

Improvement in the standard treatment for experimental glioma by fusing antibody Fc domain to endostatin

Laboratory investigation

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Object. Brain tumors pose many unique challenges to treatment. The authors hypothesized that Fc-endostatin may be beneficial. It is a newly synthesized recombinant human endostatin conjugated to the Fc domain of IgG with a long half-life (weeks) and unknown toxicity. The authors examined the efficacy of Fc-endostatin using various delivery methods.

Methods. Efficacy was assessed using the intracranial 9L gliosarcoma rat model treated with Fc-endostatin for use in rodents (mFc-endostatin), which was administered either systemically or locally via different delivery methods. Oral temozolamide (TMZ) was administered in combination with mFc-endostatin to determine if there was a beneficial synergistic effect.

Results. Intracranial delivery of mFc-endostatin via a polymer or convection-enhanced delivery 5 days after tumor implantation increased median survival, compared with the control group ($p = 0.0048$ and 0.003 , respectively). Animals treated weekly with subcutaneous mFc-endostatin (started 5 days post-tumor implantation) also had statistically improved survival as compared with controls ($p = 0.0008$). However, there was no statistical difference in survival between the local and systemic delivery groups. Control animals had a median survival of 13 days. Animals treated either with subcutaneous mFc-endostatin weekly or with polymer had a median survival of 18 and 15 days, respectively, and those treated with oral TMZ for 5 days (Days 5–9) had a median survival of 21 days. Survival was further increased with a combination of oral TMZ and mFc-endostatin polymer, with a median survival of 28 days ($p = 0.029$, compared with TMZ alone). Subcutaneous mFc-endostatin administered every week starting 18 days before tumor implantation significantly increased median survival when compared with controls ($p = 0.0007$), with 12.5% of the animals ultimately becoming long-term survivors (that is, survival longer than 120 days). The addition of TMZ to either weekly or daily subcutaneous mFc-endostatin and its administration 18 days before tumor implantation significantly increased survival ($p = 0.017$ and 0.0001 , respectively, compared with TMZ alone). Note that 12.5% of the animals treated with weekly subcutaneous mFc-endostatin and TMZ were long-term survivors.

Conclusions. Systemically or directly (local) delivered mFc-endostatin prolonged the survival of rats implanted with intracranial 9L gliosarcoma. This benefit was further enhanced when mFc-endostatin was combined with the oral chemotherapeutic agent TMZ. (DOI: 10.3171/2011.8.JNS11125)

KEY WORDS • Fc-endostatin • brain tumor • angiogenesis • survival • oncology

GLIOMA multiforme is the most common primary brain tumor in adults.²³ With the use of multimodal treatment in the last decade, the disease prognosis has improved, and median survival has increased from 9 to 21 months.²¹ Hence, seeking new

Abbreviations used in this paper: CED = convection-enhanced delivery; FITC = fluorescein isothiocyanate; hFc- and mFc-endostatin = Fc-endostatin for use in humans and in rodents, respectively; p(CPP:SA) = polyanhydride poly(1,3-bis-[*p*-carboxyphenoxy]propane-*co*-sebacic anhydride) polymer; TMZ = temozolamide.

agents and approaches to improve outcomes in patients with malignant gliomas is crucial.

Endostatin, at 20 kD, is the C-terminal fragment of collagen 18. It is an endogenous inhibitor of angiogenesis and was isolated from murine hemangioendothelioma supernatant by O'Reilly et al. in 1997.²² Many types of tumors in mice have shown growth inhibition by endostatin without toxicity,^{5,16,22} however, its full mechanism of action is still not clear.^{8,22} Given its promising high potency in preclinical studies, human endostatin's limited response in phase I and II clinical trials was un-

expected.^{7,11,17,26} Apparently, this clinical formulation of endostatin had two major flaws. The half-life of the protein in circulation was very short (42.3 minutes), and 50% of the injected endostatin used in the original clinical trials lacked four amino acids at the NH₂ terminus of the molecule.²⁵ Deletion of these four amino acids gave rise to a molecule that did not bind zinc and, consequently, showed decreased antitumor activity.²⁸

To overcome these deficiencies, a molecule of endostatin that was fused to an antibody (IgG) Fc domain was constructed and named "Fc-endostatin" ("mFc-endostatin" for rodent use and "hFc-endostatin" for human use).^{1,6,19} The presence of Fc increases endostatin's molecular weight by 30 kD (Fc-endostatin is a dimer with a molecular weight of approximately 100 kD), raises the half-life to a few weeks, and improves its pharmacokinetic parameters. Furthermore, it shows significant antitumor activity when administered at doses that are 100-fold lower than the endostatin that lacks the Fc.¹⁹

In this study we explored the efficacy of the local and systemic delivery of mFc-endostatin in treating a brain tumor by using an intracranial 9L gliosarcoma model in rats.

Methods

Treatment Agents and Antibodies

Temozolomide (Temozar, Schering Corp.) was purchased from the Johns Hopkins pharmacy. The mFc-endostatin was provided by BioXcell. Methylene chloride was obtained from Fisher Chemical. Polyanhydride poly(1,3-bis-[*p*-carboxyphenoxy]propane-*co*-sebacic anhydride) polymer, that is, p(CPP:SA), was provided by Eisai Co., Ltd. The FITC-labeled polyclonal antibody against mouse Fc fragment was purchased from Sigma-Aldrich Co., LLC. Biotinylated goat anti-rat secondary antibody was purchased from Vector Laboratories, Inc., and Alexa 594-labeled streptavidin was purchased from Molecular Probes, Life Technologies.

Tumor Cells

The 9L rodent gliosarcoma was obtained from the Brain Tumor Research Center (University of California, San Francisco). Every 3 to 4 weeks, 9L tumor pieces measuring 2 mm³ were passaged in the flank of F344 rats. For intracranial implantation, the 9L gliosarcoma tumor was surgically excised from the carrier animal, cut into 1-mm³ pieces, and placed in sterile 0.9% NaCl on ice.

Animals and Anesthetic Protocol

Two hundred one male F344 rats weighing 150–200 grams, purchased from Harlan Bioproducts (Harlan Sprague-Dawley, Inc.), were housed in standard facilities and given free access to food and water. The Johns Hopkins Animal Care and Use Committee approved the study, and all animals were treated in accordance with their policies and guidelines. Moreover, all procedures were conducted within compliance of their regulations.

Rats were anesthetized with an intraperitoneal injection of 0.6 ml of a stock solution containing ketamine HCl

(75 mg/kg, 100 mg/ml), xylazine (7.5 mg/kg, 100 mg/ml), and ethanol (14.25%) in a sterile 0.9% NaCl solution.

Intracranial Glioma Model

For intracranial implantation of the 9L gliosarcoma, rats were anesthetized and their scalps were shaved and prepared with alcohol and Prepodyne solution (West Penetone, Inc.). A midline scalp incision was made, exposing the sagittal and coronal sutures. Using an electric drill with a 2-mm round cutting bur, a small hole was made in the skull, centered 3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture. Care was taken to avoid the sagittal sinus. Under microscopic magnification, a dural and then a cortical opening were made. A small area of cortex and white matter was resected, and once hemostasis was achieved, a single tumor piece was placed in the resection cavity. The skin was then closed with surgical staples.

Polymer Formation

The mFc-endostatin was incorporated into a p(CPP:SA)20:80 at concentrations of 40% (w/w) by methods described previously.^{9,16} The p(CPP:SA) and mFc-endostatin were dissolved in 0.5 ml of methylene chloride and placed in a vacuum desiccator for 2 hours. The resultant polymer mixture was pressed into 14-mg wafers and stored at -20°C.

We used 40% polymer based on the results of Pradilla et al.,²⁴ in which no local or systemic toxicity was observed in their in vivo survival and histological examinations. Five days after 9L tumor implantation, 16 rats were anesthetized and polymer was placed through the same bur hole. Animals were observed daily and survival was recorded.

Convection Enhanced Delivery of mFc-Endostatin

Five days after 9L tumor implantation, 16 rats were anesthetized and a stereotactically guided 32-gauge needle attached to a 50-μl syringe (Gastight, Hamilton Co.) was placed into the tumor bed using the same coordinates as those for tumor implantation (3 mm lateral to the sagittal suture and 5 mm posterior to the coronal suture). Five minutes after needle placement, 25-μl mFc-endostatin (4 mg) was infused at a rate of 1 μl/minute. The needle was kept in place for 5 minutes following the infusion and then withdrawn at a rate of 1 mm/minute. The animals were observed daily and survival was recorded.

Pilot Study to Determine Optimal Administration of mFc-Endostatin

A pilot study was done to evaluate the optimal administration of mFc-endostatin. From Day 0, the day that treatment was started and tumor was implanted, tumor-bearing rats (8 animals per group) were treated with mFc-endostatin subcutaneously every day (0.67 mg/kg daily for 30 days), subcutaneously every week (4.02 mg/kg every 6 days for 30 days), or intravenously every week (4.02 mg/kg every 6 days for 30 days). All of the treatments listed above were administered as monotherapy and in combination with oral gavage of TMZ (50 mg/kg on Days

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5–9 following tumor implantation). Control rats did not receive any treatment. Three additional groups (3 rats per group) were treated with mFc-endostatin as monotherapy via various administration routes 18 days before tumor implantation. The mFc-endostatin was administered subcutaneously every day (0.67 mg/kg), subcutaneously every week (4.02 mg/kg every 6 days), or intravenously every week (4.02 mg/kg every 6 days).

The dose of mFc-endostatin in rats was 0.67 mg/kg/day based on the study results of Lee et al.,¹⁹ who reported an optimal antitumor effect of mFc-endostatin at this dosage.

Efficacy of Treatment Administered Before Tumor Implantation

Based on the encouraging results of the pilot study, we decided to examine the efficacy of treatment received before tumor implantation. Tumor-bearing rats were randomized into groups of 8 animals. Treatment with mFc-endostatin started 18 days prior to tumor implantation. The animals received no treatment (control, 8 rats), oral gavage of TMZ as monotherapy (50 mg/kg on Days 5–9, 8 rats), weekly subcutaneous mFc-endostatin alone (4.02 mg/kg, 8 rats) or in combination with oral gavage of TMZ (50 mg/kg on Days 5–9, 8 rats), or daily subcutaneous mFc-endostatin alone (0.67 mg/kg, 8 rats) or in combination with oral gavage of TMZ (50 mg/kg on Days 5–9, 8 rats). We did not give any rats weekly intravenous treatments because we found that it was not superior to the weekly subcutaneous mFc-endostatin. At Day 120, all surviving rats were deemed long-term survivors and were killed.

Efficacy of Systemically and Locally Delivered Treatment

To determine the efficacy of locally delivered mFc-endostatin with and without TMZ, tumor-bearing rats were randomized into groups of 8 (total of 80 rats) and all treatments were started 5 days after tumor implantation. Animals received no treatment (controls), weekly subcutaneous mFc-endostatin (4.02 mg/kg), 40% mFc-endostatin polymer, 4 mg of mFc-endostatin via CED, and 4 mg of mFc-endostatin via direct injection into the tumor bed for 5 minutes. The mFc-endostatin was given alone or combined with oral gavage of TMZ (50 mg/kg on Days 5–9), and another group was treated with TMZ as monotherapy (50 mg/kg on Days 5–9).

For all survival studies the animals were observed for neurological and systemic toxicity, and survival was recorded. Any animals appearing moribund were killed, and the date of death was recorded.

Immunohistological Analysis

To measure Fc-endostatin in the tumor site, 4 animals from each of 2 treatment groups (weekly or daily subcutaneous mFc-endostatin started on Day 0) were killed 12 days after tumor implantation. Their brains were fixed with 4% paraformaldehyde at 4°C for a minimum of 72 hours followed by incubation in 30% sucrose in phosphate-buffered saline for 48 hours or until the brains sunk, and then they were frozen. Next, 20-mm-thick cryo-

stat sections were cut and processed for histological studies. Brain sections were treated with proteinase K (20 Ag/ml) for 20 minutes before staining. The mFc-endostatin was detected by FITC-labeled polyclonal antibody against mouse Fc fragment. The primary antibody was detected with biotinylated goat anti-rat secondary antibody followed by Alexa 594-labeled streptavidin. The sections were visualized by microscopy (model DM IRE2, Leica).

Statistical Analysis

For all efficacy studies, death was the primary end point. The distribution of the intervals until death was determined using the Kaplan-Meier method. Statistical analysis was completed using Prism 4 software (GraphPad Software, Inc.).

Results

Our initial pilot study showed a significantly increased median survival for animals that had received the once weekly injection of mFc-endostatin 18 days prior to tumor implantation ($p = 0.007$, compared with controls). Therefore, we repeated and expanded our studied animal population from 3 to 8 animals per group.

In Vivo Efficacy of Systemic mFc-Endostatin Alone and in Combination With TMZ Before Tumor Implantation

The systemic administration of mFc-endostatin starting 18 days before tumor implantation significantly increased median survival and produced long-term survivors when compared with the control group (Table 1 and Fig. 1). Control animals had a median survival of 14 days. Animals treated with weekly subcutaneous mFc-endostatin 18 days before tumor implantation had improved survival as compared with controls, with a median survival of 19 days ($p = 0.0007$). Note that 12.5% of the animals in this treatment group survived longer than 120 days without any evidence of tumor after euthanizing and subsequent H & E staining. By contrast, there was no statistical difference in survival between the animals treated with daily subcutaneous mFc-endostatin starting 18 days before tumor implantation and controls ($p = 0.42$).

The median survival of the group that had received TMZ alone was 24 days ($p < 0.0001$, compared with controls). Survival was further increased with the combination of oral TMZ and weekly or daily subcutaneous mFc-endostatin started 18 days before tumor implantation, with a median survival of 28 and 27 days, respectively ($p = 0.0017$ and 0.0001, respectively, compared with oral TMZ alone). Note that 12.5% of the animals treated with weekly subcutaneous mFc-endostatin plus TMZ lived more than 120 days without any evidence of tumor after euthanizing and subsequent H & E staining.

In Vivo Efficacy of Systemic and Locally Delivered mFc-Endostatin Alone and in Combination With TMZ

Control animals had a median survival of 13 days. Intracranial delivery of mFc-endostatin as a polymer or via CED 5 days after tumor implantation increased median survival when compared with the control group ($p = 0.0048$ and 0.003, respectively). Animals treated with

TABLE 1: Administration of mFc-endostatin alone or in combination with TMZ before tumor implantation in a 9L gliosarcoma model in rats*

Treatment Group	Median Survival (no. of days)	p Value
control	14	<0.0001 vs TMZ
daily sc mFc-endostatin	17	0.42 vs control, 0.0003 vs TMZ
weekly sc mFc-endostatin	19	0.0007 vs control, 0.21 vs TMZ
oral TMZ	24	<0.0001 vs control
daily sc mFc-endostatin + oral TMZ	27	<0.0001 vs control, 0.0001 vs TMZ
weekly sc mFc-endostatin + oral TMZ	28	<0.0001 vs control, 0.0017 vs TMZ

* Each treatment group consisted of 8 rats. Abbreviation: sc = subcutaneous.

weekly subcutaneous mFc-endostatin started 5 days after tumor implantation had statistically improved survival as compared with controls ($p = 0.0008$), with a median survival of 18 days. However, there was no statistical difference between the local and systemic delivery groups (Fig. 2 upper).

Animals treated with oral TMZ for 5 days (Days 5–9) had a median survival of 21 days.

Survival was further increased with the combination of oral TMZ and mFc-endostatin polymer, with a median survival of 28 days ($p = 0.029$, compared with TMZ alone).

The median survival of animals receiving weekly subcutaneous mFc-endostatin plus TMZ or mFc-endostatin via CED combined with oral TMZ had a median survival of 25 and 23 days, respectively, which was not statistically significantly different from survival in the TMZ-alone group ($p = 0.055$ and 0.059, respectively).

Direct injection of mFc-endostatin alone or combined with oral TMZ did not increase median survival as compared with controls ($p = 0.49$, compared with controls; and $p = 0.13$, compared with TMZ alone; Table 2 and Fig. 2 lower).

Throughout all of the survival studies described in this paper, no toxicity was observed with any of the various routes of mFc-endostatin delivery.

Accumulation of mFc-Endostatin in Brain Tumor

To verify the existence of exogenous mFc-endostatin, histological sections of the mouse brains were incubated with FITC-labeled anti-mouse Fc fragment antibody (green). The FITC-labeled antibody reacted with the histological sections of tumors from rats treated with mFc-endostatin but not with the sections from the control group (Fig. 3). The injected exogenous mFc-endostatin was detected within the intracranial tumor.

Discussion

We report on the effect of mFc-endostatin in a brain tumor model. We showed that the systemic administration of mFc-endostatin can improve survival in a 9L glioma model in rats. Among all of the systemic administration routes, the weekly injection of subcutaneous mFc-end-

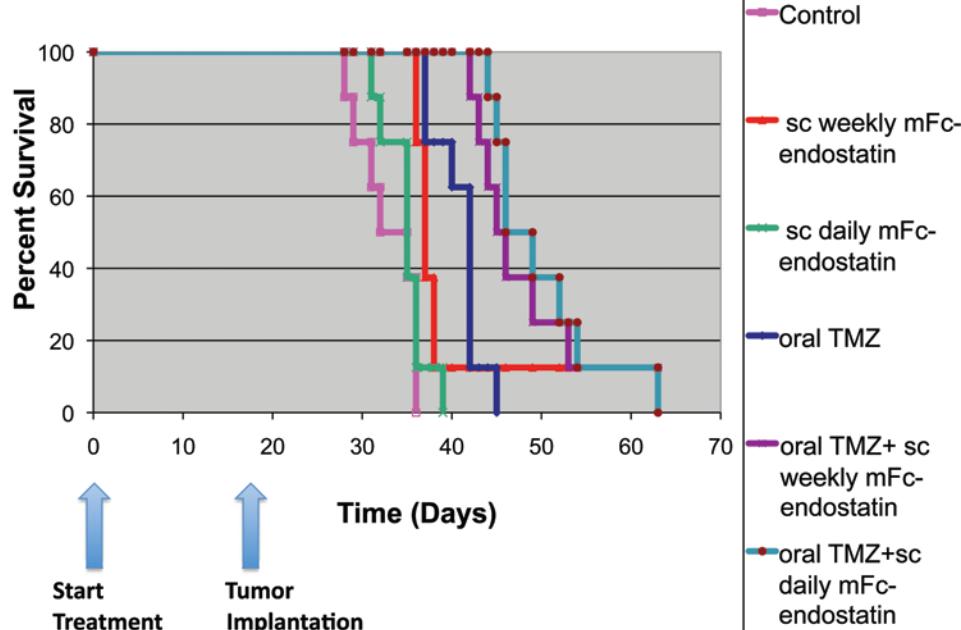


Fig. 1. Graph showing survival in rats bearing intracranial 9L gliosarcoma that were treated with mFc-endostatin 18 days before tumor implantation. Subcutaneous drug was administered either weekly or daily with and without TMZ. Those treated weekly with subcutaneous mFc-endostatin survived significantly longer, compared with controls ($p = 0.0007$). When combined with TMZ, subcutaneous mFc-endostatin administered both weekly and daily conferred survival benefits, as compared with TMZ alone ($p = 0.0017$ and 0.0001, respectively).

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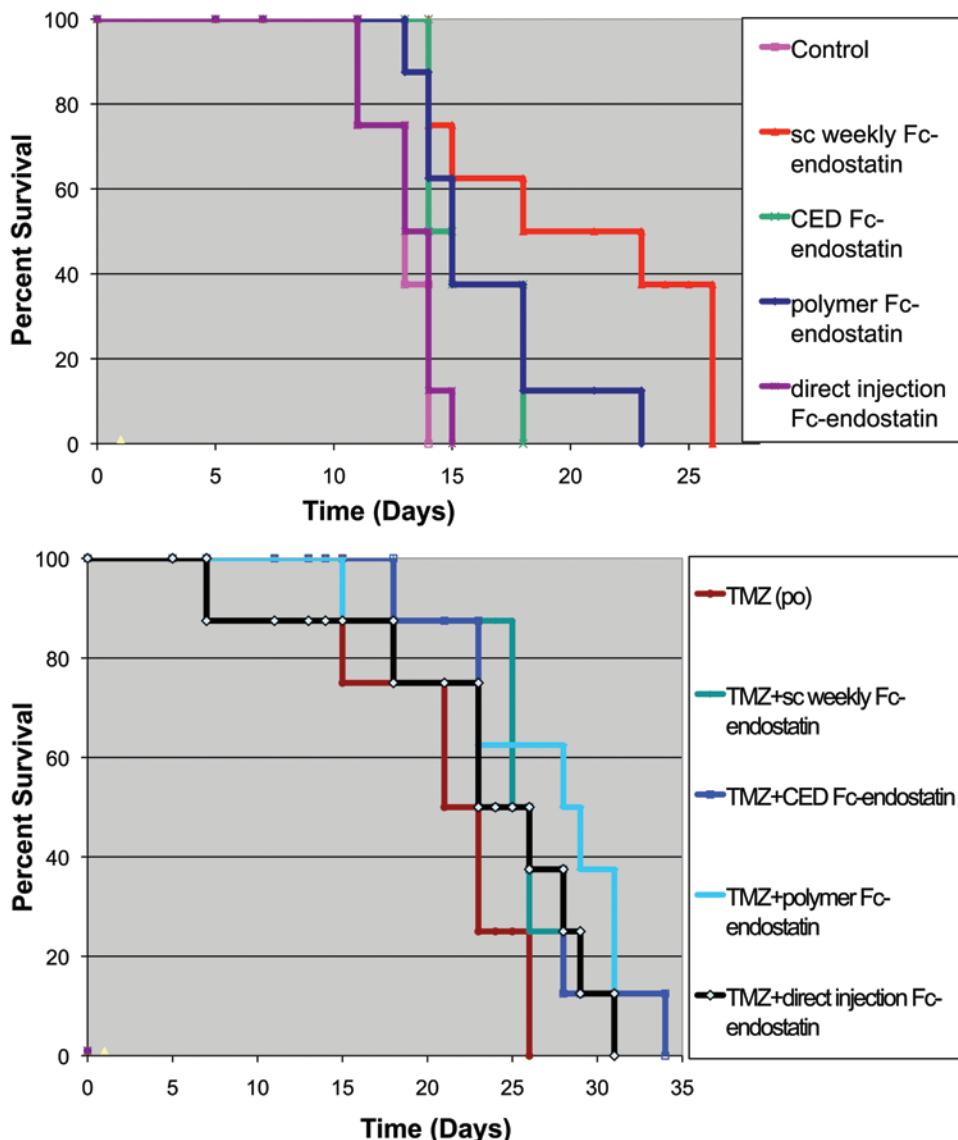


Fig. 2. Graphs showing survival of F344 rats after treatment with locally delivered mFc-endostatin. The treatment was started 5 days after intracranial 9L gliosarcoma tumor implantation. Animals treated with weekly subcutaneous endostatin had a median survival of 18 days ($p = 0.0008$), those treated with polymer or via CED had median survivals of 14 and 15 days, respectively ($p = 0.003$ and 0.0048 , respectively, **upper**). Animals that received 5 days of oral gavage of TMZ had a median survival of 21 days. When mFc-endostatin polymer was given with oral TMZ, the median survival was increased to 28 days ($p = 0.029$, compared with TMZ alone, **lower**). po = oral delivery.

ostatin was associated with increased survival compared with the daily subcutaneous injection. This finding is in line with the U-shaped efficacy curve of endostatin as reported in several studies in the past^{3,19,27} and specifically mirrors the findings described by Lee et al.¹⁹ that is, that Fc-endostatin has biphasic antitumor activity and that melanoma and pancreatic tumor models have a U-shaped curve for efficacy. In both tumor models the maximum antitumor activity was achieved at a dose of 0.67 mg/kg/day. Thus, in our study we based the dose on this value.

The action mechanism of Fc-endostatin is still unknown; however, exogenous Fc-endostatin has been detected in melanoma and pancreatic cancer cell lines, inoculated into the dorsal skin of mice.¹⁹ In our study, the

histological tumor sections incubated with FITC-labeled anti-mouse Fc fragment antibody clearly showed (for the first time) exogenous mFc-endostatin at the tumor site inside the brain (Fig. 3), as compared with the brains of controls, which were not treated with mFc-endostatin.

The blood-brain barrier is a physical and physiological barrier that restricts the entry of many exogenous compounds into the brain, limiting the access of systemically delivered drugs to gliomas. One of the techniques for overcoming this barrier is through local drug delivery. There are various options for the local delivery of drugs into the brain. Drug impregnated into a controlled release polymer matrix was successfully utilized with the local delivery of the chemotherapeutic polymer 1,3-bis(2-

TABLE 2: Locally and systemically delivered mFc-endostatin alone and in combination with TMZ in a 9L gliosarcoma model in rats*

Treatment Group	Median Survival (no. of days)	p Value
control, no Rx	13	<0.0001 vs TMZ
weekly sc mFc-endostatin	18	0.0008 vs control, 0.978 vs TMZ
mFc-endostatin via CED	14	0.003 vs control, 0.0032 vs TMZ
mFc-endostatin via polymer	15	0.0048 vs control, 0.0206 vs TMZ
mFc-endostatin via direct injection	13	0.49 vs control, 0.0002 vs TMZ
oral TMZ	21	<0.0001 vs control
weekly sc mFc-endostatin + oral TMZ	25	<0.0001 vs control, 0.055 vs TMZ
mFc-endostatin via CED + oral TMZ	23	<0.0001 vs control, 0.059 vs TMZ
mFc-endostatin via polymer + oral TMZ	28	<0.0001 vs control, 0.029 vs TMZ
mFc-endostatin via direct injection + oral TMZ	23	0.0021 vs control, 0.13 vs TMZ

* Each treatment group consisted of 8 rats.

chloroethyl)-1-nitrosourea (Gliadel, MGI Pharma Inc., now Eisai Co., Ltd.)^{21,29} and TMZ.² Convection-enhanced delivery is another promising technique that uses bulk flow to distribute infusate throughout the interstitial spaces of brain parenchyma.⁴

In the current study, survival in animals treated with the local delivery of mFc-endostatin via biodegradable polymer or CED was superior to that in the untreated group (controls), although it was not better compared with

survival in animals treated with weekly subcutaneous injections of mFc-endostatin. In fact, median survival in the latter group was 3 days longer compared with that in the polymer group. This result may be explained again by the U-shape efficacy curve of mFc-endostatin, which shows better results in smaller drug concentrations. Furthermore, the long half-life of Fc-endostatin, which is up to several weeks,¹⁹ may prolong exposure of the target (that is, the tumor site) to the drug.

Interestingly, no significant survival differences were found in our study between the polymer and CED groups ($p = 0.11$). Similar findings were recently reported by the PRECISE study group.¹⁸ In a phase III randomized trial on the survival of patients with recurrent glioblastoma multiforme treated with CED of cintredekin besudotox (IL-13 PE38QQR) compared with Gliadel, there were no survival differences and outcomes in both treatment groups were similarly superior to those in control patients.

Rats treated with mFc-endostatin did not exhibit any prominent manifestations of local toxicity. This finding is consistent with several other reports.^{17,19,24} In a phase II clinical study of doses of $90 \text{ mg/m}^2/\text{day}$ (2.5 mg/kg/day),¹⁷ recombinant human endostatin showed minimal or no toxicity. Furthermore, when incorporated into controlled-release polymers, the fragment maintained its biological activity and again exhibited no sign of local or systemic toxicity.¹⁹

Gliomas are highly infiltrative tumors. The tumor cells spread along the white matter tracts and can eventually lead to local recurrence.¹⁰ Malignant glioma rarely results in metastases; however, it does progress or recur, most often within a radius of 2 cm of the original site.⁹ Even if these invasive tumor cells do not induce a tumor recurrence in every patient, they are a potential source of tumor spread in the long term.

On the basis of this knowledge, we examined the effect of mFc-endostatin on the development of tumors when treatment was started 2.5 weeks before the tumor

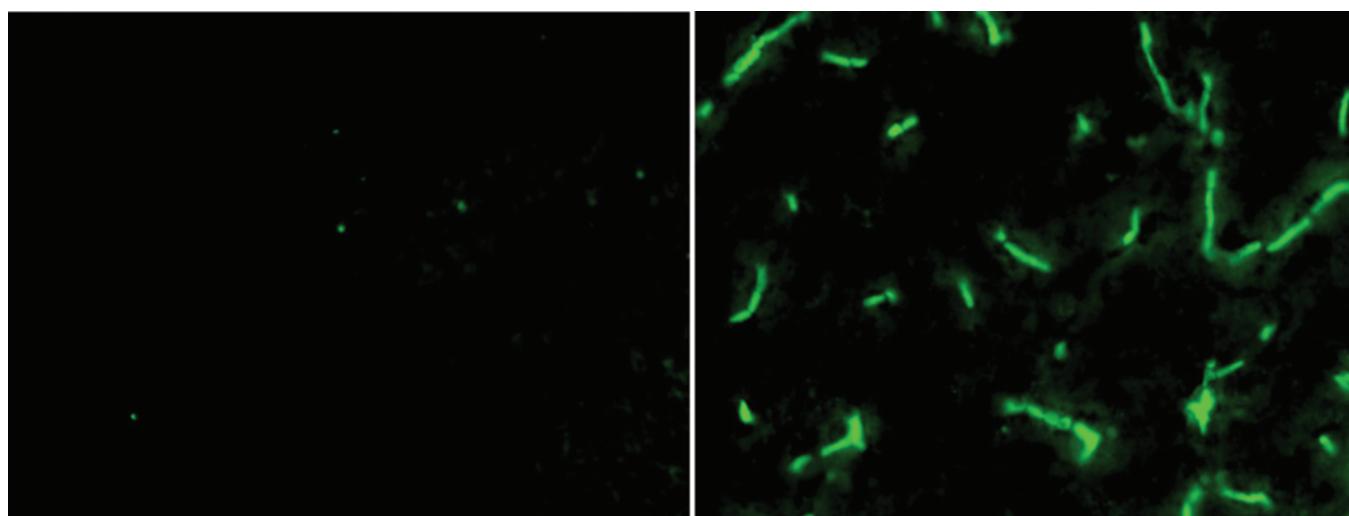


FIG. 3. Photomicrographs demonstrating immunohistochemical analysis of tissue from a rodent brain treated with subcutaneous mFc-endostatin (4.02 mg/kg) weekly (**left**) and an untreated control brain (**right**). The FITC-labeled polyclonal antibody against the mFc fragment reacted with the histological sections of tumors from rats treated with mFc-endostatin but not with the histological sections of the control group.

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was implanted. The question was whether the application of mFc-endostatin prevents tumor recurrence. We showed that the therapeutic effect was more beneficial when pretreatment was applied. The weekly injection of mFc-endostatin was associated with the longest survival, compared with controls ($p = 0.0007$) and even compared with daily mFc-endostatin ($p = 0.038$).

Current treatment modalities have provided only a modest survival benefit among patients with a variety of cancers. Many preclinical and clinical studies examining the value of combination therapies have shown an additive effect, especially when cytostatic drugs were combined with cytotoxic chemotherapies for maximizing therapeutic activity.^{12,15,20} For example, many antiangiogenic drugs can be administered over extended time periods safely and with manageable toxicity compared with standard maximum-tolerated-dose chemotherapies, which are often accompanied by severe adverse effects. Combining antiangiogenic agents with chemotherapy in high-grade gliomas may allow improved drug delivery to the tumor by decreasing tumor vessel permeability and interstitial fluid pressure in a process of vessel “normalization.”²⁰ The resulting normalized vasculature has more efficient perfusion,¹⁴ aiding the therapeutic benefit.^{13,14}

Through the survival studies presented here, we showed a synergistic effect between mFc-endostatin and the chemotherapeutic agent TMZ, which is part of the standard of care for patients with high-grade gliomas. We showed that oral TMZ plus the subcutaneous delivery of mFc-endostatin weekly ($p = 0.055$) or the polymeric delivery of mFc-endostatin ($p = 0.029$) significantly increased survival in rats with 9L gliosarcoma.

Conclusions

In this study we established that mFc-endostatin is a safe and effective drug against intracranial 9L gliosarcoma in rodents. The survival of 9L tumor-bearing animals was improved after treatment with mFc-endostatin via either systemic or local delivery routes, as compared with controls. The mFc-endostatin in combination with TMZ did not cause additional toxicity and significantly prolonged survival, compared with TMZ alone.

Disclosure

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Author contributions to the study and manuscript preparation include the following. Conception and design: Grossman, Javaherian, Brem. Acquisition of data: Tyler, Grossman, Hwang, Zadnik, Lal. Analysis and interpretation of data: Tyler, Grossman, Lal. Drafting the article: Grossman. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Tyler. Statistical analysis: Tyler, Grossman. Study supervision: Tyler, Grossman, Brem.

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