics14102183
13. Oraiopoulou ME, Tzamali E, Psycharakis SE, Tzedakis G, Makatounakis T, Manolitsi K, Drakos E, Vakis AF, Zacharakis G, Papamatheakis J, Sakkalis V. The Temozolomide-Doxorubicin paradox in Glioblastoma *in vitro-in silico* preclinical drug-screening. *Scientific Reports*. 2024;14(1):3759. https://doi.org/10.1038/s41598-024-53684-y
14. Rottenberg S, Disler C, Perego P. The rediscovery of platinum-based cancer therapy. Nature Reviews. *Cancer*. 2021;21:37-50. https://doi.org/10.1038/s41568-020-00308-y
15. Mao Y, Wei D, Fu F, Wang H, Sun Z, Huang Z, Wang Y, Zhang G, Zhang X, Jiang B, Chen H. Development of a MMAE-based antibody-drug conjugate targeting B7-H3 for glioblastoma. *European Journal of Medicinal Chemistry*. 2023;257:115489. https://doi.org/10.1016/j.ejmech.2023.115489
16. Wu X, Xu L, Li X, Zhou Y, Han X, Zhang W, Wang W, Guo W, Liu W, Xu Q, Gu Y. A HER2-targeting antibody-MMAE conjugate RC48 sensitizes immunotherapy in HER2-positive colon cancer by triggering the cGAS-STING pathway. *Cell Death and Disease*. 2023;14(8):550. https://doi.org/10.1038/s41419-023-06073-8
17. Zhou YS, Wang W, Chen N, Wang LC, Huang JB. Research progress of antiglioma chemotherapeutic drugs (Review). *Oncology Reports*. 2022;47(5):101. https://doi.org/10.3892/or.2022.8312
18. Sonabend AM, Gould A, Amidei C, Ward R, Schmidt KA, Zhang DY, Gomez C, Bebawy JF, Liu BP, Bouchoux G, Desseaux C, Helenowski IB, Lukas RV, Dixit K, Kumthekar P, Arrieta VA, Lesniak MS, Carpentier A, Zhang H, Muzzio M, Canney M, Stupp R. Repeated blood-brain barrier opening with an implantable ultrasound device for delivery of albumin-bound paclitaxel in patients with recurrent glioblastoma: a phase 1 trial. *Lancet Oncology*. 2023;24(5):509-522. https://doi.org/10.1016/S1470-2045(23)00112-2
19. Iwata H, Xu B, Kim SB, Chung WP, Park YH, Kim MH, Tseng LM, Chung CF, Huang CS, Kim JH, Chiu JWY, Yamashita T, Li W, Egorov A, Nishijima S, Nakatani S, Nishiyama Y, Sugihara M, Cortés J, Im SA. Trastuzumab deruxtecan versus trastuzumab emtansine in Asian patients with HER2-positive metastatic breast cancer. *Cancer Science*. 2024; 115(9):3079-3088. https://doi.org/10.1111/cas.16234

Received 19.10.2025
Accepted 26.11.2025