HHS Public Access

Author manuscript

Receptors Clin Investig. Author manuscript; available in PMC 2017 March 08.

Published in final edited form as:

Receptors Clin Investig. 2016 ; 3(4): . doi:10.14800/rci.1430.

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

Xuhui Bao[1], **Ira Pastan**[2], **Darell D. Bigner**[1], and **Vidyalakshmi Chandramohan**[1]

[1]Preston Robert Tisch Brain Tumor Center at Duke and Department of Pathology, Duke University Medical Center, Durham, NC, 27710, USA

[2]Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, USA

Abstract

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.

Correspondence: Vidyalakshmi Chandramohan, vidyalakshmi.chandramohan@duke.edu.

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.

Keywords

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

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 glycine residue created at position 6 through the fusion of 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 K_D 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 (IC_{50} = 0.18 ng/mL), 43MG (IC_{50} = 2.28 ng/mL), D08-0493MG (IC_{50} = 2.5 ng/mL), D2159MG (IC_{50} = 0.204 ng/mL), and D270MG (IC_{50} = 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 ^{124}I-labeled human serum albumin (^{124}I-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 ^{124}I-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.

Acknowledgments

We thank Jenna Lewis for her editorial assistance. The study was funded by the following grants from the National Institutes of Health (NIH) of the United States: R35 CA197264 (to D.D. Bigner), P01 CA154291 (to D.D. Bigner), and P30 CA014236 (to M.B. Kastan). This research was supported in part by grants from the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research (to I. Pastan).

References

1. Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005-2009. Neuro Oncol. 2012; (suppl5):v1–49. [PubMed: 23095881]
2. Louis, DN.Ohgaki, H.Wiestler, OD., Cavenee, WK., editors. World Health Organization Histological Classification of Tumours of the Central Nervous System. Lyon: International Agency for Research on Cancer; 2016. Diffuse astrocytic and oligodendroglial tumours; p. 28
3. Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical review. JAMA. 2013; 310:1842–1850. [PubMed: 24193082]
4. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med. 2008; 359:492–507. [PubMed: 18669428]
5. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol. 1995; 19:183–232. [PubMed: 7612182]
6. Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. Nature. 1985; 313:144–147. [PubMed: 2981413]
7. Wikstrand CJ, Reist CJ, Archer GE, Zalutsky MR, Bigner DD. The class III variant of the epidermal growth factor receptor (EGFRvIII): characterization and utilization as an immunotherapeutic target. J Neurovirol. 1998; 4:148–158. [PubMed: 9584952]
8. Pedersen MW, Meltorn M, Damstrup L, Poulsen HS. The type III epidermal growth factor receptor mutation. Biological significance and potential target for anti-cancer therapy. Ann Oncol. 2001; 12:745–760. [PubMed: 11484948]
9. Schwechheimer K, Huang S, Cavenee WK. EGFR gene amplification--rearrangement in human glioblastomas. Int J Cancer. 1995; 62:145–158. [PubMed: 7622287]
10. Schlegel J, Merdes A, Stumm G, Albert FK, Forsting M, Hynes N, et al. Amplification of the epidermal-growth-factor-receptor gene correlates with different growth behaviour in human glioblastoma. Int J Cancer. 1994; 56:72–77. [PubMed: 8262681]

11. Ekstrand AJ, Sugawa N, James CD, Collins VP. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. Proc Natl Acad Sci U S A. 1992; 89:4309–4313. [PubMed: 1584765]
12. Ekstrand AJ, James CD, Cavenee WK, Seliger B, Pettersson RF, Collins VP. Genes for epidermal growth factor receptor, transforming growth factor alpha, and epidermal growth factor and their expression in human gliomas in vivo. Cancer Res. 1991; 51:2164–2172. [PubMed: 2009534]
13. Yamazaki H, Ohba Y, Tamaoki N, Shibuya M. A deletion mutation within the ligand binding domain is responsible for activation of epidermal growth factor receptor gene in human brain tumors. Jpn J Cancer Res. 1990; 81:773–779. [PubMed: 2168866]
14. Humphrey PA, Wong AJ, Vogelstein B, Zalutsky MR, Fuller GN, Archer GE, et al. Anti-synthetic peptide antibody reacting at the fusion junction of deletion-mutant epidermal growth factor receptors in human glioblastoma. Proc Natl Acad Sci U S A. 1990; 87:4207–4211. [PubMed: 1693434]
15. Bigner SH, Humphrey PA, Wong AJ, Vogelstein B, Mark J, Friedman HS, et al. Characterization of the epidermal growth factor receptor in human glioma cell lines and xenografts. Cancer Res. 1990; 50:8017–8022. [PubMed: 2253244]
16. Moscatello DK, Holgado-Madruga M, Godwin AK, Ramirez G, Gunn G, Zoltick PW, et al. Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. Cancer Res. 1995; 55:5536–5539. [PubMed: 7585629]
17. Wong AJ, Ruppert JM, Bigner SH, Grzeschik CH, Humphrey PA, Bigner DD, et al. Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proc Natl Acad Sci U S A. 1992; 89:2965–2969. [PubMed: 1557402]
18. Batra SK, Castelino-Prabhu S, Wikstrand CJ, Zhu X, Humphrey PA, Friedman HS, et al. Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant EGFRvIII gene. Cell Growth Differ. 1995; 6:1251–1259. [PubMed: 8845302]
19. Choi BD, Archer GE, Michell DA, Heimberger AB, McLendon RE, Bigner DD, et al. EGFRvIII-targeted vaccination therapy of malignant glioma. Brain Pathol. 2009; 19:713–723. [PubMed: 19744042]
20. Chandramohan V, Sampson JH, Pastan I, Bigner DD. Toxin-based targeted therapy for malignant brain tumors. Clin Dev Immunol. 2012; doi: 10.1155/2012/480429
21. Ahmad ZA, Yeap SK, Ali AM, Ho WY, Mohamed Alitheen NB, Hamid M. scFv antibody: principles and clinical application. Clin Dev Immunol. 2012; doi: 10.1155/2012/980250
22. Shapira A, Benhar I. Toxin-based therapeutic approaches. Toxins. 2010; 2:2519–2583. [PubMed: 22069564]
23. Pastan I, Hassan R, Fitzgerald DJ, Kreitman RJ. Immunotoxin therapy of cancer. Nat Rev Cancer. 2006; 6:559–565. [PubMed: 16794638]
24. Piao H, Kuan CT, Chandramohan V, Keir ST, Pegram CN, Bao X, et al. Affinity-matured recombinant immunotoxin targeting gangliosides 3′-isoLM1 and 3′,6′-isoLD1 on malignant gliomas. mAbs. 2013; 5:748–762. [PubMed: 23924792]
25. Zalutsky MR, Boskovitz A, Kuan CT, Pegram CN, Ayriss J, Wikstrand CJ, et al. Radioimmunotargeting of malignant glioma by monoclonal antibody D2C7 reactive against both wild-type and variant III mutant epidermal growth factor receptors. Nucl Med Biol. 2012; 39:23–34. [PubMed: 21958852]
26. Chandramohan V, Bao X, Keir ST, Pegram CN, Szafranski SE, Piao H, et al. Construction of an immunotoxin, D2C7-(scdsFv)-PE38KDEL, targeting EGFRwt and EGFRvIII for brain tumor therapy. Clin Cancer Res. 2013; 19:4717–4727. [PubMed: 23857604]
27. Blanchette M, Fortin D. Blood-brain barrier disruption in the treatment of brain tumors. Methods Mol Biol. 2011; 686:447–463. [PubMed: 21082387]
28. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci U S A. 1994; 91:2076–2080. [PubMed: 8134351]

29. Grossi PM, Ochiai H, Archer GE, McLendon RE, Zalutsky MR, Friedman AH, et al. Efficacy of intracerebral microinfusion of trastuzumab in an athymic rat model of intracerebral metastatic breast cancer. Clin Cancer Res. 2003; 9:5514–5520. [PubMed: 14654531]

30. Heimberger AB, Archer GE, McLendon RE, Hulette C, Friedman AH, Friedman HS, et al. Temozolomide delivered by intracerebral microinfusion is safe and efficacious against malignant gliomas in rats. Clin Cancer Res. 2000; 6:4148–4153. [PubMed: 11051269]

31. Mehta AI, Choi BD, Ajay D, Raghavan R, Brady M, Friedman AH, et al. Convection enhanced delivery of macromolecules for brain tumors. Curr Drug Discov Technol. 2012; 9:305–310. [PubMed: 22339074]

32. Sampson JH, Raghavan R, Brady M, Friedman AH, Bigner D. Convection-enhanced delivery. Neurosurg. 2011; 115:463–466.

33. Sampson JH, Akabani G, Archer GE, Berger MS, Coleman RE, Friedman AH, et al. Intracerebral infusion of an EGFR-targeted toxin in recurrent malignant brain tumors. Neuro Oncol. 2008; 10:320–329. [PubMed: 18403491]

34. Chandramohan V, Bao X, Kato Kaneko M, Kato Y, Keir ST, Szafranski SE, et al. Recombinant anti-podoplanin (NZ-1) immunotoxin for the treatment of malignant brain tumors. Int J Cancer. 2013; 132:2339–2348. [PubMed: 23115013]

35. Bao X, Chandramohan V, Keir ST, Pegram CN, McLendon RE, Kuan C, et al. Antitumor efficacy of D2C7-(scdsFv)-PE38KDEL, a novel immunotoxin targeting EGFRwt and EGFRvIII, by convection-enhanced delivery in orthotopic brain tumor mouse models. J Immunother Cancer. 2013; 1(Suppl 1):P126.

36. Bao X, Chandramohan V, Reynolds RP, Norton JN, Wetsel WC, Rodriguiz RM, et al. Preclinical toxicity evaluation of a novel immunotoxin, D2C7-(scdsFv)-PE38KDEL, administered via intracerebral convection-enhanced delivery in rats. Invest New Drugs. 2016; 34:149–158. [PubMed: 26728879]