2013

# A DNA Computer for Glioblastoma Multiforme Diagnosis and Drug Delivery

Sumaiya F. Hashmi
*Claremont McKenna College*

# A DNA Computer for Glioblastoma Multiforme Diagnosis and Drug Delivery

A Thesis Presented

by

Sumaiya Fatima Hashmi

To the Keck Science Department

Of Claremont McKenna, Pitzer, and Scripps Colleges

In partial fulfillment of

The degree of Bachelor of Arts

Senior Thesis in Neuroscience

22 April 2013

TABLE OF CONTENTS

# ABSTRACT

Glioblastoma multiforme (GBM) is a debilitating malignant brain tumor with expected patient survival of less than a year and limited responsiveness to most treatments, often requiring biopsy for diagnosis and invasive surgery for treatment. We propose a DNA computer system, consisting of input, computation, and output components, for diagnosis and treatment. The input component will detect the presence of three GBM biomarkers: vascular endothelial growth factor (VEGF), caveolin-1α (CAV), and B2 receptors. The computation component will include indicator segments for each of these genes, and ensure that output is only released if all the biomarkers are present. The output component will consist of the therapeutic agent interleukin-12 (IL-12).

This study will designate four groups of animals: untreated tumor-free (control), tumor-inoculated (RG2), treated and tumor-free (DNA), and treated and tumor-inoculated (RG2/DNA). In the RG2 and RG2/DNA groups, we will inoculate adult male Fischer rats with RG2 cells into the striatum to induce tumor growth. Rats in the DNA and RG2/DNA groups will be implanted with the DNA system at the same location via recombinant adeno-associated viral vectors. The effectiveness of the DNA system will be evaluated through tumor size measurements, collected from brain slices stained with hematoxylin and eosin, and survival curve. Additionally, IL-12 localization will confirm the release of the output component.

We anticipate that the DNA treatment will result in a decrease in tumor size, leading to smaller tumor size in the RG2/DNA group versus the RG2 group. The control group is expected to survive the longest, followed by the DNA group, then the RG2/DNA group, and

finally the RG2 group. In the DNA group, IL-12 is expected to stay localized to the implantation site, remaining in its unreleased stem-loop form. On the other hand, it is expected to be released and active in the RG2/DNA group.

This study provides a proof of concept to demonstrate the viability and effectiveness of a DNA system using VEGF, CAV, and B2 receptors as biomarkers and IL-12 as a therapeutic output component in the RG2 model. Further research may include varying several of the parameters used in this study, including amount of RG2 administered, choice of biomarkers, quantity and choice of output component, and choice of animal model. This system provides a promising and innovative new approach that is less invasive than surgery yet is still effective in diagnosing, targeting, and treating GBM.

# INTRODUCTION

## Motivation

Glioblastoma multiforme (GBM) is a debilitating malignant brain tumor with an expected patient survival of less than a year and limited responsiveness to most treatments (Holland, 2000). Diagnosis is difficult as well, with a biopsy required for confirmation and grading of the tumor. Pharmacological treatment is complicated by limited permeability of the blood-brain barrier, which only allows small lipid-soluble molecules to pass through (Nathanson and Mischel, 2010). As a result, invasive surgery is often necessary (AANS 2013). A novel approach to diagnosis and treatment of GBM involves DNA computing. DNA computing simply refers to a form of computation that relies on DNA and associated biological material for performing computations rather than the silicon chips of traditional computers. Currently, much of the work with DNA computing is still in the experimental stage. It has been used to solve the traveling salesperson optimization problem and in building pattern-recognizers and simple circuits, and more recently it is being evaluated in disease diagnosis. DNA-based computing has advantages over silicon in that its raw materials are readily available at lowering costs. Additionally, DNA can store much more information per unit of area than can silicon. Further, DNA computation is suited to operate in parallel rather than serial, meaning it can perform many computations at the same time; this greatly increases speed and potential computing power (Hodge, 2009). Finally, DNA computing is more suited for computation in biological environments than is silicon, which, similar to many foreign bodies, has potential for immunogenic response and complications

(Felländer-Tsai, 2008). These factors make DNA computing a promising and exciting approach to disease diagnosis and treatment.

**The DNA computer**

The proposed DNA computer is based on a prototype by Benenson et al., 2001, which was made of DNA nucleotide sequences and enzymes. The computer was combined with input molecules in solution, and release of the output component was verified through PCR and gel electrophoresis. The DNA system has since been demonstrated *in vitro* for diagnosis and treatment of small-cell lung and prostate cancers (Benenson et al., 2004). It consists of input, computation, and output components, all made of DNA sequences, with the latter two combined into one unit (Figure 1). The purpose of the DNA computer is to detect whether specified biomarkers for the disease in question are present, and, if and only if they are all present, to release a therapeutic output component.

The input component detects levels of certain mRNA sequences that correspond to proteins that are typically over- or underexpressed in the disease in question. That is, it detects known biomarkers for a given disease, and to be effective, it requires the disease to have signature biomarkers. The computation component, or diagnostic molecule, is attached to the output component, which is an inactive drug treatment for the disease in question, encoded in a stem-loop shape. The diagnostic molecule contains short DNA sequences to represent each of the biomarkers the system checks for. As each biomarker is found to be present, that section of the diagnostic molecule is cut off, which brings the diagnostic molecule closer to activation of the output drug. Eventually, only the output component remains, which is now active and free to be released and act on its disease target.

For each biomarker to be detected, the input component includes two separate 'software' strands, one of them ('software strand 2') bound to a protector strand (Figure 1A). The protector strand has an affinity for the overexpressed mRNA ('disease-associated mRNA'), and if this biomarker mRNA is present in sufficient quantity, the protector strand will bind to it, freeing software strand 2. The two software strands then become free to bind to each other, creating a 'software molecule' with a sticky end, indicating presence of the first biomarker.

This molecule joins with the Fok1 enzyme to form a 'hardware-software complex.' (Figure 1B). Fok1 is a restriction enzyme which binds double-stranded DNA at a particular recognition site (GGATG), then cleaves the DNA sequence at 9 and 13 nucleotides away from the recognition site. The software molecule described above contains this recognition site, causing Fok1 to bind to it. The sticky end of the software molecule is complementary to a portion of the diagnostic molecule which represents overexpression of the first biomarker ('Gene 1'). The hardware-software complex will then bind the diagnostic molecule, and Fok1 will cleave the portion of the diagnostic molecule indicating overexpression of the first biomarker (Figure 1C).

For each gene to be detected, there is another portion of the diagnostic molecule, and a different set of software strands. If all the genes to be tested are overexpressed, parts of the diagnostic molecule will be progressively cleaved until only the output component remains. The output component, a stem-loop drug that is inactive until the rest of the diagnostic molecule has been cleaved (Figure 1C), will then be free to be released and act on its target (Shapiro and Benenson, 2006). This system has not yet been tested *in vivo* nor in brain

tumors, so this proposal is intended as a proof of concept emphasizing the potential viability of such a system *in vivo* in the brain.

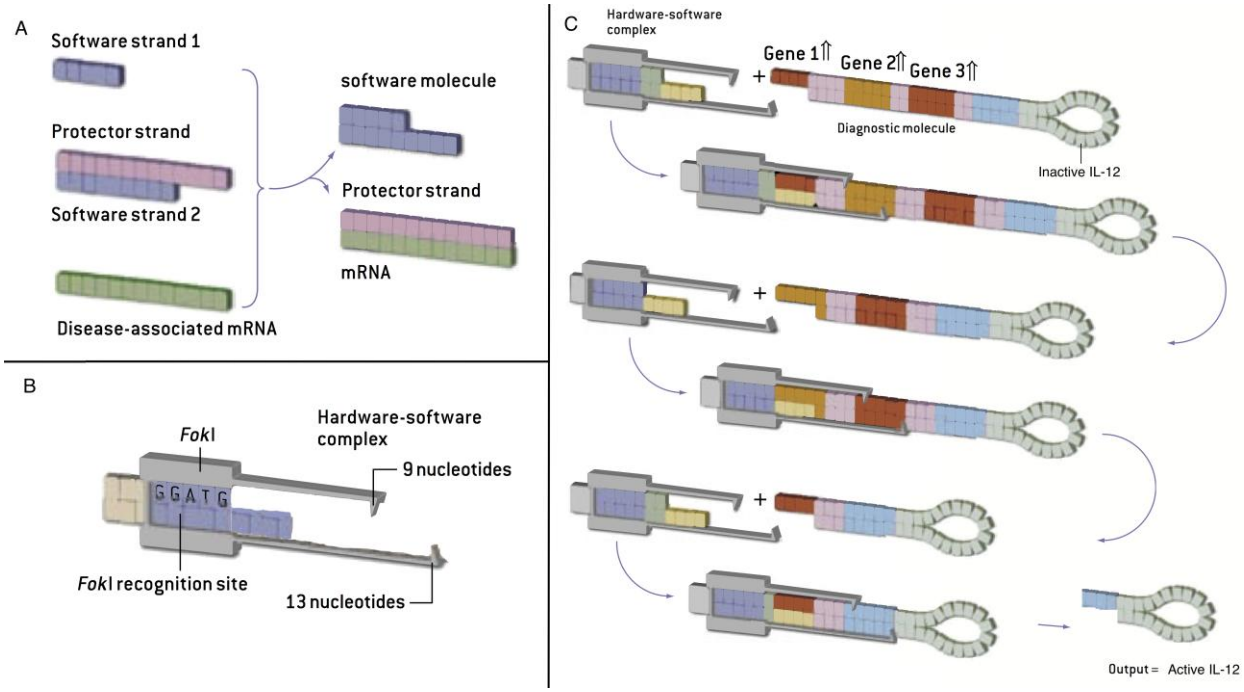


***Figure 1.*** *Schematic of DNA computer.* ***(A)*** *The input component includes two separated software strands, one of which is bound to a protector strand. The protector strand has a high affinity for the biomarker mRNA and binds it, allowing the two software strands to bind to one another.* ***(B)*** *The active software molecule contains a binding site for Fok1 enzyme. Fok1 binds at that site, forming a hardware-software complex, and is able to cleave the DNA at 9 and 13 nucleotides from that site.* ***(C)*** *The sticky end of the software molecule is complementary to the indicator for Gene 1 in the diagnostic molecule. It binds at this location and Fok1 cleaves the indicator, exposing the next portion of the diagnostic molecule. A different software-hardware complex forms, corresponding to Gene 2. The process continues until the output component, a drug coded in a stem-loop, is released. This model was initially described in Benenson et al., 2001, and this figure and description are adapted from Shapiro and Benenson, 2006.*

## GBM models

Several glioma models exist in both mice and rats. We propose using the RG2 Fischer rat model, which was created at the Koestner laboratory at The Ohio State University in 1971, for several reasons. The model has a growth pattern similar to that of human GBM in

that it spreads invasively throughout the brain and is not encapsulated in any one area (Barth et al., 2009). Additionally, if we were to use a tumor implant from a different species, there would be greater potential for an immunogenic response. Since we are not using xenografts, immunocompromised rodents are not required. The RG2 model is non-immunogenic, compared to the weakly immunogenic F98 model and strongly immunogenic C6 and 9L models (Adam et al., 2006). As compared to mouse models, RG2 provides a larger area for stereotaxic implants and evaluation of tumor progression via MRI and histology (Jacobs et al., 2011). Thus, it is an ideal model for administrating and evaluating our proposed treatment.

The RG2 cell line was established in Fischer-344 rats using ethylnitrosourea, a potent mutagen (Matsukado et al., 1998). It is commercially available and can be stereotaxically injected into the striatum in various quantities. Introduction of 500 RG2 cells has shown minimal tumor growth, which is ineffective for evaluating treatment efficacy. Inoculation with 1000 cells results in a mean survival of no more than 1 month, limiting the amount of data that can be collected (Aas et al., 1995). Therefore, based on prior experiments and personal correspondence with authors, we propose inoculation with 800 RG2 cells (Ceberg, Foray).

**DNA system components**

The input component of the DNA system will detect mRNA levels of proteins that are typically overexpressed in our GBM model. These include vascular endothelial growth factor (VEGF), caveolin-1α (CAV), and B2 receptors (Suzuki et al., 2004, Beaumont et al., 2009, Sibenaller et al., 2005, Valable et al., 2008). VEGF is a signal protein that stimulates

generation of blood vessels and is often overexpressed in tumors. CAV is a gene for membrane proteins called caveolae, and has been found to be up-regulated in several tumors and cancer cell lines (Shatz and Liscovitch, 2008). B2 receptors are G-protein coupled receptors for the peptide bradykinin, and have been implicated in tumor growth (Ikeda et al., 2004). The input component will include protector strands with strong binding affinity to the mRNA associated with the genes for these proteins.

The computation component will include a segment for each of these three genes, and will end in a stem-loop output component. The output drug will be interleukin-12, a strong anti-tumor agent shown to be a useful treatment for RG2 (Roche et al., 2010). IL-12 stimulates immunogenicity and inhibits tumor angiogenesis, and has been effective in many tumor models (Xia et al., 2013), though it has had inconclusive results on tumors in humans, and clinical trials are still in progress.

Administration of the combined computation and output components will be mediated by the vector recombinant adeno-associated virus type 2 (rAAV2), as described previously (Chiu et al., 2012). rAAV2 is a vector for gene delivery that has been used in studies involving a variety of diseases (Coura and Nardi, 2008). It is capable of targeting neurons, is nonpathogenic, and has very effective purification methods (Burger et al., 2005), making it an ideal vector for our purposes.

**Aims**

Our aims are threefold: (1) determine if the components of this DNA system can be successfully introduced *in vivo* via vectors, (2) evaluate the effect of this system on glioblastoma size as seen in the RG2 model, and (3) evaluate the effect of the system on

survival curve of RG2 rats.

# METHODS

## Animal model

Adult male Fischer 344 rats inoculated with 800 RG2 cells will be used as the animal model. Forty-two animals will be divided into 4 groups: untreated, tumor-free controls ("Control", n=10**),** untreated RG2-inoculated controls ("RG2", n=12), tumor-free DNA-treated controls ("DNA", n=10), and DNA-treated RG2-inoculated experimental group ("RG2/DNA", n=10), where treatment consists of being administered the DNA system. This will allow us to control for any adverse effects the DNA system implantation may have caused, by comparing DNA animals against Control animals. We will also be able to ensure that the output module is only released when tumor indicators are present, by comparing DNA animals against RG2/DNA animals.

Two animals from the inoculated untreated group will be sacrificed 14 days post-inoculation and their brains analyzed histologically, as described below, to confirm tumor growth. Additionally, 5 animals from each group will be sacrificed at a time point 14 days post DNA system implantation (21 days post tumor inoculation) and their tumor sizes will be measured. The remaining animals in each group will contribute to the survival curve data and will be analyzed histologically post mortem.

## RG2 inoculation

Pentobarbital at a dose of 45 mg/kg administered intraperitoneally is expected to provide anesthesia for 1 to 2 h. An intracerebroventricular injection of 800 RG2 cells will be administered to the striatum using the following stereotaxic coordinates: Bregma -0.5 mm,

midline ±3.0 mm, depth -4.6 mm below dura (Greenwood et al., 2012). Past studies have successfully administered RG2 cells to various brain regions including the striatum, which we have selected for ease of stereotaxic tumor-cell delivery. The tumor will be unilateral, with half the animals receiving it on the right side and half on the left. Control and DNA group animals will receive sham saline injections to control for effect of injection. The area will be sealed with bone wax and the incision sutured. Animals will be monitored until full recovery from anesthesia and given buprenorphine at a dose of 0.05 mg/kg for pain. Animals will be given 7 days of recovery time post inoculation before DNA system implantation, which has been previously shown to be sufficient for tumor uptake prior to treatment (Matsukado et al., 1998). Body weight will be monitored throughout the study to ensure that animals remain healthy.

**DNA system implantation**

The DNA system will be implanted using rAAV2, with separate vectors for each of the three input components as well as for the computation-output module. This vector was selected based on the size of the DNA components to be implanted. The computation-output module is about 13 Kbp (Roche et al., 2010) and the three input components are 30, 30, and 10 Kbp (NCBI Gene), making it impossible to include all the components in a single vector. The vectors will be injected stereotaxically at the tumor inoculation coordinates indicated above, following a similar protocol, with sham injections for the Control and RG2 groups.

**Tumor size measurement**

Tumors may be visualized using the contrast agent gadoteridol and MRI (Behrens et

al., 2000), but our emphasis will be on histological evaluation. Following sacrifice of selected animals, as described in the Animal Model section, coronal brain slices will be cryostatically sectioned and stained with hematoxylin and eosin (H&E), a staining agent consisting of hematoxylin, which dyes nuclei blue, and eosin, which provides a red counterstain. This will be done as described previously (Aas et al., 1995). Tumor size will be calculated using the brain slice from each animal with the largest tumor representation. The largest and smallest diameters across the tumor will be measured (both will be the same if the tumor is a circle) and then a surface size estimated with the following equation:

$$1/6\ \pi\ s\ L$$

where s and L are the small and large diameters, respectively (Adam et al., 2005).

**Interleukin-12 localization**

Several coronal brain slices obtained from each animal as described above will be stained for IL-12 as previously described (Timoshanko et al., 2011). Briefly, we will incubate the sections with anti-IL-12 p40 monoclonal antibody and Alexa Fluor 594 dye. Slices will be analyzed under confocal microscopy to visually determine IL-12 localization relative to tumor localization. See Figure 2 for an example of IL-12 staining. This will show whether or not the IL-12 was released from the DNA system, and is a way to check if the input and computation components are working correctly.

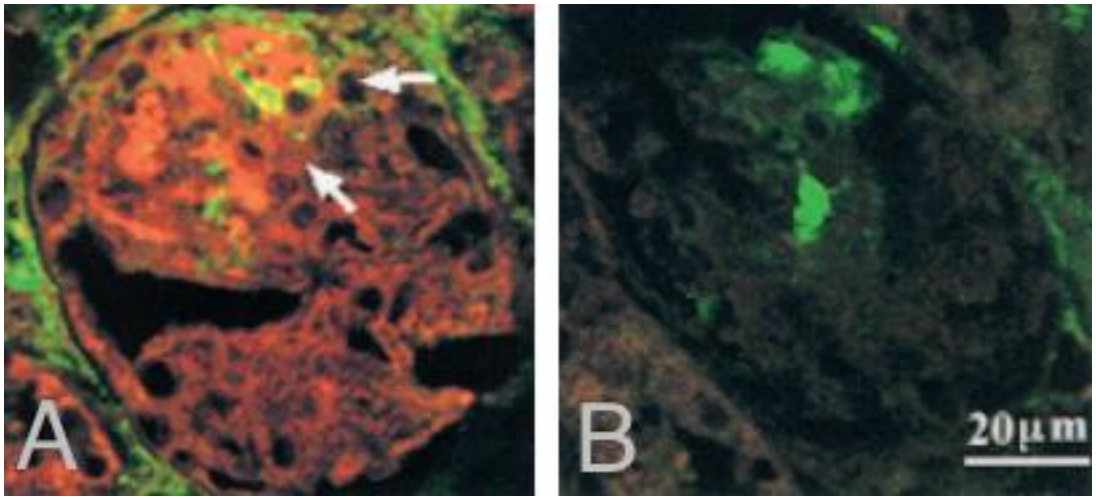


***Figure 2.*** *A demonstration of staining for Interleukin-12 under confocal microscope in mouse glomeruli.* ***(A)*** *IL-12 expression (red) is visible in WT mice that have been administered IL-12.* ***(B)*** *No IL-12 expression is visible in IL-12-deficient mice. Modified from Timoshanko et al., 2001.*

**Survival curve**

The survival curve will be based on how many animals from each group survive at each day post tumor inoculation. Survival numbers will be measured daily for each of the groups at 1100 hrs.

# RESULTS

We expect tumor visualization using H&E stain to demonstrate tumor growth in the inoculated groups (RG2 and RG2/DNA) due to uptake and growth of the RG2 tumor cells (Figure 3). Tumor size is expected to be greater in the RG2 group than in the RG2/DNA group (Figure 4), because the treatment with the DNA system is expected to be effective. We anticipate that the Control group will have a survival curve similar to that of the DNA group, because expression of the DNA system in itself is not expected to affect survival. These two groups are expected to survive longer than the other two groups, with lowest survival for the RG2 group (Figure 5), because we expect tumor inoculation to have a detrimental effect on survival, but we expect the treatment to mitigate these effects to some degree. Localization of IL-12 is expected to be different in the two groups that were administered treatment with the DNA system. In the DNA group, we anticipate that the DNA system's output component will not be released, since the biomarkers that cause the DNA system to release IL-12 are not expected to be present. Thus, IL-12 is expected to be located at the same stereotactic coordinates where it was implanted, whereas it will be released in the RG2/DNA group. Due to the presence of biomarkers in the RG2/DNA group causing the DNA system's computation component to result in a release of IL-12, we expect that the expression pattern of IL-12 will be widespread in these animals, throughout the extent of the tumor. This is expected to have the effect of reducing tumor size and prolonging survival. These results will be verified qualitatively by staining for IL-12.

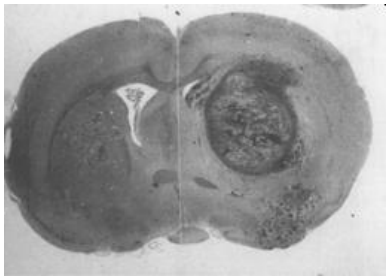

***Figure 3**. A tumor visible in the right caudate nucleus using H&E staining, following stereotaxic inoculation of RG2 cells into the head of the right caudate nucleus. (Aas et al., 1995).*

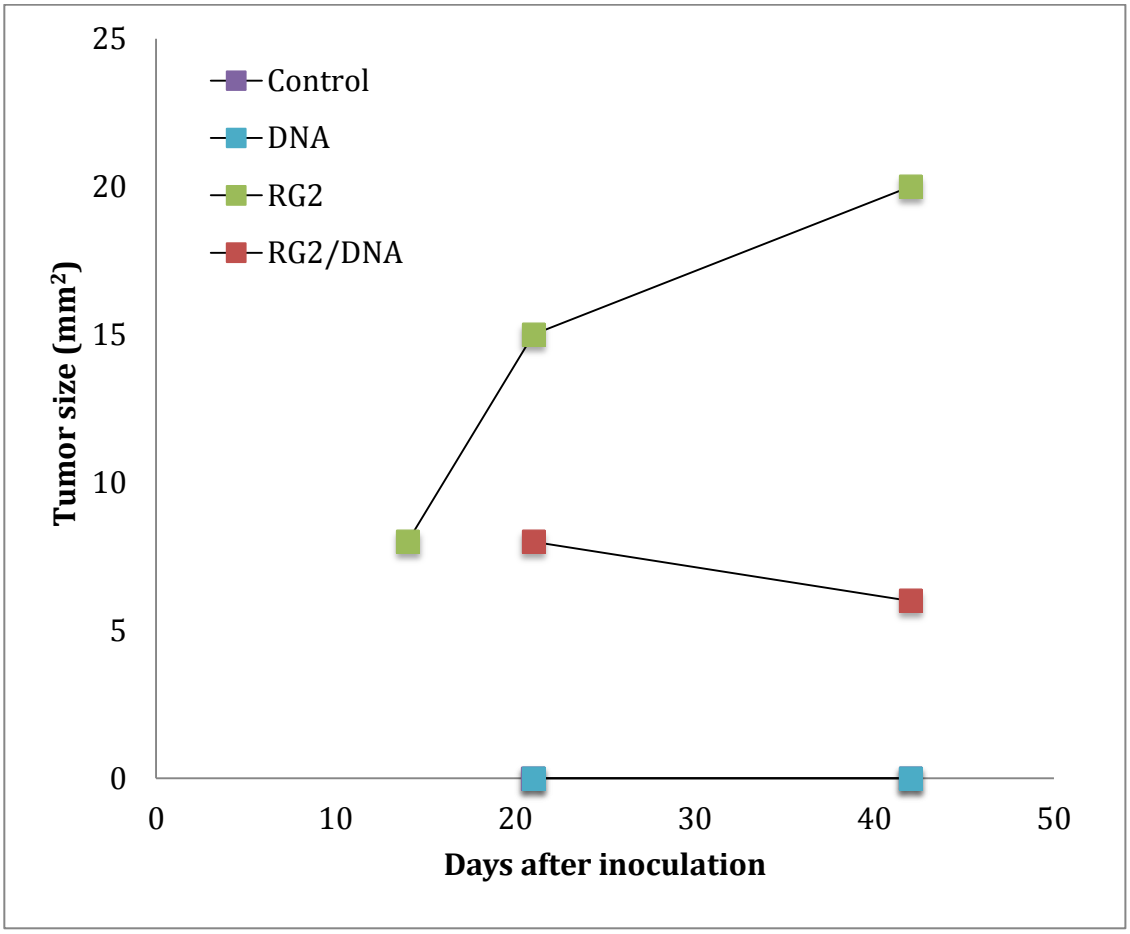


***Figure 4.** Expected tumor size after inoculation for the four groups: no tumor, no treatment (Control); no tumor, treatment (DNA); tumor, no treatment (RG2); tumor, treatment (RG2/DNA). n=2 at the 14 day timepoint, n=5 at 21 days, and n=5 at endpoint. The Control data points are hidden behind the DNA data points. Data is based on similar work by Aas et al., 1995.*

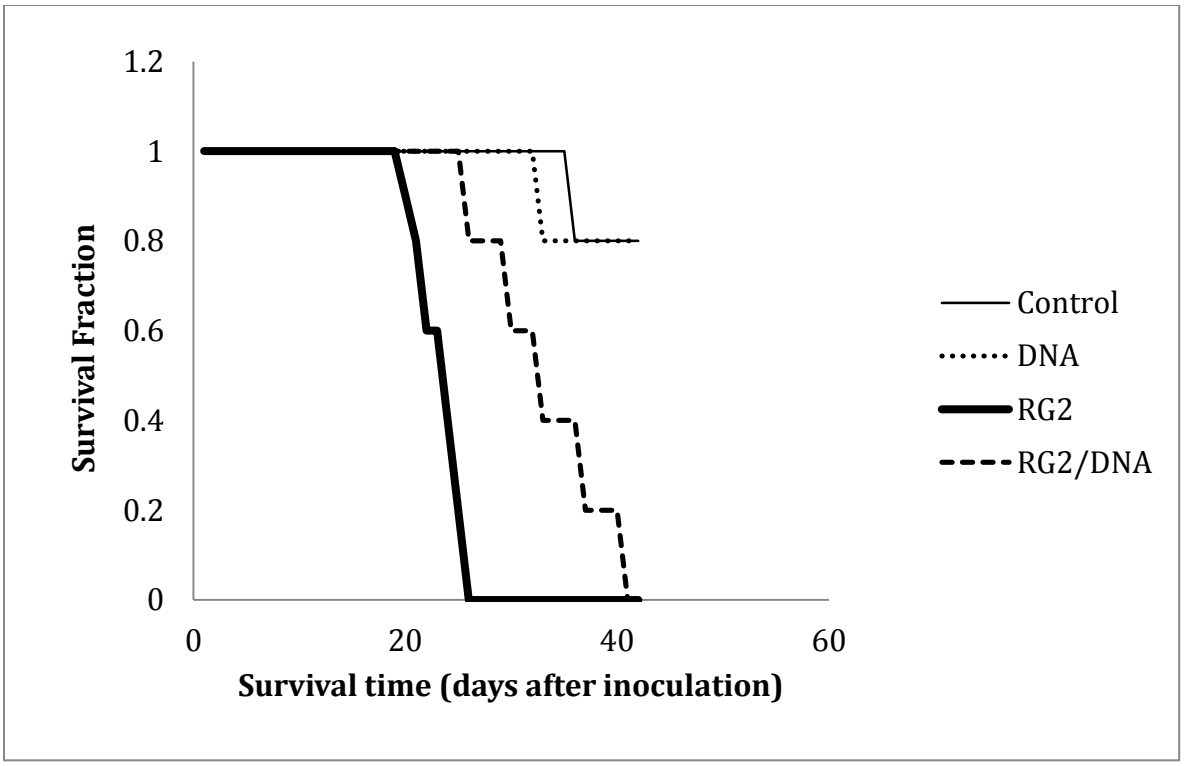


***Figure 5.*** *Expected survival curve, based on similar work by Ceberg et al., 2012, measured as the fraction of animals surviving at each day after inoculation from each of four groups: no tumor, no treatment (Control); no tumor, treatment (DNA); tumor, no treatment (RG2); tumor, treatment (RG2/DNA).*

# DISCUSSION

This system presents a novel treatment for a condition that has traditionally been very difficult to treat. Due to limited permeability of the blood-brain barrier, many purely pharmacological approaches face challenges in targeting gliomas, resulting in limited drug delivery and minimal therapeutic effect (Agarwal et al., 2013). As a result, highly invasive surgical intervention is commonly the only effective treatment option. Additionally, diagnosis often depends on biopsies, which are also invasive. This system provides an approach that is less invasive than surgery yet could still be effective in diagnosing, targeting, and treating GBM.

The system is potentially very powerful in both its ability to diagnose specific areas of the brain and to strategically release the treatment component when needed. This study demonstrates the effectiveness of IL-12 as a treatment for the RG2 GBM model, but is somewhat limited by its specificity; it is difficult to extrapolate this treatment to other models without additional investigation. Extrapolation would require demonstrating the effectiveness of this approach on other GBM models in addition to GBM as induced by RG2. Additionally, IL-12 has had mixed results in humans. One phase I clinical trial of IL-12 injections found a reduction in tumor size in about half the cases (Kang et al., 2001). However, another found limited responsiveness and severe toxicities and side effects (Atkins et al., 1997). Given IL-12's inconclusive effectiveness in treating human GBM, alternative therapeutic agents should also be considered when applying this system to other models or species.

Further research may include varying several of the parameters used in this study. If initial tumor histology does not demonstrate sufficient growth for experimentation purposes,

larger quantities of RG2 may be administered. Alternatively, if the survival curve does not allow adequate time for evaluating treatment, due to animals dying off too quickly, animals could be inoculated with fewer RG2 cells. Provided the tumor growth and survival curves of RG2-inoculated animals are satisfactory, we can evaluate effects of the DNA system. If the size of the system causes damage to brain tissue, due to the large volumes of viral vector required to deliver the system's components, we may consider finding input and output components that are fewer base pairs in length, along with a smaller viral vector.

Assuming the DNA system implantation proceeds without complication, we can examine its components. If interleukin-12 localization in the tumor-inoculated animals demonstrates that IL-12 was not released, the DNA system's input components should be reconsidered. Lack of IL-12 release could suggest that the mRNA sequences we predicted to be overexpressed were not present at high levels, or were not in close proximity to the DNA system. Other combinations of biomarkers for this disease may be used, as well as different injection sites for the DNA system. Alternative biomarkers may be determined by examining the gene expression profile of RG2. If IL-12 was released from the DNA system in the tumor-free animals, it is possible that the DNA system's input and computation components did not work as expected, causing inappropriate release of the output component. In this case, the components' proper functioning should first be verified in a controlled *in vitro* setting, and depending on those results, alternate biomarkers may need to be used.

Assuming IL-12 release proceeded as expected, with it being released in only the tumor-inoculated animals implanted with the DNA system, we can consider its effectiveness in treating tumor size and survival. If tumor size in IL-12 treated animals did not decrease,

we might experiment with greater doses of IL-12, bearing in mind space constraints for total injection volume. If this is not effective, alternate anti-tumor agents may be used.

If all the results are as expected, the next step is to apply this approach to different GBM models to ascertain its generalizability prior to any clinical trials. This study is meant as a proof of concept to demonstrate the viability and effectiveness of a DNA system using VEGF, CAV, and B2 receptors as biomarkers and IL-12 as a therapeutic output component in the RG2 model of GBM.

**Potential impact**

Within the domain of GBM, this study has a large potential impact. Following successful studies with varying doses, models, and input components, it has many clinical applications. The system could be implanted in patients predisposed to or suspected of having GBM. Traditionally, diagnosis via imaging is inconclusive and only confirmed through biopsy. This system provides a less invasive alternative which is only used when needed. The anti-tumor drug would only be released when all the diagnostic conditions are met, meaning all the relevant biomarkers of the disease are found to be present. Similar approaches using the DNA system could be applied to other tumor types as well. This methodology provides a promising and innovative new approach to diagnosis and treatment of glioblastoma multiforme.

BIBLIOGRAPHY


1. AANS (American Association of Neurological Surgeons) Conditions and Treatments. "Glioblastoma Multiforme." 10 Mar. 2013.
2. Aas, A T et al. "The RG2 Rat Glioma Model." *Journal of neuro-oncology*23.3 (1995): 175–183. Print.
3. Adam, Jean-François et al. "Prolonged Survival of Fischer Rats Bearing F98 Glioma After Iodine-enhanced Synchrotron Stereotactic Radiotherapy." *International Journal of Radiation Oncology*Biology*Physics* 64.2 (2006): 603–611. Web. 18 Mar. 2013.
4. Agarwal, Sagar et al. "Function of the blood-brain barrier and restriction of drug delivery to invasive glioma cells: findings in an orthotopic rat xenograft model of glioma." *Drug metabolism and disposition: the biological fate of chemicals* 41.1 (2013): 33–39.
5. Atkins, M B et al. "Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies." *Clinical cancer research: an official journal of the American Association for Cancer Research* 3.3 (1997): 409–417. Print.
6. Barth, R. F., and B. Kaur. "Rat Brain Tumor Models in Experimental Neuro-oncology: The C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 Gliomas." *Journal of neuro-oncology* 94.3 (2009): 299–312. Web. 1 Apr. 2013.
7. Beaumont, Marine et al. "Characterization of Tumor Angiogenesis in Rat Brain Using Iron-based Vessel Size Index MRI in Combination with Gadolinium-based Dynamic Contrast-enhanced MRI." *Journal of Cerebral Blood Flow & Metabolism* 29.10 (2009): 1714–1726. Web. 18 Mar. 2013.

8. Behrens, P F et al. “Extracellular Glutamate and Other Metabolites in and Around RG2 Rat Glioma: An Intracerebral Microdialysis Study.”*Journal of neuro-oncology* 47.1 (2000): 11–22. Print.
9. Benenson, Yaakov et al. “Programmable and autonomous computing machine made of biomolecules.” *Nature* 414.6862 (2001): 430–434.
10. Benenson, Yaakov et al. “An autonomous molecular computer for logical control of gene expression.” *Nature* 429.6990 (2004): 423–429.
11. Biston, Marie-Claude et al. “Cure of Fisher Rats Bearing Radioresistant F98 Glioma Treated with cis-Platinum and Irradiated with Monochromatic Synchrotron X-Rays.” *Cancer Research* 64.7 (2004): 2317–2323. Web. 22 Feb. 2013.
12. Burger, Corinna, Kevin Nash, and Ronald J Mandel. “Recombinant adeno-associated viral vectors in the nervous system.” *Human gene therapy*16.7 (2005): 781–791.
13. Ceberg, Crister. Question About RG2 Model. 14 Mar. 2013. E-mail.
14. Ceberg, Crister et al. “Photon activation therapy of RG2 glioma carrying Fischer rats using stable thallium and monochromatic synchrotron radiation.” *Physics in medicine and biology* 57.24 (2012): 8377–8391.
15. Coura, Renata dos Santos, and Nance Beyer Nardi. “A Role for Adeno-associated Viral Vectors in Gene Therapy.” *Genetics and Molecular Biology* 31.1 (2008): 1–11. Web. 3 Apr. 2013.
16. Von Eckardstein, K L et al. “Short-term Neuropathological Aspects of in Vivo Suicide Gene Transfer to the F98 Rat Glioblastoma Using Liposomal and Viral Vectors.” *Histology and histopathology* 16.3 (2001): 735–744. Print.

17. Felländer-Tsai, L, F P Reinholt, and I Turan. “Complications with infection and foreign body reaction after silicon implant arthroplasty in the second metatarsophalangeal joint in an adolescent: a case report.” *The Journal of foot and ankle surgery: official publication of the American College of Foot and Ankle Surgeons* 36.6 (1997): 452–456. Print.
18. Foray, Nicolas. Question About RG2 Model. 3 Mar. 2013. E-mail.
19. Greenwood, Benjamin N. et al. “5-HT2C Receptors in the Basolateral Amygdala and Dorsal Striatum Are a Novel Target for the Anxiolytic and Antidepressant Effects of Exercise.” *PLoS ONE* 7.9 (2012): e46118. Web. 18 Mar. 2013.
20. Hodge, Russ. “DNA Computers.” *The Molecules of Life: DNA, RNA, and Proteins*. 2009. 72–74. Print.
21. Holland, Eric C. “Glioblastoma Multiforme: The Terminator.” *Proceedings of the National Academy of Sciences of the United States of America* 97.12 (2000): 6242–6244. Web. 18 Mar. 2013.
22. Ikeda, Yasuhiro et al. “Host stromal bradykinin B2 receptor signaling facilitates tumor-associated angiogenesis and tumor growth.” *Cancer research*64.15 (2004): 5178–5185.
23. Jacobs, Valerie L, Pablo A Valdes, et al. “Current Review of in Vivo GBM Rodent Models: Emphasis on the CNS-1 Tumour Model.” *ASN NEURO*3.3 (2011): 171–181. Web. 18 Mar. 2013.
24. Jacobs, Valerie L., Russell P. Landry, et al. “Propentofylline Decreases Tumor Growth in a Rodent Model of Glioblastoma Multiforme by a Direct Mechanism on Microglia.” *Neuro-Oncology* 14.2 (2012): 119–131. Web. 22 Feb. 2013.

25. Kang, W K et al. “Interleukin 12 gene therapy of cancer by peritumoral injection of transduced autologous fibroblasts: outcome of a phase I study.” *Human gene therapy* 12.6 (2001): 671–684.

26. Matsukado, Koichiro, Masao Sugita, and Keith L Black. “Intracarotid Low Dose Bradykinin Infusion Selectively Increases Tumor Permeability Through Activation of Bradykinin B2 Receptors in Malignant Gliomas.” *Brain Research* 792.1 (1998): 10–15. Web. 22 Feb. 2013.

27. Møller, Heidi G. et al. “A Systematic Review of MicroRNA in Glioblastoma Multiforme: Micro-modulators in the Mesenchymal Mode of Migration and Invasion.” *Molecular Neurobiology* 47.1 (2013): 131–144. Web. 22 Feb. 2013.

28. Nathanson, David, and Paul S. Mischel. “Charting the Course Across the Blood-brain Barrier.” *Journal of Clinical Investigation* 121.1 (2011): 31–33. Web. 1 Apr. 2013.

29. NCBI. “Gene.” 12 Mar. 2013.

30. Roche, F P et al. “Semliki Forest Virus-mediated Gene Therapy of the RG2 Rat Glioma.” *Neuropathology and applied neurobiology* 36.7 (2010): 648–660.

31. Rodriguez-Madoz, Juan R., Jesus Prieto, and Cristian Smerdou. “Semliki Forest Virus Vectors Engineered to Express Higher IL-12 Levels Induce Efficient Elimination of Murine Colon Adenocarcinomas.”*Molecular Therapy* 12.1 (2005): 153–163. Web. 25 Feb. 2013.

32. Shapiro, Ehud and Benenson, Yaakov. “Bringing DNA Computers to Life.” *Scientific American* 2006: 45–51.

33. Shatz, Maria, and Mordechai Liscovitch. “Caveolin-1: a tumor-promoting role in human cancer.” *International journal of radiation biology* 84.3 (2008): 177–189.

34. Sibenaller, Zita A et al. “Genetic Characterization of Commonly Used Glioma Cell Lines in the Rat Animal Model System.” *Neurosurgical focus* 19.4 (2005): E1. Print.
35. Suzuki, Toyofumi et al. “Imaging Endogenous Gene Expression in Brain Cancer In Vivo with 111In-Peptide Nucleic Acid Antisense Radiopharmaceuticals and Brain Drug-Targeting Technology.” *Journal of Nuclear Medicine* 45.10 (2004): 1766–1775. Web. 18 Mar. 2013.
36. Timoshanko, Jennifer R. et al. “Interleukin-12 from Intrinsic Cells Is an Effector of Renal Injury in Crescentic Glomerulonephritis.” *Journal of the American Society of Nephrology* 12.3 (2001): 464–471. Web. 15 Mar. 2013.
37. Valable, Samuel et al. “Assessment of blood volume, vessel size, and the expression of angiogenic factors in two rat glioma models: a longitudinal in vivo and ex vivo study.” *NMR in biomedicine* 21.10 (2008): 1043–1056.
38. Xia, Xiangwen et al. “Intra-arterial interleukin-12 gene delivery combined with chemoembolization: anti-tumor effect in a rabbit hepatocellular carcinoma (HCC) model.” *Acta radiologica (Stockholm, Sweden: 1987)* (2013): n. pag.