

## Local delivery of angiogenesis-inhibitor minocycline combined with radiotherapy and oral temozolomide chemotherapy in 9L glioma

### Laboratory investigation

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**Object.** Over the past several years, there has been increasing interest in combining angiogenesis inhibitors with radiotherapy and temozolomide chemotherapy in the treatment of glioblastoma. Although the US FDA approved bevacizumab for the treatment of glioblastoma in 2009, the European Medicines Agency rejected its use due to its questionable impact on patient survival. One factor contributing to the failure of angiogenesis inhibitors to increase overall patient survival may be their inability to cross the blood-brain barrier. Here the authors examined in a 9L glioma model whether intracranial polymer-based delivery of the angiogenesis inhibitor minocycline potentiates the effects of both radiotherapy and temozolomide chemotherapy in increasing median survival. The authors also investigated whether the relative timing of minocycline polymer implantation with respect to radiotherapy affects the efficacy of radiotherapy.

**Methods.** Minocycline was incorporated into the biodegradable polymer poly(1,3-bis-[p-carboxyphenoxy propane]-co-[sebacic anhydride]) (CPP:SA) at a ratio of 50:50 by weight. Female Fischer 344 rats were implanted with 9L glioma on Day 0. The minocycline polymer was then implanted on either Day 3 or Day 5 posttumor implantation. Cohorts of rats were exposed to 20 Gy focal radiation on Day 5 or were administered oral temozolomide (50 mg/kg daily) on Days 5–9.

**Results.** Both minocycline polymer implantations on Days 3 and 5 increased survival from 14 days to 19 days ( $p < 0.001$  vs control). Treatment with a combination of both minocycline polymer and radiotherapy on Day 5 resulted in a 139% increase in median survival compared with treatment with radiotherapy alone ( $p < 0.005$ ). There was not a statistically significant difference in median survival between the group that received minocycline implanted on the same day as radiotherapy and the group that received minocycline polymer 2 days prior to radiotherapy. Lastly, treatment with a combination of minocycline polymer with oral temozolomide resulted in a 38% extension of median survival compared with treatment of oral temozolomide alone ( $p < 0.001$ ).

**Conclusions.** These results show that minocycline delivered locally potentiates the effects of both radiotherapy and oral temozolomide in increasing median survival in a rodent glioma model. More generally, these results suggest that traditional therapy in combination with local, as opposed to systemic, delivery of angiogenesis inhibitors may be able to increase median survival for patients with glioblastoma. (<http://thejns.org/doi/abs/10.3171/2013.11.JNS13556>)

**KEY WORDS** • glioblastoma • angiogenesis • minocycline •  
radiotherapy • temozolomide • 9L glioma • oncology

**G**LIOMA is the most common primary brain tumor in adults, with approximately 10,000 patients in the US receiving this diagnosis annually.<sup>8</sup> Despite treatment advances made over the past several decades, glioblastoma remains one of the most challenging cancers to treat, with an average life expectancy of

up to 21 months from diagnosis.<sup>1,12,21</sup> Current treatment options typically include a combination of radiotherapy, temozolomide chemotherapy, and debulking surgery.<sup>31</sup>

Glioblastomas actively recruit and stimulate new blood vessels for tumor growth.<sup>16</sup> In the microscopic angiogenesis grading system proposed in 1972 by Brem et al. glioblastomas were consistently rated among the most angiogenic due to the number of blood vessels per high power field, degree of endothelial cell hyperplasia, and endothelial cytology.<sup>4</sup> Building on this observation, one

Abbreviations used in this paper: BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea; CPP:SA = poly(1,3-bis-[p-carboxyphenoxy propane]-co-[sebacic anhydride]).

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method to slow down the growth of glioblastomas may be to combine angiogenesis inhibitors with currently used modalities in treating glioblastoma.

This approach has proven successful in the treatment of tumors outside the brain, both clinically and in animal models.<sup>23</sup> For example, in colon cancer, adding the angiogenesis inhibitor bevacizumab to chemotherapy agents irinotecan, fluorouracil, and leucovorin increased median survival in patients from 15.6 months to 20.3 months.<sup>13</sup> Similarly in lung cancer, adding bevacizumab to paclitaxel-carboplatin increased median survival in patients from 10.3 months to 12.3 months.<sup>28</sup> Another example involves the significant growth inhibition of human glioblastoma implanted subcutaneously in the flanks of mice by combining angiogenesis inhibitor TNP-470 (an inactivator of methionine aminopeptidase-2 [MetAP2], which results in endothelial cell cycle arrest) with radiation.<sup>19</sup>

Despite successes both clinically and in animal models of systemically administered angiogenesis inhibitors combined with other modalities in the treatment of tumors outside the brain, it is unclear at present whether the same approach will work to increase survival in patients with tumors inside the brain.

The combination of systemically administered angiogenesis inhibitors combined with radiation has yielded inconclusive results for intracranial tumors both in clinical trials<sup>11</sup> and in animal models.<sup>36</sup> For example, in the experiment mentioned earlier involving TNP-470, the treatment had no effect in increasing survival of mice with intracranial tumors.<sup>19</sup> Similarly, the combination of systemically administered angiogenesis inhibitors combined with chemotherapy has also yielded inconclusive results for intracranial tumors. In one study, the intraperitoneal delivery of the angiogenesis inhibitor minocycline did not have an effect on potentiating the effects of intra-peritoneal DNA-alkylating agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU).<sup>41</sup> In a clinical trial, combining systemically administered bevacizumab with irinotecan resulted in improvement of progression-free survival at 6 months.<sup>37</sup> However, it is questionable whether overall survival improved and whether bevacizumab influenced the radiological results that were used in evaluating progression-free survival rather than changing the structure of the actual tumor. There have also been several Phase II trials examining the addition of systemic bevacizumab administration to temozolomide and radiation therapy.<sup>22,38</sup> However, these studies lacked a control arm, and a placebo-controlled Phase III trial is necessary before definitive conclusions can be drawn.

One challenge to the success of systemically administered angiogenesis inhibitors in the treatment of intracranial tumors may be the blood-brain barrier. One method to overcome this challenge involves the use of polymers implanted intracranially for local drug delivery; this method has been demonstrated to be clinically effective for the delivery of carmustine in the form of the Gliadel wafer.<sup>3,21</sup> The concept of using a polymer to deliver chemotherapy drugs intracranially has also been shown in animal models to be effective for the delivery of many other compounds,<sup>25</sup> including temozolomide,<sup>5</sup> doxorubicin,<sup>18</sup> O-6 benzylguanine,<sup>27</sup> taxol,<sup>40</sup> and campto-

thecin.<sup>42</sup> Here we evaluate in an animal model whether local delivery of an angiogenesis inhibitor combined with radiation or oral temozolomide can effectively increase median survival.

The angiogenesis inhibitor we test is minocycline. Minocycline is one of the most frequently prescribed drugs in the tetracycline class and is used clinically to treat a variety of bacterial infections. In addition to its antibacterial properties, it has also been shown to be an inhibitor of angiogenesis in a rabbit cornea model<sup>32</sup> and to prolong survival in a rat glioma model when delivered locally.<sup>9,41</sup>

We first evaluate whether local delivery of minocycline using biodegradable polymers potentiates the effect of radiation therapy in prolonging the median survival of rats implanted with 9L glioma. It has been suggested that the combination of radiation and angiogenesis inhibitor therapies is only synergistic during a “normalization window” when tissue hypoxia is greatly diminished.<sup>2,20,44</sup> Despite these results, the effect of the relative timing of angiogenesis inhibitor and radiotherapy on median survival, as opposed to morphological, radiological, or histological response, is unclear at present. Based on these studies, in our experiments we time implantation of the minocycline polymer either simultaneous with radiotherapy or 2 days prior to radiotherapy and evaluate median survival.

In addition to examining whether local delivery of minocycline potentiates the effects of radiotherapy, we also look at whether local delivery of minocycline potentiates the effects of the oral DNA alkylating agent temozolomide on median survival. As we discussed previously, it is unclear at present whether systemic delivery of angiogenesis inhibitors potentiates the effects of temozolomide. Previously, it was established that minocycline polymer potentiates the effects of the DNA alkylating agent BCNU, by increasing median survival 93% compared with BCNU alone.<sup>42</sup> In this study, we evaluate whether local delivery of an angiogenesis inhibitor potentiates the effects of oral temozolomide in increasing median survival.

## Methods

### Study Materials

Minocycline (Sigma-Aldrich) was incorporated into a polyanhydride poly(1,3-bis-[p-carboxyphenoxy propene]-co-[sebacic anhydride]) (CPP-SA) polymer (generously supplied by Eisai Co., Ltd.) at concentrations of 50% (wt/wt) by methods described previously.<sup>33</sup> Minocycline polymers were stored at -20°C until use.

### Tumor Cells

The 9L gliosarcoma cells were originally obtained from the Brain Tumor Research Center (University of California at San Francisco). For tumor piece implantation, 9L tumor pieces measuring 2 mm<sup>3</sup> were passaged in the flank of Fischer 344 rats every 2–3 weeks. At the time of intracranial implantation, the 9L gliosarcoma tumor was surgically excised from the carrier animal, cut into 2-mm<sup>3</sup> pieces, and placed in sterile 0.9% saline on ice.

### *Study Animals*

Female Fischer 344 rats, weighing 125–175 g each, were purchased from Harlan Bioproducts and were used for all studies. Animals were housed in standard facilities and given free access to food and water. All animals were treated in accordance with the policies and guidelines of the Johns Hopkins University Animal Care and Use Committee.

### *Anesthesia*

Rats were anesthetized with a 0.6-ml intraperitoneal injection 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 Gliosarcoma Model*

For intracranial implantation of the 9L gliosarcoma, 110 F344 female rats were anesthetized. The head was shaved and prepared with alcohol and Prepodyne solution (DeLaval, 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 superior sagittal sinus. Forceps were used to lift off the remaining bone. Under microscopic magnification, a dural opening and then a cortical opening were made. A small area of cortex and white matter was resected. Once hemostasis was achieved, a single 2-mm<sup>3</sup> tumor piece was placed in the resection cavity. The skin was then closed with surgical staples.

### *Efficacy Study*

For the efficacy study, the 110 rats received tumor as described above and then were divided into the following groups (Table 1): control (no treatment) (n = 16); oral temozolomide (50 mg/kg) given on Days 5–9 (n = 16); 20 Gy radiation therapy alone on Day 5 (n = 15); 50% minocycline CPP:SA polymer implanted on Day 5 (n = 16); 50% minocycline CPP:SA polymer implanted on Day 5 plus oral temozolomide (50 mg/kg) given on Days 5–9 (n = 16); 50% minocycline CPP:SA polymer implanted on Day 5 plus 20 Gy radiation therapy on Day 5 (n = 15); 50% minocycline CPP:SA polymer implanted

on Day 3 (n = 8); and 50% minocycline CPP:SA polymer implanted on Day 3 plus 20 Gy radiation therapy on Day 5 (n = 8). The experiment was continued for 120 days, and animals surviving to that point were deemed to be long-term survivors. Prior experiments have established that there is not a statistically significant difference in median survival between control cohorts of animals implanted with only tumor and those implanted with both tumor and blank polymer.<sup>10,18,42</sup> For this reason, a cohort of animals bearing empty polymers was not included in these experiments. Prior experiments have also established that minocycline delivered systemically has no effect on survival in a 9L rat glioma model.<sup>41</sup>

### *Radiation Therapy*

Anesthetized rats were placed at a fixed distance from the radiation source in a Cs137 laboratory irradiator (Mark 1 Irradiator, model 68; J. L. Shepherd Associates), and shielded with a square primary collimator (1 cm in diameter aperture) centered over the tumor implantation site. The dose rate at this setting was 2.94 Gy/min, and the body was externally shielded from the radiation. External-beam single-dose radiation treatment was subsequently delivered at a dose of 20 Gy to animals receiving radiation therapy. All of this occurred 5 days after tumor implantation.

### *Statistical Analysis*

The primary outcome variable measured was the time to death from the day of tumor implantation (Day 0). Kaplan-Meier survival curves were generated, and groups were compared using Kruskal-Wallis ANOVA followed by the Wilcoxon rank-sum test, since the data are nonparametric. GraphPadPrism (version 4.0, GraphPad Software, Inc.) was used for all analyses. A p value < 0.05 was considered statistically significant for all comparisons.

## **Results**

### *Timing of Minocycline Polymer Implantation*

All control animals died within 19 days of tumor implantation, with a median survival of 14 days. Minocycline polymer implanted on Day 3 resulted in a median survival of 19 days. Minocycline polymer implanted on Day 5 also resulted in a median survival of 19 days. There

TABLE 1: Details regarding the different experiment groups, median survival, and number of long-term survivors\*

Group	No. of Animals	Median Survival (days)	Long-Term Survivors
control (no treatment)	16	14	0
oral TMZ, Days 5–9	16	21	0
XRT, Day 5	15	31	1 (6.7%)
minocycline, Day 5	16	19	0
minocycline, Day 5+oral TMZ, Days 5–9	16	29	1 (6.3%)
minocycline, Day 5+XRT, Day 5	15	74	7 (46.7%)
minocycline, Day 3	8	19	0
minocycline, Day 3+XRT, Day 5	8	62	1 (12.5%)

\* TMZ = temozolomide; XRT = radiotherapy.

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was no difference in median survival between animals treated with minocycline polymer on Days 3 and 5. The increase in median survival for both groups compared with the control group was statistically significant ( $p < 0.001$ ). Histological analysis showed that all animals died of a large tumor mass.

### Effect of Minocycline Polymer Combined With Radiotherapy

Minocycline polymer implanted on Day 5 was added to focal radiotherapy on Day 5 to determine whether the addition of the minocycline polymer would potentiate the effects of radiotherapy in increasing median survival (Fig. 1). The median survival of animals treated with radiotherapy alone was 31 days, with 1 long-term survivor from a cohort of 15 animals. The median survival of animals treated on Day 5 with minocycline polymer alone was 19 days. The median survival of animals treated with both radiotherapy and minocycline was 74 days, with 7 of 15 animals deemed long-term survivors (surviving for 120 days). This dual treatment represented increases of 139% compared with radiotherapy alone ( $p < 0.005$ ), 289% compared with Day 5 implantation of minocycline polymer alone ( $p < 0.001$ ), and 429% when compared with untreated controls ( $p < 0.001$ ).

### Timing of Minocycline Polymer Implantation With Respect to Radiotherapy

The median survival of animals treated with Day 3 implantation of minocycline polymer combined with Day 5 radiotherapy was 62 days, with 1 of 8 animals being a long-term survivor (Fig. 2). The median survival of animals treated with Day 5 implantation of minocycline polymer combined with Day 5 radiotherapy was 74 days,

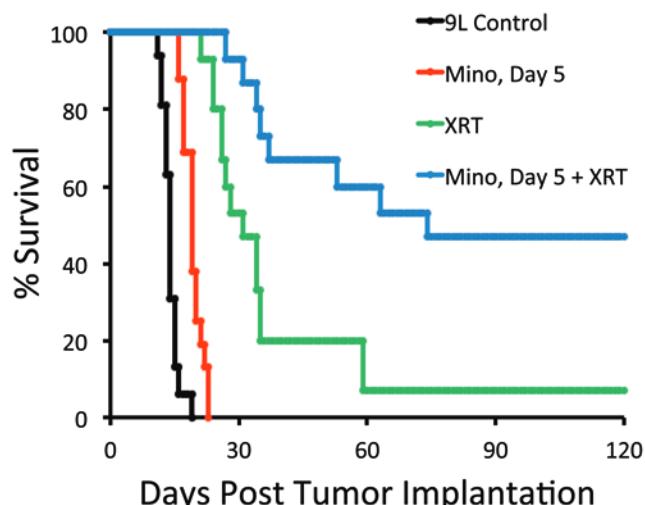


Fig. 1. Kaplan-Meier survival curves for control animals (black), animals treated with minocycline polymer (mino) alone on Day 5 (red), radiotherapy (XRT) alone on Day 5 (green), and minocycline polymer on Day 5 in combination with radiotherapy on Day 5 (blue). Median survival for the control group was 14 days, minocycline alone was 19 days, radiotherapy alone was 31 days, and the combined group was 74 days. The increase in median survival when Day 5 minocycline was added to radiotherapy was statistically significant ( $p < 0.005$ ).

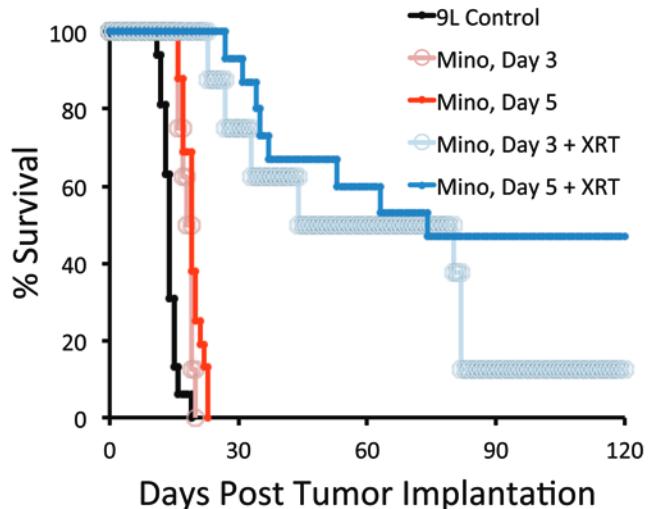


Fig. 2. Kaplan-Meier survival curves for control animals (black) and animals treated with radiotherapy on Day 5 combined with either minocycline polymer on Day 3 (light blue) or Day 5 (dark blue). Median survival for the control group was 14 days, Day 3 minocycline combined with radiotherapy was 62 days, and Day 5 minocycline combined with radiotherapy was 74 days. The difference in median survival between the two combined treatments was not statistically significant.

with 7 of 15 animals being long-term survivors. However, the difference in median survival between these two groups was not statistically significant ( $p = 0.197$ ).

### Effect of Minocycline Polymer Combined With Oral Temozolomide

Minocycline polymer implanted on Day 5 was added to oral temozolomide on Days 5–9 to determine whether the addition of the minocycline polymer would potentiate the effects of oral temozolomide in increasing median survival (Fig. 3). The median survival of controls was 14 days. The median survival of animals treated with minocycline polymer implanted on Day 5 was 19 days ( $p < 0.001$ , compared with controls). The median survival of animals treated with oral temozolomide on Days 5–9 was 21 days ( $p < 0.001$ , compared with controls). The median survival for the combination of Day 5 minocycline polymer and oral temozolomide was 29 days. This dual treatment represented increases of 38% when compared with oral temozolomide alone ( $p < 0.001$ ) and 53% when compared with Day 5 minocycline alone ( $p < 0.001$ ). All treatment groups resulted in median survival increases from that of untreated controls ( $p < 0.001$ ).

## Discussion

Systemic administration of angiogenesis inhibitors added to traditional therapy has been shown to extend median survival clinically in cancers outside the brain, such as colon cancer<sup>13</sup> and non–small cell lung cancer.<sup>28</sup> Even though glioblastoma is a highly vascular tumor,<sup>4,16</sup> systemic administration of angiogenesis inhibitors added to traditional therapy has not yet been shown to increase median survival for glioblastoma. One challenge may be for the angiogenesis inhibitors to cross the blood-brain

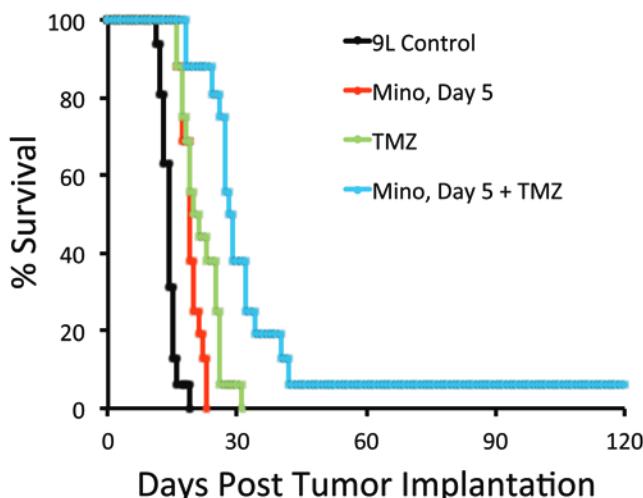


Fig. 3. Kaplan-Meier survival curves for control animals (black), minocycline polymer alone on Day 5 (red), animals treated with temozolamide on Days 5–9 (green), or minocycline polymer on Day 5 in combination with oral temozolamide on Days 5–9 (blue). Median survival for the control group was 14 days, oral temozolamide alone was 21 days, minocycline alone was 19 days, and the combined group was 29 days. The increase in median survival when Day 5 minocycline was added to oral temozolamide was statistically significant ( $p < 0.001$ ).

barrier, and an approach to overcome this challenge is the use of polymers for drug delivery inside the brain.

This study in the 9L gliosarcoma model investigated the potential benefits of adding intracranial minocycline polymer therapy to either radiotherapy or oral temozolamide chemotherapy, 2 commonly used methods for treating glioblastoma in the clinic. It also examined whether the timing of the angiogenesis inhibitor minocycline relative to radiotherapy affected median survival, as previous studies have suggested the possibility of an optimal time window for radiotherapy after starting an angiogenesis inhibitor.<sup>44</sup> Previous studies have already demonstrated that minocycline delivered systemically has little to no effect on survival of rats in a 9L glioma model.<sup>41</sup>

#### Timing of Minocycline Polymer Implantation

Past studies have compared survival between minocycline polymers implanted on Day 0 (simultaneous with tumor implantation) and Day 5 (after the tumor has already been established). In one study involving minocycline-impregnated biodegradable polymers, all rats were long-term survivors when the minocycline polymer was implanted on Day 0, while there was an increase in median survival from 14 days to 20 days when the minocycline polymer was implanted when the tumor was more established, on Day 5 posttumor implantation.<sup>9</sup>

In our current studies, we used a minocycline-impregnated biodegradable polymer and observed an increase in median survival from 14 days to 19 days when the minocycline polymer was implanted on Day 5 posttumor implantation ( $p < 0.001$ ). This result is consistent with the increase in median survival from 14 to 20 days reported previously by Frazier et al.<sup>9</sup> The same increase in median survival of 5 days was observed when the minocycline polymer was implanted on Day 3 posttumor

implantation ( $p < 0.001$ ). The lack of a statistically significant difference in median survival between minocycline polymer implantation on Days 3 and 5 posttumor implantation lays the foundation for examining whether delivering minocycline earlier with respect to radiotherapy would result in potentiation of radiotherapy.

#### Combination of Minocycline Polymer and Radiotherapy

Several studies have examined whether systemic delivery of angiogenesis inhibitors potentiates the effects of radiotherapy on survival for intracranial tumors. It has been shown that systemic administration of the angiogenesis inhibitor TNP-470 can significantly enhance the effects of radiotherapy in slowing the growth of subcutaneous tumors in mice.<sup>19</sup> However, in the same study, systemic TNP-470 had no effect either alone or in addition to radiotherapy on the survival of mice with intracranial tumors.<sup>19</sup> Similarly, intraperitoneal injections of the rat anti-mouse VEGFR-2 antibody DC101 did not increase the effects of local radiotherapy on survival in an intracranial tumor model in mice.<sup>36</sup> One study in humans examined whether systemic administration of the anti-VEGF antibody bevacizumab improves glioma response to radiotherapy.<sup>11</sup> However, due to a lack of randomization and the small size of the study, it is inconclusive whether the combination resulted in increased survival. To our knowledge, systemic administration of angiogenesis inhibitors has not been shown to potentiate the effects of radiotherapy on survival for intracranial tumors.<sup>19,36,43</sup>

Systemic administration of minocycline has been investigated in animal models for potentiating the effects of radiotherapy for tumors outside the brain.<sup>30,34</sup> Intraperitoneal minocycline injections caused a nearly 2-fold increase in tumor growth delay when combined with either single-dose or fractionated radiation treatment in the subcutaneous Lewis lung carcinoma model.<sup>30</sup> Similarly, intraperitoneal minocycline injections caused a tumor growth delay after treatment with radiotherapy for mouse mammary carcinoma implanted in the hind leg.<sup>34</sup> The conclusion we drew from these studies is that systemic delivery of minocycline has the ability to potentiate the effects of radiotherapy for extracranial tumors.

In our experiments, we showed that intracranial delivery of minocycline potentiated the effects of radiotherapy on median survival for intracranial tumors. Specifically, survival of the minocycline and radiation combination group was increased to 74 days from 31 days for radiotherapy alone ( $p < 0.005$ ) and from 19 days for the minocycline alone group ( $p < 0.001$ ). In an animal study by Verhoeff et al., adding intracranial controlled-release pegaptanib (an RNA aptamer against VEGF) to radiotherapy further increased progression-free survival from that of localized radiotherapy alone.<sup>36</sup> The results from both the Verhoeff et al. experiments and the data we present here suggest that intracranial delivery of angiogenesis inhibitors may be an effective way of potentiating the effects of radiotherapy for intracranial tumors.

#### Timing of Minocycline Polymer Implantation With Respect to Radiotherapy

Oxygen-deficient hypoxic areas in tumors may be ra-

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dioresistant.<sup>24,29</sup> In the past 10 years, it was proposed that angiogenesis inhibitors may normalize the tumor vasculature, resulting in better oxygen delivery and response to radiotherapy.<sup>14,15</sup> However, the optimal relative timing of radiation and angiogenesis inhibitor therapy still remains to be clarified.<sup>39</sup>

It has been suggested that the combination of radiation and angiogenesis inhibitor therapies is only synergistic during a “normalization window” when tissue hypoxia is greatly diminished.<sup>44</sup> In that study by Winkler et al., which involved human glioblastoma xenografts growing orthotopically in the mouse brain, a synergistic effect on tumor growth delay was seen only when radiotherapy was initiated 4 days after the start of treatment with the VEGFR-2 monoclonal antibody DC101. In 4 other combinations (radiotherapy initiated 9 days prior, 2 days prior, 1 day after, and 7 days after the start of angiogenesis inhibitor therapy), only an additive effect was seen. In another study, administering bevacizumab 2 days prior to radiotherapy was synergistic as evaluated by bioluminescent imaging, while administration either simultaneously with or 5 days prior to radiotherapy was considered additive.<sup>20</sup> Lastly, it was demonstrated that thalidomide (an inhibitor of VEGF and basic fibroblast growth factor) induces maximal tumor oxygenation at 2 days, compared with Days 1, 3, 4, and 5.<sup>2</sup>

Based on these studies, we investigated whether delivery of intracranial minocycline 2 days prior to radiotherapy would result in better median survival compared with simultaneous delivery of minocycline and radiotherapy. We believed that the mechanism for minocycline to potentiate the effects of radiotherapy would be similar to that of agents used in the other studies, as minocycline has been shown to increase the oxygenation of tumors.<sup>34</sup> Our results showed that there was not a statistically significant difference in median survival between minocycline polymer implantation on Day 3 or 5. Adding radiotherapy on Day 5 to both scenarios increased median survival substantially for both groups. However, the difference in median survival between radiotherapy added to either Day 3 or Day 5 minocycline was not statistically significant. One possibility for this lack of difference in median survival is that the “normalization window” may be narrow and that 2 days may not have been captured in it.

### Combination of Minocycline Polymer and Oral Temozolomide

Previously, it had been established in animal models that minocycline potentiates the action of several alkylating agents in the treatment of cancer.<sup>9,30,34,41</sup> It was shown that implantation of minocycline polymer on Day 5 combined with intraperitoneal administration of the alkylating agent BCNU resulted in median survival that was substantially increased compared with that of intraperitoneal injection of BCNU alone.<sup>9,41</sup> In another experimental model, addition of minocycline to treatment with cyclophosphamide resulted in a 2.1-fold increase in tumor growth delay for multiple-dose regimens of the alkylating agent cyclophosphamide in a Lewis lung carcinoma model.<sup>30</sup> Similarly, addition of minocycline to treatment with cyclophosphamide resulted in a 2-fold growth delay in a mammary carcinoma model.<sup>34</sup>

In our experiments, we have shown that similar to the case with other alkylating agents, minocycline polymer also potentiates the effect of temozolomide, a frequently used DNA-alkylating agent for intracranial tumors. Specifically, treatment with oral temozolomide alone resulted in median survival of 21 days, while the addition of minocycline polymer to oral temozolomide increased survival to 29 days ( $p < 0.001$ ).

We did not plan an experiment involving the combination of minocycline with the combination of both radiotherapy and oral temozolomide, as it has been established in the 9L glioma model that the combination of radiotherapy and oral temozolomide resulted in  $\geq 50\%$  long-term survivors.<sup>35</sup> Despite the overall significance of this study, further studies involving human xenografts and larger animals are necessary to support these conclusions.

## Conclusions

Over the past several years, there has been increasing interest in combining angiogenesis inhibitors with other modalities in the treatment of glioblastoma.<sup>6,7,26</sup> Notably, when bevacizumab was first approved for the treatment of metastatic colorectal cancer in 2004, the approval was based on clinical trials that added bevacizumab to irinotecan, fluorouracil, and leucovorin.<sup>13</sup> In 2009, the US Food and Drug Administration approved bevacizumab for the treatment of glioblastoma.<sup>7</sup> However, the European Medicines Agency rejected bevacizumab’s use for glioblastoma in 2009, as it was unclear whether its systemic administration resulted in a survival benefit.<sup>43</sup>

Several challenges exist for the systemic administration of drugs in the treatment of intracranial tumors, including the presence of the blood-brain barrier and reaching systemic toxicity before a therapeutic concentration within the brain can be attained.<sup>17</sup> In this work, we demonstrated that local delivery of the antiangiogenesis agent minocycline potentiates the effects of radiotherapy and systemic temozolomide in increasing median survival in a rat glioma model. More generally, we suggest that intracranial, as opposed to systemic, delivery of antangiogenic agents may be more effective in potentiating the effects of other modalities in achieving a survival benefit.

## Disclosure

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Author contributions to the study and manuscript preparation include the following. Conception and design: Bow. Acquisition of data: Tyler, Bow, Hwang, Schildhaus, Xing, Murray, Salditch, Zhang. Analysis and interpretation of data: Tyler, Bow. Drafting the article: Bow. 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, Ye. Study supervision: Tyler, Brem.

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