

# New therapeutic approach for brain tumors: Intranasal delivery of telomerase inhibitor GRN163

Rintaro Hashizume, Tomoko Ozawa, Sergei M. Gryaznov, Andrew W. Bollen, Kathleen R. Lamborn, William H. Frey II, and Dennis F. Deen

*Brain Tumor Research Center of the Department of Neurological Surgery, University of California San Francisco, San Francisco, CA (R.H., T.O., K.R.L., D.F.D.); Geron Corporation, Menlo Park, CA (S.M.G.); Department of Pathology, University of California San Francisco, San Francisco, CA (A.W.B.); Alzheimer's Research Center, HealthPartners Research Foundation at Regions Hospital, St. Paul, MN (W.H.F.); USA*

The blood-brain barrier is a substantial obstacle for delivering anticancer agents to brain tumors, and new strategies for bypassing it are greatly needed for brain-tumor therapy. Intranasal delivery provides a practical, noninvasive method for delivering therapeutic agents to the brain and could provide an alternative to intravenous injection and convection-enhanced delivery. We treated rats bearing intracerebral human tumor xenografts intranasally with GRN163, an oligonucleotide N3'→P5'thio-phosphoramidate telomerase inhibitor. 3'-Fluorescein isothiocyanate (FITC)-labeled GRN163 was administered intranasally every 2 min as 6  $\mu$ l drops into alternating sides of the nasal cavity over 22 min. FITC-labeled GRN163 was present in tumor cells at all time points studied, and accumulation of GRN163 peaked at 4 h after delivery. Moreover, GRN163 delivered intranasally, daily for 12 days, significantly prolonged the median survival from 35 days in the control group to 75.5 days in the GRN163-treated group. Thus, intranasal delivery of GRN163 readily bypassed the blood-brain barrier, exhibited favorable tumor uptake, and inhibited tumor growth, leading to a prolonged lifespan for treated rats compared to controls. This delivery approach appears to kill tumor cells selectively, and

no toxic effects were noted in normal brain tissue. These data support further development of intranasal delivery of tumor-specific therapeutic agents for brain tumor patients. *Neuro-Oncology* 10, 112–120, 2008 (Posted to *Neuro-Oncology* [serial online], Doc. D06-00225, February 20, 2008. URL <http://neuro-oncology.dukejournals.org>; DOI: 10.1215/15228517-2007-052)

Keywords: brain tumors, GRN163, intranasal delivery, telomerase inhibitor, xenografts

Despite the development of drugs that preferentially target tumor cells without harming normal tissues, delivery of these drugs to brain tumors remains a major challenge because of difficulty in penetrating the blood-brain barrier (BBB). Malignant gliomas are the most common primary tumors that occur in the human brain. The 5-year survival rate for patients with glioblastoma (GBM), the most aggressive form of malignant glioma, is less than 5% even with surgery followed by radiation therapy and chemotherapy.<sup>1</sup> Clearly there is a great need for new therapeutic strategies that will provide efficient drug delivery to the brain tumors.

Intranasal delivery provides a practical, noninvasive method for delivering therapeutic agents to the brain because of the unique anatomic connection provided by the olfactory and trigeminal nerves. These nerves connect the nasal mucosa and the CNS, allowing them to detect odors and other chemical stimuli.<sup>2,3</sup> Intranasally administered drugs reach the parenchymal tissues of the brain and spinal cord and/or cerebrospinal fluid (CSF)

Received December 20, 2006; accepted August 17, 2007.

Address correspondence to Rintaro Hashizume, Brain Tumor Research Center, Department of Neurological Surgery, University of California San Francisco, San Francisco, CA 94143-0520, USA (rintaro.hashizume@ucsf.edu).

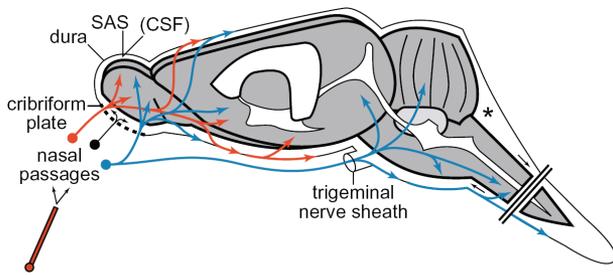


Fig. 1. The anatomical extraneuronal pathways provided by the olfactory and trigeminal nerves following intranasal administration. Intranasally applied drugs are rapidly transported into the CNS tissue by the peripheral olfactory system (shown in red), connecting the nasal passages and olfactory bulb/rostral brain, and peripheral trigeminal system (shown in blue), connecting the nasal passages and the brainstem/spinal cord. Cisternal sampling in rats (asterisk) has demonstrated that some molecules, mostly smaller molecular weight solutes, can rapidly enter the cerebrospinal fluid (CSF) after intranasal administration (shown in black). Abbreviation: SAS, subarachnoid space. This figure was originally published in Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience*. 2004;127:481–496 (Copyright Elsevier, 2004).

within minutes using an extracellular route through perineural channels (Fig. 1).<sup>3–5</sup> In addition to bypassing the BBB, the advantages of intranasal delivery include rapid delivery to the CNS, avoidance of hepatic first-pass drug metabolism, and elimination of the need for systemic delivery, thereby reducing unwanted systemic side effects. Intranasal delivery also provides painless and convenient self-administration by patients, features that encourage its use for delivering therapeutic agents into the CNS.<sup>2,3</sup>

Recently, anticancer agents such as methotrexate,<sup>6,7</sup> 5-fluorouracil,<sup>8</sup> and raltitrexed<sup>9</sup> have been delivered to the CNS and/or CSF using intranasal delivery. However, these chemotherapeutic agents do not discriminate between tumor and normal tissues. Thus, the concentrations of drug required to kill tumor cells can also lead to toxicity in normal neural tissues. To achieve therapeutic efficacy without toxicity to normal tissues, the drugs must preferentially target brain tumor while sparing normal tissues from damage.

Because telomerase is expressed in the vast majority of GBMs but not in normal brain tissues,<sup>10</sup> inhibition of telomerase provides a therapeutic strategy for selectively targeting malignant gliomas. We have previously shown that GRN163, a 13-mer oligonucleotide N3'→P5' thio-phosphoramidate telomerase inhibitor (Geron Corporation, Menlo Park, CA, USA), inhibited the growth rate of subcutaneous human U-251 MG GBM xenografts in athymic mice and prolonged survival of athymic rats bearing U-251 MG intracerebral tumor xenografts.<sup>11</sup> To maximize the dose of drug to the tumor cells, GRN163 was administered directly into the tumor site using intratumoral injection for the subcutaneous tumors

and convection-enhanced delivery (CED) for the intracerebral tumors. CED uses convective flow to distribute drug through a surgically implanted catheter.<sup>12,13</sup> Thus, administering GRN163 directly into human patients' tumors via CED is a promising approach, but one that would require surgically invasive procedures. In contrast, intranasal drug delivery has the advantage of being noninvasive.

Here, we tested the hypothesis that intranasal delivery of the telomerase inhibitor GRN163 enables the compound to reach intracerebral tumors and inhibit tumor growth *in vivo* without neurotoxic side effects. Our results suggest that intranasal delivery of GRN163 provides a promising noninvasive approach for the treatment of malignant gliomas.

## Materials and Methods

### Cell Cultures

U-251 MG human GBM cells were obtained from the Department of Neurological Surgery Tissue Bank at the University of California, San Francisco (UCSF). Cells were maintained as exponentially growing monolayers in complete minimal essential medium (CMEM) consisting of Eagle's minimal essential medium supplemented with 10% fetal calf serum and nonessential amino acids. Cells were cultured at 37°C in a humidified atmosphere containing 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Cells were seeded into culture flasks 2 days before tumor implantation. For implantation, cells were harvested by trypsinization, washed once, and resuspended in Hank's balanced salt solution (HBSS) without Ca<sup>2+</sup> and Mg<sup>2+</sup>.

### Oligonucleotides

GRN163 is complementary to a part of the template region of hTR and has the nucleotide sequence 5'-TAG-GGTTAGACAA-3'. The mismatch control thio-phosphoramidate oligonucleotide (MM-Control) has the same nucleotide composition but the sequence 5'-TAG-GTGTAGCAA-3', in which the four mismatched nucleotides are underlined.<sup>14</sup> The thio-phosphoramidate oligonucleotides were prepared as described previously.<sup>15</sup> 3'-fluorescein isothiocyanate (FITC)-labeled GRN163 was prepared according to the published procedure.<sup>16</sup> A working concentration of 0.65 μmol GRN163 in 65 μl phosphate-buffered saline (PBS) was used for both tumor-distribution and efficacy studies.

### Animals

Six-week-old male athymic rats (rnu/rnu, homozygous) were purchased from Harlan (Indianapolis, IN, USA) and housed under aseptic conditions, which included filtered air and sterilized food, water, bedding, and cages. The UCSF Institutional Animal Care and Use Committee approved all animal protocols.

### *U-251 MG Human GBM Intracerebral Tumor Model*

Tumor cells were implanted into the brains of athymic rats as previously described.<sup>17</sup> Briefly, rats were anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg) and injected slowly with the U-251 MG cell suspension ( $2 \times 10^6$  cells in 10  $\mu$ l HBSS) into the right caudate-putamen using an implantable guide-screw system. Typically, this procedure results in a 100% tumor take and a median survival time of animals of approximately 35 days after tumor implantation.<sup>17</sup>

### *Intranasal Delivery of GRN163 in Athymic Rats*

Rats were anesthetized with inhalation of 2%–2.5% isoflurane and placed in a supine position in an anesthesia chamber. PBS (6  $\mu$ l) containing GRN163 (0.65  $\mu$ mol/65  $\mu$ l) was administered intranasally as drops with a small pipette every 2 min into alternating sides of the nasal cavity, followed by 5  $\mu$ l for the last dose (for a total of 22 min). A total volume of 65  $\mu$ l was delivered into the nasal cavity. After delivery, the animals were removed from the anesthesia chamber; they regained consciousness and were ambulatory within 3 min.

### *Distribution Study of GRN163 in Athymic Rats*

FITC-labeled GRN163 was delivered as above over 22 min into the nasal cavity of non-tumor-bearing rats and tumor-bearing rats on day 25 after implantation, when the intracerebral tumors were approximately 50 mg in size as determined from earlier growth curves.<sup>17</sup> Two rats each were euthanized at 10 min and 0.5, 1, 2.5, 4, and 24 h after delivery by perfusion with PBS, and their brains were dissected and frozen in ethanol and dry ice. The brains were sectioned coronally, and 10- $\mu$ m-thick sections were placed on microscope slides. Ten to 15  $\mu$ l of mounting medium containing 2  $\mu$ g/ml of 4',6-diamidino-2'-phenylindole dihydrochloride (DAPI, Boehringer Mannheim Biochemica, Mannheim, Germany) was added over the sections, and a cover glass was placed over the samples. The resulting slides were immediately viewed and photographed using a fluorescence microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA,  $\times 10$  magnification). An ultraviolet-light transmission filter was used for DAPI to visualize the nuclei of cells. A blue-light transmission filter was used for FITC to visualize the green signal that indicates cells containing GRN163.

### *In Vitro Telomerase-Activity Inhibition Study*

Cells ( $2.5 \times 10^4$ ) in log-phase growth were seeded into T-25 plastic flasks containing CMEM, and 24 h later GRN163 was added to final concentration of 0, 3, 7, 10, or 20  $\mu$ M. Cells were exposed to the oligonucleotides for 3 days at 37°C and then were trypsinized, counted, and washed twice with PBS. Cells were pelleted and stored at  $-80^\circ\text{C}$ .

### *Telomere Repeat Amplification Protocol Assay*

Telomere repeat amplification protocol (TRAP) assays were performed with the TRAPeze Telomerase Detection System (Chemicon International, Inc. Temecula, CA, USA) according to the manufacturer's instructions. Briefly, cells were lysed with 1 $\times$  CHAPS lysis buffer (10 mM Tris-HCl [pH 7.5], 1 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.1 mM benzamidine, 5 mM  $\beta$ -mercaptoethanol, 0.5% CHAPS, 10% glycerol) for 30 min on ice. The lysates were centrifuged at 12,000g for 20 min at 4°C. When preparing extracts from tumor tissues, a small portion of the tumor was minced in ice-cold 1 $\times$  CHAPS lysis buffer containing 200 U/ml RNase inhibitor (Promega, Madison, WI, USA), incubated for 30 min on ice, and centrifuged at 12,000g. The protein concentrations of the samples were determined by using Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA); 125 ng protein extract was used per reaction for the TRAP assay. Samples were mixed with TRAPeze kit reagents according to the manufacturer's instructions, using 30 min of telomerase extension at 30°C, and inactivating telomerase at 94°C for 2 min. The telomerase extension products were amplified by 33 PCR cycles (94°C for 30 s, 59°C for 30 s, 72°C for 1 min). A standard batch of U-251 MG cell line was used as a positive control, and lysis buffer was used as a negative control for each run. PCR samples were resolved on a 12.5% polyacrylamide gel. The gel was stained with SYBER Green (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and scanned using a Kodak image station 4000MM (Eastman Kodak Company, Scientific Imaging Systems, New Haven, CT, USA). Activity of TRAP products was quantified using ImageJ 1.32j software (National Institutes of Health, <http://rsb.info.nih.gov/ij>).

### *Treatment of Tumors in Athymic Rats with Intranasal GRN163*

Tumor-bearing rats were treated intranasally with daily doses of 0.21, 0.325, and 0.65  $\mu$ mol GRN163 or MM-Control in PBS or PBS-Control for 12 consecutive days excluding weekends over a 3-week period. Treatment was initiated on day 14 when intracerebral tumors were approximately 20 mg in size. The investigators treating the animals were fully blinded with regard to treatment. All rats were monitored every day and were euthanized when they exhibited neurological symptoms indicative of impending death.<sup>17</sup> Their brains were sectioned coronally for histological examination using standard hematoxylin and eosin staining ( $\times 5$  magnification). For telomerase activity assay, tumor tissues were dissected after 12 days, quick-frozen in ethanol and dry ice, and stored at  $-80^\circ\text{C}$ .

### *Statistical Analysis*

Comparison of survival times was done using the exact Wilcoxon rank sum test. Each control group was compared separately to the GRN163 group. Because the GRN163 group was required to demonstrate improved

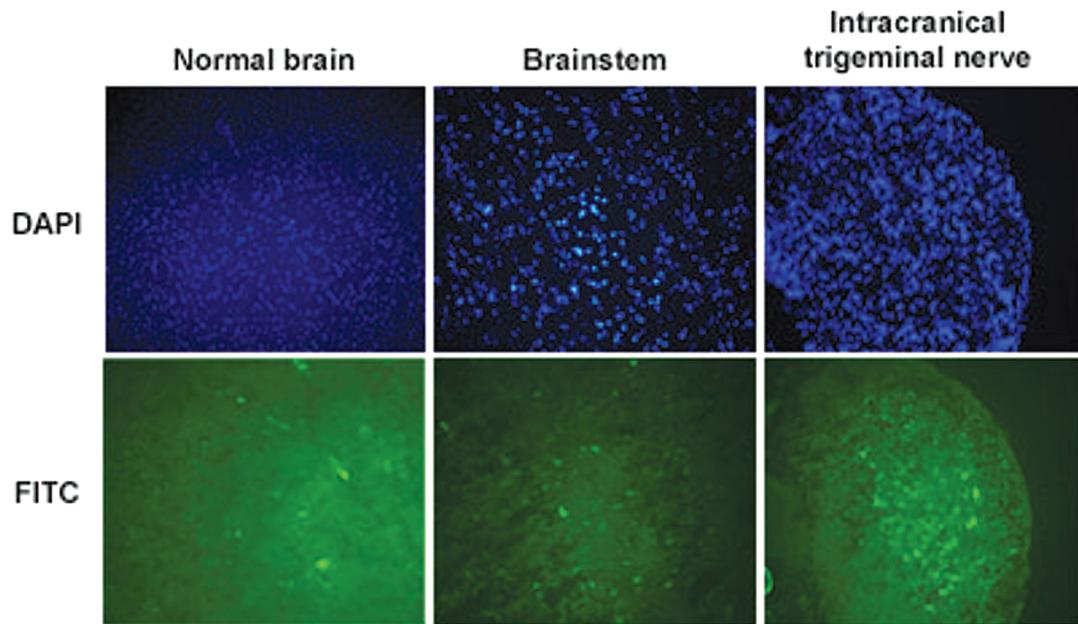


Fig. 2. Distribution of fluorescein-labeled GRN163 by intranasal delivery into normal brain in athymic rats. Faint fluorescence of 3'-fluorescein isothiocyanate (FITC)-labeled GRN163 was detected diffusely in normal brain, brainstem, and intracranial trigeminal nerves at 10 min after intranasal delivery into non-tumor-bearing rats. 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI) indicates nuclear staining in blue; FITC staining reveals fluorescently labeled GRN163 in green.

survival against each of the control groups to be considered effective, no adjustment was made for multiple comparisons.

## Results

### *Intranasally Administered GRN163 Rapidly Distributes throughout the Brains of Non-Tumor-Bearing Rats*

To determine whether GRN163 can be transported intranasally into the brain, we first administered FITC-labeled GRN163 into non-tumor-bearing rats. FITC-labeled GRN163 (0.65  $\mu\text{mol}/65 \mu\text{l}$ ) was administered as 10 6- $\mu\text{l}$  drops spaced 2 min apart and one 5- $\mu\text{l}$  drop into alternating sides of the nasal cavity with a small pipette. Rats were euthanized 10 min after intranasal delivery, and their brains were examined for the presence of the compound using a fluorescence microscope. Faint fluorescence of the compound was observed diffusely in the brain, brainstem, and intracranial trigeminal nerves at this time, suggesting that delivery of GRN163 to the brain occurred rapidly (Fig. 2). No apparent toxicity or behavioral abnormalities were observed in any of the rats during this brief experiment. Moreover, there was no detectable fluorescence from GRN163 in lung or liver tissue obtained from the rats.

### *Intranasally Delivered GRN163 Preferentially Accumulates in Intracerebral Tumors*

We next investigated distribution of intranasal FITC-labeled GRN163 into rats bearing human U-251 MG brain tumors at various times after intranasal delivery. Rats were given 0.65  $\mu\text{mol}$  FITC-labeled GRN163 over a 22-min time period, as above, and were euthanized 0.5 to 24 h later. Fluorescence was visible at the edge of the tumor 0.5 h after intranasal delivery and was visible throughout the tumor at 4 h (Fig. 3A). Fluorescence remained visible in the tumor cells 24 h after delivery. In contrast, very little or no fluorescence was detected in normal brain cells adjacent to the tumor (Fig. 3A), and no fluorescence was detected in other normal brain regions at any time point studied (Fig. 3B). The animals did not exhibit any apparent toxicities or behavioral abnormalities during the course of this experiment.

### *GRN163 Inhibits Telomerase Activity in U-251 MG Cells In Vitro*

Prior to in vivo GRN163 treatment, the effect of GRN163 at various concentrations on telomerase activity of U-251 MG cells was studied initially in a short-term cell-based TRAP assay. GRN163 showed a dose-dependent inhibition of telomerase activity; exposure of cells to 3, 7, 10, or 20  $\mu\text{M}$  for 3 days inhibited telomerase activity by 21%, 31%, 51%, and 81%, respectively, relative to the activity seen in untreated U-251 MG cells (Fig. 4A).

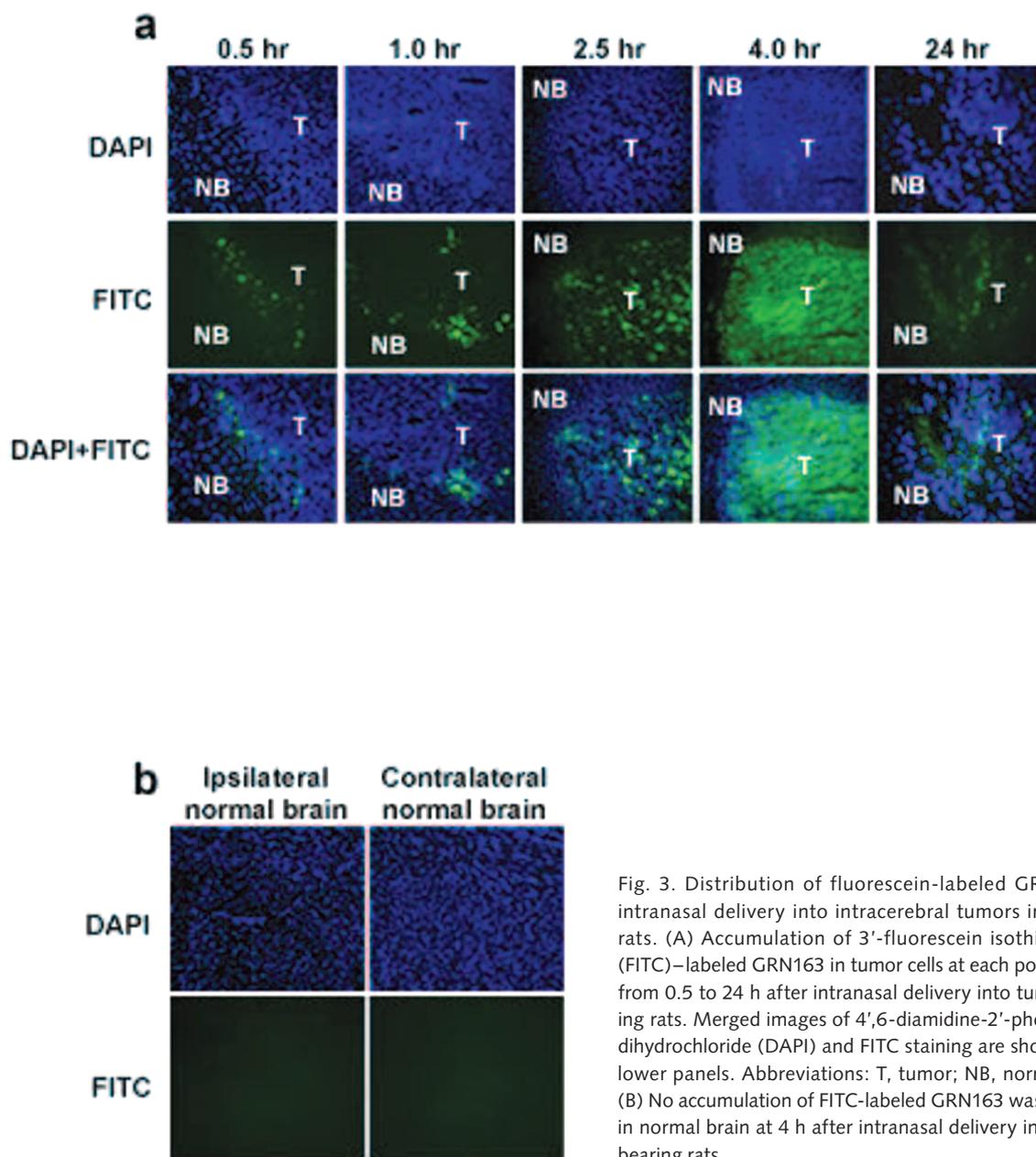


Fig. 3. Distribution of fluorescein-labeled GRN163 by intranasal delivery into intracerebral tumors in athymic rats. (A) Accumulation of 3'-fluorescein isothiocyanate (FITC)-labeled GRN163 in tumor cells at each point in time from 0.5 to 24 h after intranasal delivery into tumor-bearing rats. Merged images of 4',6-diamidino-2'-phenylindole dihydrochloride (DAPI) and FITC staining are shown in the lower panels. Abbreviations: T, tumor; NB, normal brain. (B) No accumulation of FITC-labeled GRN163 was detected in normal brain at 4 h after intranasal delivery into tumor-bearing rats.

#### *Intranasally Delivered GRN163 Inhibits Brain-Tumor Growth and Prolongs the Lifespan of Rats*

To test the potential therapeutic value of delivering GRN163 via the nasal cavity, we treated small groups of rats (three to five animals per group) bearing intracerebral tumors with graded concentrations of GRN163 and PBS-Control for 12 days beginning on day 14 after tumor cell implantation. The maximum attainable concentration of GRN163, due to solubility constraints, was 0.65  $\mu\text{mol}/65 \mu\text{l}$ . GRN163 uptake in the tumor was confirmed by fluorescein staining of FITC-GRN163 at 4 h after final delivery of the 12-day treatment. The median survival in the PBS-Control group was 36 days; the groups treated with 0.21, 0.325, and 0.65  $\mu\text{mol}$

GRN163 had median survival times of 48, 47, and 99 days, respectively. Inhibition of telomerase activity of intracerebral xenografts by GRN163 was confirmed by TRAP assay. When the rats were treated with 0.21, 0.325, and 0.65  $\mu\text{mol}$  GRN163 for 12 days, telomerase activity was inhibited by 15%, 43%, and 54%, respectively (Fig. 4B).

Because it corresponded to the longest survival time, a daily dose of 0.65  $\mu\text{mol}$  GRN163 or MM-Control was selected for intranasal treatment of a larger number of rats. Ten rats in each group received treatment for 12 days. Survival times of PBS-Control and MM-Control animals ranged from 32 to 43 days, with a median survival time of 35 days for each control group (Fig. 5). In

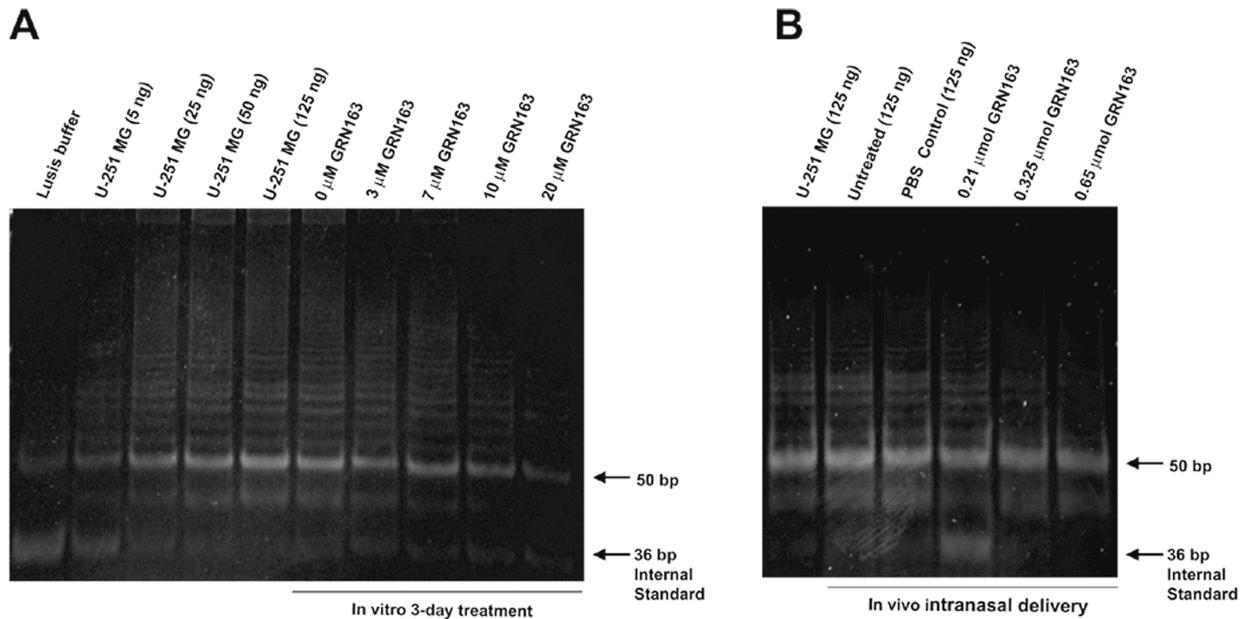


Fig. 4. Inhibition of telomerase activity by GRN163 in glioblastoma cells and intracerebral xenografts. (A) U-251 MG cells were treated with a range of doses of GRN163 for 3 days. Cells were collected and telomerase activity was measured for 125 ng protein extract per lane using the telomere repeat amplification protocol (TRAP) assay. A standard batch of U-251 MG cell line was used as a positive control, and lysis buffer was used as a negative control. (B) Rats were treated intranasally with daily doses of 0.21, 0.325, and 0.65  $\mu$ mol GRN163 for 12 days. The intracerebral tumors were collected and telomerase activity was measured for 125 ng protein extract per lane using the TRAP assay. Tumor tissue from untreated or PBS-Control rats was used as a positive control.

contrast, GRN163 treatment significantly prolonged the survival of animals ( $p < 0.01$ ), with a median survival time of 75.5 days (Fig. 5). Moreover, three GRN163-treated rats were alive and showed no neurological symptoms at day 104, when they were euthanized. There was no evidence of a tumor at the original implantation site in these three rats; rather, the injection site showed astrogliosis with a macrophage infiltrate and foci of hemosiderin and calcium, along with several small vessels (Fig. 6A). However, all the other GRN163-treated rats that were euthanized due to neurological symptoms had a large tumor at the site of implantation (Fig. 6B). All animals that received MM-Control (Fig. 6C) or PBS-Control (Fig. 6D) also had large tumors at the site of implantation when they were euthanized. None of the GRN163-treated animals exhibited evidence of toxicity or behavioral abnormalities during the 12-day treatment period. Rats in all three groups gained weight during the treatment period, but later those with symptoms of tumor typically began to lose weight a few days before being euthanized. An autopsy was performed on four rats selected randomly from the three groups that were euthanized when they showed symptoms of tumor. All organs were grossly normal, and lungs, livers, hearts, spleens, and kidneys were normal on histological examination.

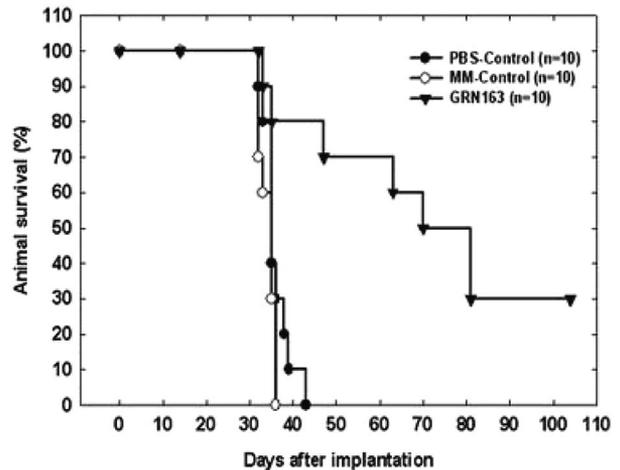


Fig. 5. Survival of rats treated with intranasal GRN163. Animals in both PBS- and MM-Control groups had median survivals of 35 days, whereas animals in the GRN163 treatment group had a median survival of 75.5 days, significantly longer than either control group ( $p < 0.01$ ).

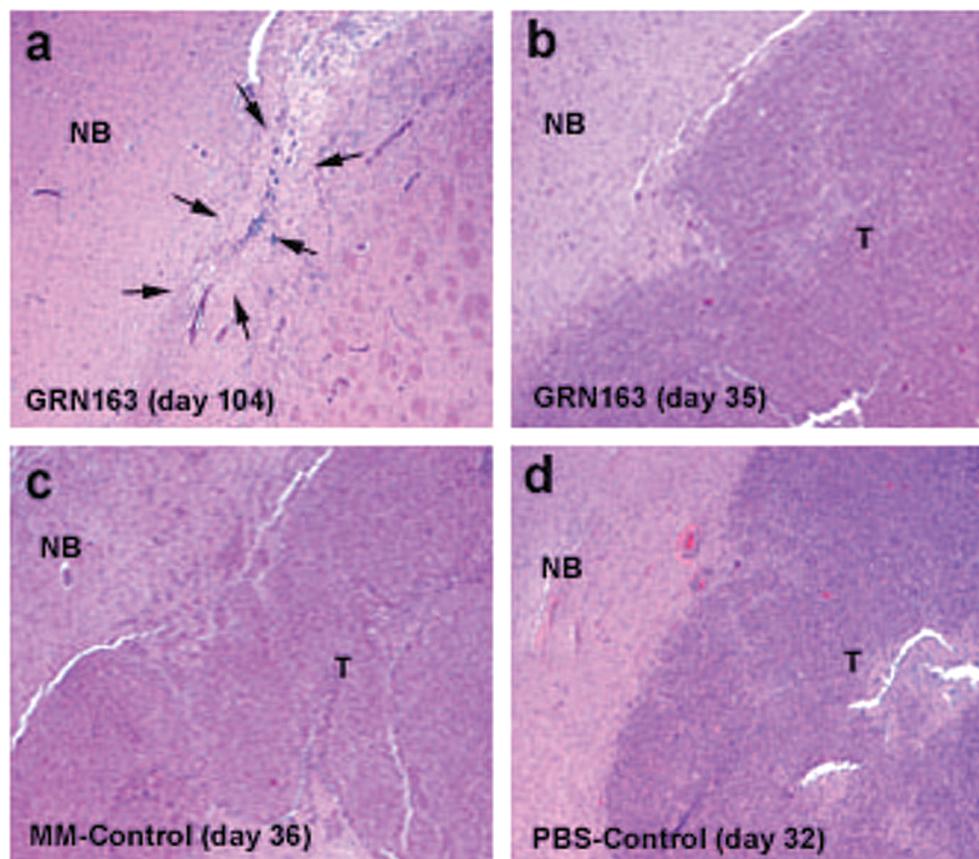


Fig. 6. Intranasal GRN163 inhibits tumor growth in athymic rats. (A) Brain from a rat that was treated with intranasal GRN163 and euthanized on day 104, with no symptoms of tumor. The injection site shows astrogliosis with a macrophage infiltrate and foci of hemosiderin and calcium, along with several small vessels (arrows). No neoplastic cells are present. (B–D) Interface of normal brain and neoplasm treated with intranasal GRN163 (B), MM-Control (C), and PBS-Control (D). Days are post-tumor implantation to euthanization due to neurological symptoms. Abbreviations: T, tumor; NB, normal brain.

## Discussion

Our data demonstrate favorable *in vivo* distribution and efficacy of the telomerase inhibitor GRN163 when applied intranasally into human intracerebral glioblastoma xenografts. In our initial distribution study, we found rapid movement of GRN163 into the brain, including the brainstem and intracranial trigeminal nerves. This finding is consistent with the extraneuronal pathway that has been proposed for transport of therapeutic agents from the nasal cavity into the brain.<sup>2,3</sup> This pathway occurs along the olfactory and trigeminal sensory neurons and likely involves extracellular bulk flow along perineuronal and/or perivascular channels, which delivers drug directly to the brain parenchyma, spinal cord, and perhaps the CSF (Fig. 1).<sup>3</sup> Thorne et al. reported that insulin-like growth factor-1 is rapidly transported into the brain and upper spinal cord within 30 min of intranasal administration via the extraneuronal pathway.<sup>3</sup> Delivery along the extraneuronal pathway is not receptor-mediated and requires only minutes for the drug to reach the brain.<sup>5</sup> It is unlikely that GRN163 is delivered via an intraneuronal pathway

along the primary olfactory sensory neurons, because this pathway involves axonal transport and requires several hours to days for the drug to reach different areas of the brain.<sup>4,18</sup>

In our intracerebral tumor model, GRN163 showed tumor-specific distribution at all time points studied (0.5 up to 24 h) and achieved highest concentration within the tumor at 4 h after delivery, indicating favorable uptake and retention of the compound in the tumor. This may be due to the specific binding affinity of this compound to telomerase in the tumor cells,<sup>19</sup> and the specificity achieved with intranasal delivery appears to be superior to the results obtained using CED, which reportedly delivers drugs beyond the tumor boundary into adjacent normal brain tissues.<sup>11,20,21</sup> This spillover of drug to adjacent brain regions may be due to a pressure gradient of convective bulk flow of CED and could lead to neural toxicity.<sup>12,22</sup> In contrast to CED, intranasal GRN163 showed very little or no accumulation in adjacent normal brain tissues surrounding the tumor. Thus, intranasal delivery may prevent unwanted damage to normal brain tissues adjacent to the tumor.

The results of our tumor efficacy study, in which rats were treated intranasally with a daily dose of 0.65  $\mu\text{mol}$  of GRN163 for 12 days, showed that rats treated with GRN163 survived significantly longer than rats treated with PBS- and/or MM-Control. Notably, three rats treated with GRN163 showed no neurological symptoms by day 104 and no evidence of tumor at the original implantation site. Telomerase inhibition is related to telomere length in a time-dependent manner. Rapid inhibition occurs in cells with shorter telomeres,<sup>23,24</sup> and U-251 MG cells have relatively short telomeres (approx. 3.9 kilobases) as compared with other brain tumor cells.<sup>11</sup> Our in vitro studies showed that a 3-day exposure of U-251 MG cells to GRN163 inhibited telomerase activity by 81% in a dose-dependent manner. In addition, intranasal treatment of GRN163 for 12 days inhibited telomerase activity of intracerebral tumors by 54%. Thus, it is likely that in this study intranasal delivery of GRN163 led to specific inhibition of telomerase activity in U-251 MG tumor cells, which inhibited their growth, resulting in prolonged lifespan for the animals. The length of the protocol used in this study was determined by the average lifespan of the control animals, which limited the time frame within which results from treatment could be considered significant to approximately 12 days. We used the maximum dose of GRN163 (0.65  $\mu\text{mol}/65 \mu\text{l}$ ) that could be dissolved in saline solution. Further work will be required to fully define the maximum tolerated dose and dosing schedule for optimal intranasal delivery. These parameters will likely be determined by the size of the tumor, the characteristics of the drug, and the type of animal model studied.

Recently, intranasal delivery of insulin has been shown to improve memory in normal adults<sup>25</sup> and in patients with early Alzheimer's disease<sup>26</sup> without changing blood levels of glucose or insulin,<sup>27</sup> implying that insulin entered the brain directly without entering the bloodstream. Intranasal treatment with perillyl alcohol given to patients with recurrent brain tumors is being investigated in combination with radiotherapy.<sup>28</sup>

In conclusion, this is the first report to demonstrate that intranasal delivery of telomerase inhibitor GRN163 achieves efficient distribution into an intracerebral tumor and inhibits tumor growth in vivo, resulting in prolonged survival of athymic rats without apparent toxicity. Our findings support further development of intranasal GRN163 as a potential therapy for brain-tumor patients and perhaps as a means for treating multifocal brain tumors and/or pediatric brainstem tumors, which are less amenable to potentially risky surgical procedures. Our results also support the investigation of delivering other tumor-specific agents intranasally for the treatment of intracranial neoplasms.

## Acknowledgments

This research was supported by grant CA107268 from the National Institutes of Health. The authors are grateful to Francis C. Szoka Jr., Lily J. Hu, and Jingli Wang for helpful discussion; Raquel A. Santos for technical assistance; Anita Lal for critically reading the manuscript; and Ilona Garner for excellent editorial assistance. The GRN163 used in these studies was provided by Geron Corporation, and Dr. Gryaznov is an employee of Geron Corporation.

## References

1. CBTRUS, Central Brain Tumor Registry of the United States. *Primary Brain Tumors in the United States: Statistical Report Tables, 1998–2002*. 2005. Available at <http://www.cbtrus.org/2005–2006/tables/2006.table18–19.pdf>.
2. Dhanda DS, Frey WH 2nd, Leopold D, Kompella UB. Approaches for drug deposition in the human olfactory epithelium. *Drug Deliv Technol*. 2005;5:64–72.
3. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience*. 2004;127:481–496.
4. Thorne RG, Emory CR, Ala TA, Frey WH 2nd. Quantitative analysis of the olfactory pathway for drug delivery to the brain. *Brain Res*. 1995;692:278–282.
5. Thorne RG, Frey WH 2nd. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. *Clin Pharmacokinet*. 2001;40:907–946.
6. Wang F, Jiang X, Lu W. Profiles of methotrexate in blood and CSF following intranasal and intravenous administration to rats. *Int J Pharm*. 2003;263:1–7.
7. Shingaki T, Sakane T, Yamashita S, Sezaki H, Yoshiharu T, Shoubu S. Transnasal delivery of anticancer drugs to the brain tumor: a new strategy for brain tumor chemotherapy. *Drug Deliv System*. 1999;14:365–371.
8. Sakane T, Yamashita S, Yata N, Sezaki H. Transnasal delivery of 5-fluorouracil to the brain in the rat. *J Drug Target*. 1999;7:233–240.
9. Wang D, Gao Y, Yun L. Study on brain targeting of raltitrexed following intranasal administration in rats. *Cancer Chemother Pharmacol*. 2006;57:97–104.
10. Le S, Zhu JJ, Anthony DC, Greider CW, Black PM. Telomerase activity in human gliomas. *Neurosurgery*. 1998;42:1120–1125.
11. Ozawa T, Gryaznov SM, Hu LJ, et al. Antitumor effects of specific telomerase inhibitor GRN163 in human glioblastoma xenografts. *Neuro-Oncology*. 2004;6:218–226.
12. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. *Proc Natl Acad Sci USA*. 1994;91:2076–2080.
13. Huynh GH, Deen DF, Szoka FC Jr. Barriers to carrier mediated drug and gene delivery to brain tumors. *J Control Release*. 2006;110:236–259.
14. Gryaznov SM, Pongracz K, Matray T, et al. Telomerase inhibitors—oligonucleotide phosphoramidates as potential therapeutic agents. *Nucleosides Nucleotides Nucleic Acids*. 2001;20:401–410.

15. Pongracz K, Gryaznov SM. Alpha-oligodeoxyribonucleotide N3'→P5' phosphoramidates: synthesis and duplex formation. *Nucleic Acids Res.* 1998;26:1099–1106.
16. Gryaznov SM, Letsinger RL. Synthesis and properties of oligonucleotides containing aminodeoxythymidine units. *Nucleic Acids Res.* 1992;20:3403–3409.
17. Ozawa T, Wang J, Hu LJ, Bollen AW, Lamborn KR, Deen DF. Growth of human glioblastomas as xenografts in the brains of athymic rats. *In Vivo.* 2002;16:55–60.
18. Balin BJ, Broadwell RD, Salzman M, el-Kalliny M. Avenues for entry of peripherally administered protein to the central nervous system in mouse, rat, and squirrel monkey. *J Comp Neurol.* 1986;251:260–280.
19. Herbert BS, Pongracz K, Shay JW, Gryaznov SM. Oligonucleotide N3'→P5' phosphoramidates as efficient telomerase inhibitors. *Oncogene.* 2002;21:638–642.
20. Mamot C, Nguyen JB, Pourdehnad M, et al. Extensive distribution of liposomes in rodent brains and brain tumors following convection-enhanced delivery. *J Neurooncol.* 2004;68:1–9.
21. Kawakami K, Kawakami M, Kioi M, Husain SR, Puri RK. Distribution kinetics of targeted cytotoxin in glioma by bolus or convection-enhanced delivery in a murine model. *J Neurosurg.* 2004;101:1004–1011.
22. Groothuis DR. The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery. *Neuro-Oncology* 2000;2:45–59.
23. Asai A, Oshima Y, Yamamoto Y, et al. A novel telomerase template antagonist (GRN163) as a potential anticancer agent. *Cancer Res.* 2003;63:3931–3939.
24. Herbert B, Pitts AE, Baker SI, et al. Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. *Proc Natl Acad Sci USA.* 1999;96:14276–14281.
25. Benedict C, Hallschmid M, Hatke A, et al. Intranasal insulin improves memory in humans. *Psychoneuroendocrinology.* 2004;29:1326–1334.
26. Reger MA, Watson GS, Frey WH 2nd, et al. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. *Neurobiol Aging.* 2006;27:451–458.
27. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci.* 2002;5:514–516.
28. da Fonseca CO, Landeiro JA, Clark SS, Quirico-Santos T, da Costa Carvalho Mda G, Gattass CR. Recent advances in the molecular genetics of malignant gliomas disclose targets for antitumor agent perillyl alcohol. *Surg Neurol.* 2006;65(suppl.):S1:2–1:8; discussion S1:8–1:9.