Intratumoral Therapy of Glioblastoma Multiforme Using Genetically Engineered Transferrin for Drug Delivery

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Abstract

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor with median survival of only 12 to 15 months under the current standard of care. To both increase tumor specificity and decrease nonspecific side effects, recent experimental strategies in the treatment of GBM have focused on targeting cell surface receptors, including the transferrin (Tf) receptor, that are overexpressed in many cancers. A major limitation of Tf-based therapeutics is the short association of Tf within the cell to deliver its payload. We previously developed two mutant Tf molecules, K206E/R632A Tf and K206E/K534A Tf, in which iron is locked into each of the two homologous lobes. Relative to wild-type Tf, we showed enhanced delivery of diphtheria toxin (DT) from these mutants to a monolayer culture of HeLa cells. Here, we extend the application of our Tf mutants to the treatment of GBM. In vitro treatment of Tf mutants to a monolayer culture of glioma cells showed enhanced cellular association as well as enhanced delivery of conjugated DT. Treatment of GBM xenografts with mutant Tf-conjugated DT resulted in pronounced regression in vivo, indicating their potential use as drug carriers.

Introduction

Malignant gliomas are the most common primary brain tumor in the United States, accounting for 70% of the 21,410 estimated new cases of brain cancer in 2008 (1–3). Although the prevalence of malignant gliomas represents only 1.5% of the 1,437,180 new cancers diagnosed in 2008, glial tumors are associated with disproportionately high morbidity and mortality (1). For instance, grade 4 astrocytomas, or glioblastoma multiforme (GBM), represent 60% to 70% of all malignant gliomas, with median survival of 12 to 15 months, despite administration of the current standard of care (described below).

The first step in the treatment of malignant gliomas typically involves a gross surgical resection of the tumor (2). Next, to potentially eradicate residual cancer cells not surgically removed, the patient is given 6 weeks of radiation therapy concomitant with the chemotherapeutic temozolomide. This treatment is followed by an additional 6 months of maintenance temozolomide (4). Although this treatment regimen significantly increases the 1- to 3-month median survival of a patient newly diagnosed with GBM, there is much room for improvement. Possible reasons for the inability to extend survival beyond ~1 year include the nonspecificity of radiation therapy and temozolomide in combination with intrinsic cellular resistance to treatment (5, 6) allowing GBM to recur after a median survival of 32 to 36 weeks (7). Consequently, a variety of agents have been investigated to combat GBM, many of which focus on proteins involved in the enhanced proliferative and migratory capacity characteristic of this disease (5, 8–10). These approaches target proteins that are selectively expressed or overexpressed in tumors to minimize nonspecific toxicity to normal cells typical of conventional therapies.

Motivated by early clinical successes of GBM therapeutics, we undertook a study to improve upon the current standard of care using the transferrin receptor (TfR) as our therapeutic target (11–13). Specifically, we were intrigued by Tf-CRM107, consisting of a human serum transferrin (Tf) molecule chemically conjugated to CRM107, a mutant variant of diphtheria toxin (DT). Although this conjugate drug has shown therapeutic promise in the treatment of progressive and recurrent GBM in both a phase I and a multicenter phase II study (5, 6), a conditional power analysis in phase III determined that TF-CRM107 was unlikely to improve overall patient survival compared with the current standard of care (14). A reasonable hypothesis to account for this poor efficacy is the rapid cycling of Tf through the cell, severely limiting the time in which to deliver its toxin payload (15).

The trafficking pathway of Tf, by receptor-mediated endocytosis, serves the crucial physiologic role of transporting iron to cells. Each Tf molecule is capable of binding two ferric (Fe³⁺) ions, one in each of its two homologous iron-binding
lobes, the NH$_2$-terminal lobe (N-lobe) and the COOH-terminal lobe (C-lobe; ref. 16). Iron-loaded Tf (holo-Tf) specifically binds to TfR residing on the surface of all actively dividing cells. It is well known that many tumors significantly overexpress TfR relative to nonneoplastic tissue, ascribed to the extra need for iron in their rapidly proliferating cells (17, 18). However, once Tf delivers its iron to the cell, iron-free Tf (apo-Tf) still bound to TfR is recycled to the cell surface, where it is released from its receptor; Tf cannot re-enter cells until it acquires more iron, a rather inefficient process at this post-delivery site (13). Therefore, each holo-Tf molecule is restricted to a single 4- to 5-minute passage through the cell, whereas 30 such successive cycles of trafficking might be required for a Tf-conjugated toxin to be delivered into the cytosol (19, 20).

To enhance the delivery of drugs conjugated to Tf, we developed a mathematical model describing the Tf/TfR trafficking pathway using the principles of mass action kinetics (15). A sensitivity analysis of the various model parameters identified decreasing the iron release rate in the endosome as a previously unreported design criterion to enhance the cellular association of Tf. By inhibiting the delivery of ferric ions by holo-Tf within the endosomal compartment, we manipulated the cell to recycle holo-Tf, rather than apo-Tf, to the cell surface. Each Tf molecule could now cycle through its trafficking pathway multiple times because holo-Tf maintains a higher affinity for TfR at the cell surface and could re-enter the cell, increasing the probability that a Tf-conjugated toxin will deliver its cytotoxic payload. Recombinant protein technology was used to develop two mutant variants of Tf exhibiting reduced iron release kinetics (21, 22). Both mutants contain two point mutations, one within each iron-binding lobe specifically targeting amino acids involved in the release of iron (21, 22). Mutations of residues in the C-lobe, Arg$_{632}$ and Lys$_{534}$ to alanine, were introduced into gene) as described previously (21). Mutations of residues in the C-lobe, Arg$_{632}$ and Lys$_{534}$ to alanine, were introduced into the plasmid already containing the N-lobe mutation, K206E, by PCR using two sets of complimentary mutagenic oligonucleotide primers. Another plasmid coding for the NH$_2$-terminal hexa His-tagged nonglycosylated recombinant Tf (wild-type Tf) served as a control (25).

Production of recombinant Tf expression vectors

Recombinant Tf mutants were generated via site-directed mutagenesis introduced into the pNUT N-His K206E hTf NG construct using the QuickChange mutagenesis kit (Stratagene) as described previously (21). Mutations of residues in the C-lobe, Arg$_{632}$ and Lys$_{534}$ to alanine, were introduced into the plasmid already containing the N-lobe mutation, K206E, by PCR using two sets of complimentary mutagenic oligonucleotide primers. Another plasmid coding for the NH$_2$-terminal hexa His-tagged nonglycosylated recombinant Tf (wild-type Tf) served as a control (25).

Production and purification of recombinant Tf

The recombinant Tf plasmids were transfected into baby hamster kidney cells for protein expression. The recombinant proteins were secreted into the tissue culture medium (25). Conversion of all recombinant Tf to the fully (and most stable) ferric form involved the addition of Fe-NTA to the tissue culture medium. Purification was accomplished by chromatography on a Ni-NTA column (Qiagen Incorporated) using a BioCad Sprint chromatography system. A Sephacryl S200HR gel filtration column was used as the final step of purification, fully exchanging the proteins into 100 mmol/L of NH$_4$HCO$_3$. The samples were stored at −20°C as concentrated stock solutions (∼2.5 mmol/L; ref. 26).

Radioiodination of recombinant Tf

Holo-Tf samples were specifically radiolabeled at tyrosine residues with Na$_{125}$I purchased from MP Biomedicals using IODO-BEADS (Pierce Biotechnology). Size exclusion chromatography through Sephadex G10 columns was used to eliminate free $^{125}$I from the labeled protein. The specific activity and concentration of each radioiodinated holo-Tf was determined by a phosphotungstic acid assay. The labeled protein was used in Tf/TfR cellular trafficking studies.

Tf/TfR cellular trafficking studies

U251 and U87 glioma cells were seeded in growth medium onto 35 mm dishes (Becton Dickinson and Company) at a density of 3.0 × 10$^4$ cells/cm$^2$ and 4.0 × 10$^4$ cells/cm$^2$, respectively. Different seeding densities were used due to differences in both the size and the proliferative rates of the
cells. Following an incubation period of 14 to 16 hours in a humidified 5% CO₂, 37°C environment, the growth medium was aspirated, and incubation medium (DMEM-HG, 20 mmol/L HEPES, 1 mmol/L sodium pyruvate, 100 units/mL penicillin, 100 μg/mL streptomycin, and 1 g/L bovine serum albumin; pH 7.4) containing 1 nmol/L of radioiodinated Tf was added to each dish. The cells were then placed in a 37°C environment for 5, 15, 30, 60, 90, and 120 minutes, after which the incubation medium was removed. The cells were washed five times with ice-cold WHIPS (20 mmol/L HEPES, 1 g/L polyvinylpyrrolidone, 130 mmol/L NaCl, 5 mmol/L KCl, 0.5 mmol/L MgCl₂, and 1 mmol/L CaCl₂; pH 7.4) to remove nonspecifically bound ligand. To separate cell surface Tf specifically bound to TfR from internalized Tf, 1 mL of ice-cold acid strip (30 mmol/L glycine-HCl, 100 mmol/L NaCl, 1 g/L polyvinylpyrrolidone, and 2 mol/L urea; pH 3.0) was added to each dish and then placed on ice for 12 minutes. Each dish was washed once more with 1 mL acid strip. The cells were solubilized by adding 1 mL of 1 N NaOH to each dish for 30 minutes followed by an additional wash with 1 mL of 1 N NaOH. The two basic washes were collected and the solution was assayed for radioactivity using a Cobra Series Auto-Gamma Counter (Packard Instrument, Co.) to determine the amount of internalized ligand. Experiments were performed thrice with triplicate time points for each Tf ligand.

**Conjugation of recombinant Tf to DT**

DT conjugates of recombinant Tf were prepared using the chemical crosslinkers 2-iodoethanolamine and N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), purchased from Pierce, to create a reducible disulfide bond. DT in PBS was thiolated with an 8-fold molar excess of 2-iodoethanolamine for 60 minutes at room temperature. The thiolated DT was separated from free 2-iodoethanolamine by size exclusion chromatography using Zeba desalting spin columns (Pierce). Recombinant Tf in PBS was reacted with a 3-fold molar excess of SPDP for 30 minutes at room temperature. This SPDP-modified Tf (TF-SPDP) compound was separated from free SPDP by size exclusion chromatography using Zeba desalting spin columns. TF-SPDP and thiolated DT (1:1 molar ratio) were mixed, diluted, and incubated overnight at 4°C. TF-DT conjugates were purified by high-pressure liquid chromatography (AKTA FPLC Chromatographic Systems, GE Healthcare Bio-Sciences) using two HiPrep 16/60 Sephadryl S200HR size exclusion columns in series (GE Healthcare). The identity of each peak was confirmed with SDS-PAGE, and the concentration of the 1:1 TF-DT conjugates was quantified using the Bradford dye binding assay.

**In vitro cytotoxicity studies**

The sulforhodamine B cell proliferation assay was used to quantify cell survival based on the measurement of cellular protein content (27). U251 and U87 glioma cells were seeded onto each well of a 96-well tissue culture plate at cell densities of 1.5 × 10⁴ cells/cm² and 3.0 × 10⁴ cells/cm², respectively. As mentioned above, different seeding densities were used due to differences in the cell sizes and their proliferation rates. After overnight incubation, growth medium was aspirated, and the cells were incubated for 48 hours with 100 μL of fresh growth medium containing concentrations of TF-DT spanning three orders of magnitude (10⁻⁴ to 10⁻¹ nmol/L). Then, 100 μL of cold 10% trichloroacetic acid was added to each well to fix the cells at 4°C for 1 hour. The trichloroacetic acid solution was removed, and the cells were washed four times with distilled water then thoroughly blow-dried. Subsequently, 50 μL of a 1% acetic acid solution containing 0.4% sulforhodamine B was added to each well for 30 minutes at room temperature. The dye solution was removed, and the cells were washed four times with a 1% acetic acid solution to remove unbound dye; following this step, the cells were again blow-dried. The dye was dissociated from the proteins and solubilized with 100 μL of a 10 mmol/L Tris base solution. The absorbance of each well was determined with an Infinite F200 plate reader (Tecan Systems Incorporated) at wavelengths of 560 and 700 nm. The survival of cells relative to a control (i.e., cells incubated in growth medium without TF-DT) was calculated by determining the ratio of the (A₅₆₀−A₇₀₀) values. Experiments were performed thrice with quadruplicate points per each concentration.

**Intratumoral therapy of mice with glioma flank tumors**

The treatment regimen presented here was modeled after the methods developed by Oldfield and coworkers for TF-CRM107 (28). Solid U87:EGFRVIII glioma flank tumors were established in 4- to 6-week-old nu/nu female mice by s.c. injection of 5 × 10⁶ cells. This cell line expresses EGFRVIII, a constitutively active mutant variant of EGFR, which leads to more rapid proliferation than the typical U87 cell line (9), allowing palpable tumors of 0.5 cm in diameter to be established in a relatively short period of 2 weeks. Flank tumors were established on both flanks of mice to maximize mouse usage. Each mouse participated in only one treatment cohort, eliminating the possibility of any systemic effect on the contralateral tumor. Once tumors reached volumes of 200 mm³, they were randomly assigned into one of four treatment groups, each containing four tumors. Each tumor received a 100 μL intratumoral injection of PBS containing no drug (the control) or 0.25 μg of one of the TF-DT samples. The injections occurred every other day starting at day 0 and ending on day 6. Following the 6th day, no further injections were given, and the tumor volume was continuously measured for a period of 4 weeks or until the mice were sacrificed due to excessive tumor growth. Tumor measurements were performed using calipers, and tumor volumes were calculated using the ellipsoid formula 1/2 (LW²), where L equals length (longest diameter) and W equals width (widest part of tumor perpendicular to L). Monitoring was performed to observe a continued regression of the tumor, a plateau in tumor size, or tumor recurrence. All mouse experiments were performed in accordance with the Institutional Animal Use Committee at the University of California, San Francisco.

**Histology and immunohistochemistry**

Additional U87:EGFRVIII flank tumors were generated and used for histology. Subcutaneous tumors were grown to volumes of 200 mm³ and treated intratumorally with two doses...
relative to wild-type Tf, were found to have an increased cellular association in a HeLa cell system (23). To determine whether these mutants showed similar increased cellular association in glioma cells, we measured the concentration of internalized Tf in U251 and U87 glioma cells over time and evaluated the cumulative exposure of cells to Tf-conjugated drug as determined by area under the curve (AUC) analysis. At the end of the 2-hour incubation period, the internalized level of wild-type Tf in U251 cells was $2.29 \times 10^6$ molecules/cell compared with values of $4.77 \times 10^5$ and $7.26 \times 10^5$ molecules/cell for K206E/R632A Tf and K206E/K534A Tf, respectively. AUC values were $2.29 \times 10^6$, $3.48 \times 10^6$, and $5.17 \times 10^6$ (molecules x minutes)/cell for wild-type Tf, K206E/R632A Tf, and K206E/K534A Tf, respectively (Fig. 1A). These values translate into an AUC increase of 54.7% and 147% over the wild-type. This trend in AUC increase was confirmed by two additional experiments yielding average AUC values of $(2.75 \pm 0.41) \times 10^6$ (molecules x minutes)/cell for wild-type Tf compared with values of $(4.15 \pm 0.65) \times 10^6$ and $(6.67 \pm 1.32) \times 10^6$ (molecules x minutes)/cell for K206E/R632A Tf and K206E/K534A Tf ($P = 0.0173$ and $P = 0.0039$), respectively, which translate to AUC increases of $50.5 \pm 23.4\%$ and $142 \pm 48\%$. Student’s $t$ test was used to show that the increase in AUC exhibited by each mutant Tf compared with wild type was statistically significant ($P < 0.05$).

Comparable results were obtained using U87 cells (Fig. 1B). At the end of the 2-hour incubation period, the internalization level of wild-type Tf was $2.87 \times 10^6$ molecules/cell compared with values of $7.04 \times 10^5$ and $11.8 \times 10^5$ molecules/cell for K206E/R632A Tf and K206E/K534A Tf, respectively. AUC values were $2.95 \times 10^6$, $4.97 \times 10^6$, and $8.43 \times 10^6$ (molecules x minutes)/cell for wild-type Tf, K206E/R632A Tf, and K206E/K534A Tf, respectively. This translates into AUC increases of 68.4% and 186%. This trend was confirmed by two additional experiments yielding average AUC values of $(2.50 \pm 0.68) \times 10^6$ (molecules x minutes)/cell for wild-type Tf compared with values of $(4.04 \pm 0.92) \times 10^6$ ($P = 0.0396$) and $(6.81 \pm 1.41) \times 10^6$ (molecules x minutes)/cell ($P = 0.0044$) for K206E/R632A Tf and K206E/K534A Tf, respectively, which translate to AUC increases of $61.5 \pm 36.7\%$ and $172 \pm 56\%$.

**In vitro toxicity of Tf-DT**

Having confirmed the increased association of the recombinant mutant Tf with glioma cells compared with wild-type Tf, we next evaluated the *in vitro* efficacy of drug delivery to glioma cells. We synthesized and administered DT conjugates of the various Tf ligands to U251 and U87 cells over a range of concentrations for 48 hours. Each mutant Tf-DT exhibited significantly enhanced drug delivery efficacy relative to wild-type Tf-DT (Fig. 2). Figure 2A and B correspond to the cytotoxic effects of Tf-DT conjugates against the U251 glioma cell line, indicating that less drug is required for the mutant conjugate to achieve the same level of cytotoxicity in comparison to the wild-type conjugate. IC$_{50}$ values, or the concentrations at which 50% inhibition of cellular growth occurs, were $9.70 \pm 0.50$ pmol/L for wild-type Tf versus $6.94 \pm 0.52$ pmol/L ($P = 0.001$) and $6.47 \pm 0.76$ pmol/L ($P = 0.002$) for K206E/R632A Tf and K206E/K534A Tf, respectively. As

**Results**

**Mutant Tf shows increased association with glioma cells**

The engineered ligands, K206E/R632A Tf and K206E/K534A Tf, with a significantly increased ability to retain iron...
shown in Fig. 2C and D, the same trend was observed with U87 cells, demonstrating IC₅₀ values of 15.0 ± 3.0 pmol/L for wild-type Tf compared with values of 7.85 ± 1.55 pmol/L (P = 0.01) and 6.82 ± 1.21 pmol/L (P = 0.006) for K206E/R632A Tf and K206E/K534A Tf, respectively. Student’s t test was used to show that the decrease in IC₅₀ exhibited by both mutant Tf-DT conjugates compared with the wild-type counterpart was statistically significant (P < 0.05).

In vivo efficacy and safety of Tf-DT delivered systemically to mice

To determine whether this in vitro efficacy of our novel Tf-based conjugates could be observed in vivo, we applied our conjugates to mice with established subcutaneous glioma flank tumors. Consistent with the data obtained in Fig. 2, the mutant Tf-DT conjugates show a significant improvement in reducing tumor bulk in flank xenografts (Fig. 3). The results for the tumors treated with the PBS control show rapid tumor growth due to a lack of drug treatment. Therefore, mice with these tumors were prematurely sacrificed, and consequently, data for these tumors did not extend beyond day 8. The results for the tumors treated with the wild-type TF-DT show a delayed growth profile. However, the data for the average of four tumors treated with this cohort display a very large standard deviation. The reason for this behavior is that, whereas two of the tumors in this treatment group displayed a delayed growth profile similar to the averaged curve, the growth of the other two tumors in the wild-type group was suppressed with evidence of tumor regression near the final days of the observation period. Although the reasons for this inconsistency are unclear at this time, it is apparent that, at the dosage used, the two mutant TF-DT conjugates outperformed the wild-type TF-DT. For each mutant TF-DT, rapid and near-complete tumor regression was observed in all four tumors. Mice were examined and weighed daily. All mice continued to gain weight at an equal rate throughout the treatment regardless of treatment cohort (data not shown), suggesting that tumor apoptosis was a specific effect of TF-DT therapy, and not a result of systemic toxicity.

DT conjugates induce apoptosis in U87:EGFRvIII xenografts

Following the above in vivo treatment and observation protocol, additional tumors were treated with two doses of each therapy. Mice were sacrificed 24 hours posttreatment and tumors harvested for histologic and immunohistochemical analyses. Immunohistochemical analysis for expression levels of cleaved caspase-3 (Fig. 4) verified that toxin conjugate treatment decreased tumor growth through an increase in apoptosis. U87:EGFRvIII flank tumors showed little baseline apoptosis (Fig. 4A), demonstrating low cleaved caspase-3 levels with an immunostain score of 0. The wild-type TF-DT induced a moderate level of apoptosis (Fig. 4B) with an immunostain score between 1 and 2. Tumors treated with either mutant TF-DT showed the greatest level of apoptosis (Fig. 4C and D) with immunostain scores of 3.
Discussion

TfR has been a cancer cell target of interest for many years because of its naturally high expression in cancer cells. Both monoclonal antibodies to TfR and the Tf ligand itself have been used to selectively target therapies to tumors whereas minimizing toxicities toward nonneoplastic cells and tissue (29). In some cases, the use of Tf monoclonal antibody–based conjugates was preferred over Tf-based conjugates because (a) Tf only has a short period of time to deliver its payload and (b) high endogenous levels of Tf might prevent the Tf-drug conjugates from reaching cancer cells (30). However, other studies have found that the alternative trafficking behavior of Tf monoclonal antibody–based conjugates, which seem to be targeted to the lysosome for degradation rather than following the conventional recycling pathway, might adversely influence the effectiveness of protein drugs such as DT and CRM107 (15).

Recently, we identified a novel design strategy to increase the time Tf spends inside a cancer cell. Through the aid of a mathematical model of the Tf/TfR trafficking pathway, we determined that decreasing the iron release kinetics of Tf in the acidic endosome is an effective means of increasing the drug delivery efficacy of Tf. With knowledge gained from recent work regarding the mechanism of iron release within the Tf/TfR cell cycle (16, 21, 22, 25, 26), we used site-directed mutagenesis to generate two mutants of Tf, K206E/R632A Tf and K206E/K534A Tf. These mutations were highly effective in decreasing Tf iron release within the acidic endosomal compartment (31); each mutant possesses a single point mutation in each of the two lobes which effectively blocks or drastically slows iron release from either lobe.

The K206E mutation comprises one half of a motif in the N-lobe known as the dilysine trigger (22, 32). The dilysine trigger is comprised of two lysine residues, K206 and K296, which facilitate the release of iron at the acidic pH of the endosome. By converting lysine at position 206 to glutamate, iron removal from this lobe is significantly inhibited by the formation of a salt bridge between the negatively charged Glu206 on one side of the binding cleft and the positively charged Lys296 on the other side. In a similar fashion, iron release from the C-lobe is influenced by a pH-sensitive triad comprised of three amino acid residues, Lys534, Arg632, and Asp634 (21). Alanine scanning revealed that substitution of an alanine residue to either Lys534 or Arg632 significantly inhibited iron removal from the C-lobe.

We therefore generated K206E/R632A Tf and K206E/K534A Tf and subjected these mutants to in vitro drug delivery efficacy studies in HeLa cells. This study provided proof-of-principle that manipulation of Tf was an effective means of altering its drug delivery efficacy and thereby helped to circumvent the problem of the short association time of wild-type Tf within cells. The study presented here substantiates this finding and extends the applicability of our approach in vitro to a second tumor type, GBM, and further translates these in vitro results into glioma xenograft models in vivo.

The restricted nature of GBM within the brain cavity confines it to a region of generally low TfR expression separated from systemic circulation via the largely impregnable blood-brain barrier (33, 34). Although this permits the possibility of specifically targeting DT to neoplastic tissue whereas minimizing toxicities toward normal brain tissue, the blood-brain barrier along with high endogenous levels of Tf precludes the intravenous application of Tf-DT. To address these issues, we intend to administer the mutant Tf-DT conjugates locally in the brain cavity.

Figure 3. Tumor volume data for female nude mice with established U87:EGFRvIII glioma flank tumors treated with PBS control or DT conjugates of wild-type Tf, K206E/R632A Tf, or K206E/K534A Tf. Points, mean tumor volume evaluated from an average of four tumors within each treatment group; bars, SE.

Figure 4. Immunostaining for cleaved caspase-3 (reddish brown regions) in U87:EGFRvIII xenograft sections from mice treated with PBS control (A), or DT conjugates of wild-type Tf (B), K206E/K534A Tf (C), or K206E/R632A Tf (D). The blue regions represent nuclei counterstained with hematoxylin. Bar, 50 μm.
the treatment of GBM. Although systemic therapies are generally preferred, the current treatment of GBM already involves an invasive surgical resection procedure; therefore, patients with GBM are expected to be more open to local therapies, as suggested by the clinical trials of TF-CRM107. Moreover, direct application allows for on-site tumor delivery of therapeutics in high concentrations and effectively provides the therapeutic agent immediate access to the tumor site. For example, convection-enhanced delivery could be used for the direct application of our novel therapeutic, as in the case of TF-CRM107 (6), in which convective mass transfer facilitates intratumoral drug distribution over a larger tumor volume, enhancing its tumor accessibility (35, 36). In this scenario, the high endogenous Tf concentration is a desirable feature because any mutant Tf-DT conjugates that diffuse away from the tumor site and into the systemic circulation would be outcompeted by endogenous Tf for available receptor sites on normal tissue. The earlier success of TF-CRM107 in phase II clinical trials, in which the conjugate was well tolerated by patients with GBM, indicates that clinical potential exists for the mutant Tf-toxin conjugates described in this study. Although phase III trials of TF-CRM107 were halted based on a conditional power analysis predicting less efficacy than the current standard of care, our mutant Tf-based conjugates show an improved therapeutic efficacy that might warrant support for continued clinical investigation of Tf as a drug carrier against GBM.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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