EGFR/EGFRvIII-targeted immunotoxin therapy for the treatment of glioblastomas via convection-enhanced delivery

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Glioblastoma is the most aggressive malignant brain tumor among all primary brain and central nervous system tumors. The median survival time for glioblastoma patients given the current standard of care treatment (surgery, radiation, and chemotherapy) is less than 15 months. Thus, there is an urgent need to develop more efficient therapeutics to improve the poor survival rates of patients with glioblastoma. To address this need, we have developed a novel tumor-targeted immunotoxin (IT), D2C7-(scdsFv)-PE38KDEL (D2C7-IT), by fusing the single chain variable fragment (scFv) from the D2C7 monoclonal antibody (mAb) with the Pseudomonas Exotoxin (PE38KDEL). D2C7-IT reacts with both the wild-type epidermal growth factor receptor (EGFRwt) and EGFR variant III (EGFRvIII), two onco-proteins frequently amplified or overexpressed in glioblastomas. Surface plasmon resonance and flow cytometry analyses demonstrated a significant binding capacity of D2C7-IT to both EGFRwt and EGFRvIII proteins. In vitro cytotoxicity data showed that D2C7-IT can effectively inhibit protein synthesis and kill a variety of EGFRwt-, EGFRvIII-, and both EGFRwt- and EGFRvIII-expressing glioblastoma xenograft cells and human tumor cell lines. Furthermore, D2C7-IT exhibited a robust anti-tumor efficacy in orthotopic mouse glioma models when administered via intracerebral convection-enhanced delivery (CED). A preclinical toxicity study was therefore conducted to determine the maximum tolerated dose (MTD) and no-observed-adverse-effect-level (NOAEL) of D2C7-IT via intracerebral CED for 72 hours in rats. Based on this successful rat toxicity study, an Investigational New Drug (IND) application (#116855) was approved by the Food and Drug Administration (FDA), and is now in effect for a Phase I/II D2C7-IT clinical trial (D2C7 for Adult Patients with Recurrent Malignant Glioma, https://clinicaltrials.gov/ct2/show/NCT02303678). While it is still too early to draw conclusions from the trial, results thus far are promising.

Keywords: Epidermal growth factor receptor; D2C7-(scdsFv)-PE38KDEL; Immunotoxin; Convection-enhanced delivery; Glioblastoma


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EGFR/EGFRvIII in glioblastomas

Glioblastomas account for 45%-50% of all primary malignant brain tumors and for 82% of malignant glioma cases. They are the most aggressive malignant brain tumors among all primary brain and central nervous system (CNS) tumors diagnosed in the United States [1, 2]. Although glioblastomas are typically confined to the CNS and do not metastasize, they do infiltrate the surrounding brain parenchyma and are highly invasive [3]. The median survival time for glioblastoma patients undergoing the current standard of care treatment of surgery, followed by radiation and chemotherapy, is less than 15 months [1, 4]. Thus, more effective therapeutic approaches are desperately needed to improve the poor survival rates of patients with glioblastoma.

Epidermal growth factor receptor (EGFR), a 170 kDa, transmembrane receptor tyrosine kinase (RTK), has been associated with a large number of human malignancies, including glioblastoma [5]. In 1985, Libermann et al. were the first to describe EGFR gene amplification in malignant brain tumors [6], and subsequent studies have confirmed that approximately 37%-58% of glioblastomas have an amplification of the EGFR gene [7]. Amplification of the EGFR gene is associated with high EGFR mRNA or protein levels, and, in most cases, gene amplification is accompanied by gene rearrangement. Extensive deletions in the EGFR gene’s coding sequence is the most common rearrangement in glioblastomas [7]. Thus, the amplification of the EGFR gene, as well as the deletions/mutations of the gene generating constitutively active mutant receptors, are two important mechanisms for EGFR oncogenicity [8].

Among the genomic variants of EGFR, the class III mutant EGFRvIII, which contains an in-frame deletion of 801 base pairs of the coding sequence resulting in the generation of a novel glycine residue at the fusion junction, is the most frequently detected deletion [9-15]. The mutant EGFRvIII protein is approximately 145 kD and has a tumor-specific primary sequence represented by the novel tumor-specific primary sequence amino acid residues 5 and 274. The EGFRvIII gene/transcript is found in over 50% of glioblastomas exhibiting EGFR gene amplification [16, 17]. EGFRvIII is a constitutively active RTK that is not further activated by EGFR ligands [18]. Like the wild-type epidermal growth factor receptor (EGFRwt), EGFRvIII is widely expressed in glioblastomas [14] and is associated with resistance to radiation and chemotherapy [19].

Since the EGFRvIII mutation is highly prevalent in glioblastomas with EGFRwt amplification, while the EGFR protein is nearly undetectable in the normal brain [5], the development of a therapeutic agent that can target both forms of the receptor—rather than only targeting a single antigen—would be advantageous for glioblastoma treatment.

EGFR/EGFRvIII-targeted immunotoxin therapy

In the past two decades, monoclonal antibody-based (mAb-based) studies have increasingly focused on immunotoxins (ITs) constructed by fusing a genetically engineered single-chain variable fragment (scFv) to bacterial or plant toxins. Since the scFv-IT fusion protein is smaller than the original mAb, it has a superior capacity for tumor penetration, which can lead to enhanced anti-tumor efficacy when it is delivered intrathecally or intratumorally [20-24]. Our study focuses on D2C7, a unique mAb that reacts with both EGFRwt and EGFRvIII proteins [25]. In comparison with the established specific mAbs (anti-EGFRwt mAb, EGFR1, or anti-EGFRvIII mAb, L8A4), D2C7 showed a significantly higher tumor localization in tumors expressing EGFRwt and/or EGFRvIII proteins [25]. Significantly, in an immunohistochemical analysis of 101 adult glioblastoma samples, the D2C7 mAb positively stained virtually all cells in 100% (50/50) of the samples that had amplification of the EGFRwt gene and in 76% (39/51) of the cases without this amplification [25]. The D2C7 mAb is reactive with a 55-amino acid (AA) region present in the extracellular domain of both EGFRwt (583-637 AAs) and EGFRvIII (292-346 AAs) proteins. We then developed a novel IT, D2C7-(scdsFv)-PE38KDEL (D2C7-IT), by fusing the scFv of the D2C7 mAb with domains II and III of Pseudomonas exotoxin A (PE38KDEL) [26]. D2C7-IT’s antigen-binding capacity was assessed by surface plasmon resonance and flow cytometry analyses. The surface plasmon resonance showed that the KD of D2C7-IT on the EGFRwt- and EGFRvIII-coated chips was 1.6 × 10^-9 and 1.3 × 10^-9 mol/L, respectively [26]. The flow cytometry analysis revealed that D2C7-IT can bind to both the EGFRwt-expressing NR6W cells and the EGFRvIII-expressing NR6M cells, but does not bind to the parental NR6 cells [26]. In vitro cytotoxicity data showed that D2C7-IT can effectively inhibit protein synthesis in a variety of EGFRwt- (43MG and A431P), EGFRvIII- (NR6M), or both EGFRwt- and EGFRvIII-expressing (D08-0493MG, D2159MG, and D270MG) glioblastoma xenograft cell lines and human tumor cell lines. D2C7-IT was highly effective in killing the A431 (IC50 = 0.18 ng/mL), 43MG (IC50 = 2.28 ng/mL), D08-0493MG (IC50 = 2.5 ng/mL), D2159MG (IC50 = 0.204 ng/mL), and D270MG (IC50 = 0.265 ng/mL) cells [26]. The favorable in vitro cytotoxicity results indicated a promising therapeutic potential for D2C7-IT in glioblastoma treatment.
Convection-enhanced delivery of the tumor-targeted immunotoxin

Current glioblastoma therapeutics are limited by their inability to efficiently cross the restrictive blood-brain barrier (BBB). The non-targeted systemic or intrathecal delivery results in systemic toxicity to surrounding tissues and produces suboptimal drug delivery to the tumor, especially for large soluble molecules such as antibodies or ITs [27]. Two decades ago, Bobo et al. investigated the convection-enhanced delivery (CED) of macromolecules in order to enhance the distribution of large molecules into the brain while achieving greater magnitude of drug concentration levels [28]. CED continues to be an innovative technique that bypasses the BBB and takes advantage of its restrictive nature to limit drug egress from the brain, therefore allowing targeted localized delivery and dramatically increasing the drug dose that can be provided at the brain tumor site [29, 30]. Since intracerebral CED for IT administration has been well established and has shown promising benefits [31-34], the intracerebral administration of D2C7-IT was performed in our orthotopic xenograft glioma mouse models via CED, in which we used an osmotic pump to deliver the ITs directly into the brain tumor site [26,35].

D2C7-IT therapy via intracerebral CED significantly prolonged survival time of immunocompromised NOD-SCID gamma (NSG) mice bearing tumor xenografts compared to control groups in three tumor models (43MG, NR6M, and D270MG). In the 43MG intracerebral glioma xenograft model overexpressing the EGFRwt protein in the absence of EGFRwt amplification, intracerebral CED of D2C7-IT prolonged survival by 310% (P=0.006) [26]. Similarly, in the EGFRvIII-expressing NR6M orthotopic tumor model, D2C7-IT treatment showed a statistically significant increase in survival by 28% (P=0.002) [26]. Furthermore, in the D270MG intracerebral glioma xenograft model expressing both EGFRwt and EGFRvIII proteins, the delivery of D2C7-IT via intracerebral CED prolonged survival by 166% (P=0.001) [26]. Hence, D2C7-IT demonstrated significant efficacy against brain tumors expressing EGFRwt and/or EGFRvIII. A subsequent preclinical study was performed under Good Laboratory Practice (GLP) regulations to evaluate the systemic toxicity of D2C7-IT administered via intracerebral CED to support an initial US Food and Drug Administration (FDA) Investigational New Drug (IND) application for a Phase I/II clinical trial in patients with glioblastoma.

In the preclinical toxicity study, D2C7-IT was co-infused with a low-molecular-weight tracer, gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA), and 124I-labeled human serum albumin (124I-HSA) to monitor the distribution of D2C7-IT via intracerebral CED with the aim of replicating the formulation for the future D2C7-IT clinical trial [35]. The systemic toxicity of D2C7-IT was examined in a rat intracerebral CED model over a 72-hour period. The following critical issues emerged during the first two trials: (1) the osmolality of the dose formulation, (2) the pump flow rate, (3) the adsorption of the ITs into the pump interior reservoir, and (4) the adverse effects in rats caused by HSA immunogenicity [36]. These issues were addressed by correcting the osmolality of the dose formulation to match the normal rat CNS osmolality, selecting a smaller osmotic pump with a slower flow rate, increasing the carrier protein (albumin) concentration in the formulation, and substituting 2% rat serum albumin (RSA) for 3% HSA [36]. Ultimately, D2C7-IT was formulated in an isotonic control formulation (potassium phosphate buffer in saline with 2% RSA, 2 μCi 124I-HSA, and 1 mM gadolinium). Dose formulations were delivered into the right caudate nucleus of individual rats via subcutaneously implanted osmotic pumps at a nominal 1.01 μL/h flow rate. The maximum tolerated dose (MTD) of D2C7-IT was determined to be between a total dose of 0.10 and 0.35 μg, while the no-observed-adverse-effect-level (NOAEL) of D2C7-IT was a total dose of 0.05 μg in rats. Both the MTD and NOAEL were utilized as references for the D2C7-IT clinical trial design [36]. Based on the preclinical toxicity study, a Phase I study has been initiated to define the MTD of D2C7-IT delivered by intracerebral CED and to determine the optimal dose for a single-arm Phase II trial in recurrent glioblastoma patients (D2C7 for Adult Patients with Recurrent Malignant Glioma, https://clinicaltrials.gov/ct2/show/NCT02303678).

Conclusions

D2C7-IT is a novel scFv immunotoxin that reacts with both EGFRwt and EGFRvIII proteins. D2C7-IT has several promising traits that make it an ideal candidate for treating glioblastoma, including its high-binding affinity to EGFRwt/EGFRvIII proteins expressed on glioblastoma cells, promising in vitro cytotoxicity against glioblastoma cells expressing EGFRwt and/or EGFRvIII proteins, and robust in vivo anti-tumor efficacy in orthotopic mouse glioma models. We believe the dual-binding capacity of D2C7-IT can significantly improve the therapeutic efficacy for glioblastoma patients expressing EGFRwt and/or EGFRvIII proteins. Subsequent to the successful GLP toxicity study in rats, an IND is now in effect for a Phase I/II D2C7-IT clinical trial (NCT02303678, D2C7 for Adult Patients with Recurrent Malignant Glioma, clinicaltrials.gov). Fourteen patients have been treated in a dose-escalation study with no significant toxicity. After a suitable period of follow-up and after the maximum tolerated dose is reached, the results will be published in a separate publication.
Author Contributions

X.B. and V.C. were involved in the writing and revision of the manuscript; I.P. and D.D.B. were involved in the critical review of the manuscript. All authors read and approved the final manuscript.

Conflicting interests

D.D. Bigner owns stock in Istari Oncology and is a consultant to Genetron Health. I. Pastan is inventor on immunotoxin patents, all of which have been assigned to N.I.H.

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