The Effect of Masitinib on Pediatric Glioblastoma

Abstract

Tumors of the Central Nervous System are the most prevalent pediatric solid tumor with gliomas being the most common and most aggressive form. The current standard of care involves a combination therapy with chemoradiation and gross tumor resection. The five-year survival rate of pediatric brain tumors is upwards of 70%, however, the combination of chemotherapy and radiation has proved to be harmful, especially to children, and in many cases significantly reduces their overall quality of life physically, socially and cognitively. As a result, finding ways to increase the efficacy of chemotherapy and/or radiation without increasing dosing is paramount in order to increase the life quality of survivors. Using a mouse model, this study investigated masitinib, a tyrosine kinase inhibitor, as a single agent and in combination with temozolomide, the current standard of care DNA alkylating agent, in the treatment of pediatric glioblastoma. This involved injection of patient derived pediatric glioblastoma xenografts into athymic nude mice and subsequent treatment of the mice with masitinib and/or temozolomide. Any possible chemosensitizing effects masitinib may have in a pediatric brain tumor xenograft model were also evaluated. The rationale for this combination is based on masitinib's ability to effectively cross through the blood brain barrier and inhibit cell division in other types of cancer. While results indicated that masitinib does not work well as a single agent, it may in fact have acted as a chemosensitizer towards temozolomide, providing increased tumor growth delay relative to either drug used as a monotherapy. This finding warrants further investigation of masitinib in combination with temozolomide against pediatric glioblastoma

Introduction

Tumors of the Central Nervous System are the most common solid tumors of childhood, affecting around 1500 patients in the United States per year (Albright, 1993). Of these tumors, gliomas are the most common and aggressive malignant primary brain tumor in children and account for 56% of all solid tumor cases (Albright, 1993). A major challenge for treating any type of brain tumor is getting therapeutic chemical agents through the blood brain barrier (BBB) (King, 2011). This barrier, which is the body's way of protecting the brain from harmful and unwanted chemicals, creates a major treatment dilemma for those who develop drugs against brain tumors.

One way scientists have engineered drugs to cross the BBB is by increasing the lipid solubility of the drug so it can more easily pass through the endothelial cells in the brain (King, 2011). However, this is no simple task or without risks, as this increases the ability of compounds to pass through every cell in the body, potentially causing harmful or increased side effects (King, 2011). Another, more direct way to break through the barrier is to hone in on sites specific to cancer cells, such as tumor necrosis factor (TNF). Being tumor specific, the agent will then only attack the cells that make up the tumor, leaving other healthy parts of the brain, and body, unharmed. For example, recent studies demonstrated that injecting TNF into the bloodstream allows trastuzumab (a breast cancer drug) to cross the BBB and reach cancer cells in the brain (Connell et al, 2013).

Even with increasing positive results in crossing the BBB, treatment of pediatric brain tumors lags behind that of adult brain tumors as a result of the initial idea that the two are both biologically and genetically similar (Fangusaro, 2012). Recent research, however, does not support this original hypothesis. For example, the amplification of an Epidermal Growth Factor

Receptor (EGFR) is much more prevalent in pediatric brain tumors than those found in adults (Pollack, 2011). Pediatric tumors also have infrequent deletion of the phosphatase and tensin homolog (PTEN), whose deletion is a prominent characteristic in adult high-grade gliomas (Pollack, 2011). As a result, development of medicinal therapies and practices targeting pediatric tumor specific mutations/abnormalities are necessary for proper treatment.

A current clinical strategy has focused on breaking down pediatric tumor types into subgroups and treating these subgroups as different tumors/diseases. Thompson et al. did so in 2006 with medulloblastomas, subdividing the cancer into five groups based on specific molecular patterns, including alterations in gene signaling pathways, the presence of different mutations, and even the clinical features of the group itself (Pollack, 2011). By breaking down this disease into subcategories, specific gene pathways or mutations can be targeted to the patient's particular medulloblastoma tumor type. Doing so brings the idea of personalized medicine to the world of brain cancer. Treating each tumor as an individual and not a generalized group, would potentially increase the chances of objective, measurable, tumor responses in both adults and children, and avoid costly and unnecessary treatments. Such treatments include harmful brain radiation that has become a standard for most effective treatments, usually resulting in long-term brain damage, which negatively effects pediatric brain cancer survivors in various physical, cognitive, social and emotional ways throughout their lifetime (Lannering et al, 1990).

Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumor in humans, accounting for 17% of all primary brain tumor cases (American Brain Tumor Association). The current standard of care treatment for GBM involves a combination of gross tumor resection, radiation and chemotherapy (Minniti, 2009). As a result of its ability to cross the

BBB, temozolomide (TMZ), a DNA alkylating agent, has become the gold standard for chemotherapeutic treatment of GBM (Gerstner and Fine, 2007). TMZ was introduced in the 1990's for use as a standard of care chemotherapy treatment of many brain cancers including GBM (Mrugala et al, 2010). This prodrug is initially administered to the body in an inactive form and becomes converted to its active form (5-(3-methyltriazen-l-yl)imidazole-4-carboxamide) spontaneously at the bodies physiologic pH of 7.4 (Barone et al, 2006). Due to its stability at gastric pH and nearly 100% oral bioavailability/rapid absorption, it works well as an oral agent (Barone et al, 2006).

As described by Zhang et al, (2012) once activated, TMZ acts by delivering a methyl group to the purine bases of DNA (O6-guanine; N7-guanine and N3-adenine). DNA methylation then changes the behavior of the cancer cells by blocking various chemical signaling pathways resulting in defects in genes associated with cell growth and survival. However, one major drawback Zhang and his colleagues found to the use of TMZ is the presence of Methylguanine Methyltransferase (MGMT). MGMT is a protein that if present in the tumor cell can remove the methyl group placed on guanine by TMZ rendering the drug ineffective. In addition it was found that mismatch repair deficient tumors (MMR-) can tolerate a guanine methylation, creating a major roadblock to effective TMZ treatment (Zhang et al, 2012).

Barone et al (2006) found that even with the promising features of TMZ, adult phase II trials did not show good results with only 8% of GBM patients showing an objective response and 53% a temporary stable disease. TMZ did show better results with recurrent or progressive low-grade gliomas and anaplastic astrocytoma, giving a 61% and 35% objective response rate respectively, proving the drug crosses the BBB. Barone et al (2006) also found that phase I clinical trials in children showed a maximum tolerated dose of 180 mg/m^2 in patients with

craniospinal irridation (CSI) and 215 mg/m^2 without. Phase II clinical trials focusing on high-grade gliomas in children showed a response rate even lower than that found in adults, with an objective response rate of 6% in one study.

These results demonstrate that even though TMZ is one of the primary chemotherapeutic agents used to fight pediatric brain tumors, it is not a good candidate for monotherapy. Nevertheless, the fact that the addition of TMZ to radiation therapy increases the median survival rate of patients from 12 to 15 months, with the two-year survival rate increasing from 10% to 26%, TMZ is a good candidate for combination therapy (TMZ + radiation) (Furnari et al, 2007).

Those children who do sustain an objective response after undergoing combination therapy although alive, can still have a reduced quality of life due to the radiation treatments placed on their developing brains. These long-term or late effects can include reduced function of various cognitive, motor, visual and hormonal pathways (Lannering et al, 1990). The effects experienced are correlated with the location of the tumor, and therefore the location of tumor radiation, which effectively kills all of cells in that area, causing a decrease in life quality to ensue up to several years after treatment (Butler et. al, 2006).

Another possible treatment strategy would be to use masitinib, a novel tyrosine kinase inhibitor (TKI) that targets stem cell factors c-Kit, Platelet Derived Growth Factor Receptors (PDGFRs) and Lyn (Humbert et al, 2010). Masitinib, however, has yet to be evaluated for its use against pediatric GBM. As described by Fraley et al (2001), tyrosine kinases are enzymes that can transfer a phosphate group from ATP to the tyrosine amino acid of a protein in a cell. This transfer causes an intracellular cascade that is critical for cell division and growth. Mutated kinases essentially become stuck in the "on" position, causing uncontrolled cell proliferation and resulting in cancer.

In vitro, masitinib has been shown to have greater activity and selectivity against c-Kit than imatinib (Gleevec), an FDA approved TKI (Published Library of Science, 2009). As a result, masitinib was recommended for in vivo trials and shown to inhibit tumor growth in c-Kit expressing mice by inhibiting mast cell degranulation, cytokine production and migration (Published Library of Science, 2009). These effects, however, were minimal and not enough to promote the use of masitinib as a monotherapy. Nevertheless, because it had some effect and is therefore known to cross the BBB, it is very possible that masitinib will work as a chemosensitizer. One study conducted on a patient with pancreatic cancer saw no positive effects with masitinib as a monotherapy but saw a synergistic effect when combined with gemcitabine (Mitry et. al, 2010). In addition, canine studies have shown masitinib to have chemosensitizing effects with vinblastine and doxorubicin (Thamm et. al, 2012).

Overall, it is widely accepted within the clinical setting that the use of TMZ is not efficient by itself, and any real current success requires the use of radiation, which can induce an array of harmful side effects. In this study I tested the efficacy of TMZ against GBM, in a mouse model, by using it in conjunction with masitinib. While neither drug has been shown to work well as a single agent, I hypothesize that masitinb will increase efficacy of TMZ by acting as a chemosensitizer due to its targeting of stem cell factors critical to tumor cell growth and development. If so, this drug may enter human clinical trials with the intention of reducing the amount of harmful brain radiation currently needed to combat pediatric GBM, so that quality of life may be preserved for patients. The use of masitinib in particular is justified by its success in other animal models and against other cancer types.

Materials and Methods

Male and female athymic nude mice were used for all studies. Animals were maintained in filter top cages in Thoren units within the Cancer Center Isolation Facility (CCIF) building at Duke University. All animal procedures conformed to Institutional Animal Care and Use Committee guidelines. All xenograft lines were maintained as previously described by Bullard and Bigner (1979).

Original animals were obtained from the Mogul Laboratories, but since May 29th 1978, the Bigner Friedman Keir (BFK) lab has bred from their own closed colony. In general, breeding animals are maintained in a barrier facility within the CCIF. All animals are stored under pathogen free conditions in sterilized plastic containers covered with polyester bacterial air filters. Breeding animals are stored in containment hoods, while experimental animals are kept in separate rooms. Food and water was provided ad libitum. All animals receive fat enriched food and vitamin-supplemented water. Stock and breeding animals receive antibiotic supplemented water, while the experimental animals are maintained on water adjusted to pH 2.2-3.0 with HCl. Food, water, and bedding are changed twice per week under sterile conditions. Animals are checked daily and euthanasia is performed with cervical dislocation or CO_2 euthanasia.

Xenograft specifics

The xenografts used in this study, 212 and 717, were maintained as described by Friedman et al, (1994). These xenografts were derived directly from patient (human) operating room samples and maintained through serial passage from one mouse generation to the next. See Table 1 for further information.

Table 1: **Specific parameters of chosen xenografts 212 and 717.**

Xenograft Line	Patient Gender	Age	Tumor Type	*Specific mutations/abnormalities	**Gene Expression Pattern
212	Female	11	Giant Cell Glioblastoma	TP53 and RB1 mutation	Mesenchymal
717	Male	11	Glioblastoma	CDKN2A deletion	Classical

*It has been found that a mutation of tumor protein p53 (TP53) on chromosome 17 or retinoblastoma 1 (RB1) on chromosome 13 disrupts activity of these tumor suppressive genes, causing a decreased regulation of cell growth and division (Genetics Home Reference C and B, 2014). Cyclin-dependent kinase inhibitor 2A (CDKN2A) is also a tumor suppressor gene located on chromosome 9 whose deletion is seen in a variety of cancer types (Genetics Home Reference A, 2014).

**As described by Verhaak et al (2010), four subtypes of GBM have been identified based on differing gene expression patterns and clinical characteristics: Proneural, Neural, Classical and Mesenchymal. Identifying subtypes lays the groundwork for personalized medicine as each subtype may require a different therapeutic approach. Mesenchymal gene expression, found in xenograft line 212, is characterized by frequent mutations of PTEN and TP53. Classical gene expression, found in xenograft line 717, is characterized by high levels of EGFR and lack of a TP53 mutation.

Xenograft Transplantation

In preparation for subcutaneous xenograft transplantation, tumors were removed from the host animal under sterile conditions under a laminar flow containment hood. Once this was done, the tumor was then segmented and placed into a modified tissue press and passed through two layers of mesh. This homogenate was then passed through a 19-gauge needle before being loaded into a repeating Hamilton syringe dispenser (250*ul*). After the animal's coat was prepped with betadine, a 19-gauge needle was attached to the dispenser and the tumor homogenate was injected subcutaneously into the right hind flank of the athymic mouse at an inoculation volume of 50*u*l (Friedman et al, 1988).

Tumor Assessment

Subcutaneous tumors were measured twice weekly with hand-held vernier calipers (Scientific Products, McGraw, IL). Tumor volume was calculated per the formula: $[(\text{width})^2 \times (\text{length})]/2 = \text{mm}^3$ tumor volume. Groups of 8 to 10 mice were randomly selected based on tumor volume and treated when the median tumor volume ranged between 100 - 400 mm^3. These treated mice were then compared with untreated, control, animals.

Assessment of Response

The response of subcutaneous xenografts was assessed by delay in tumor growth and by tumor regression. Growth delay, expressed as T-C, is defined as the difference in days between the median time required for tumors in the treated (T) and the control (C) animals to reach a volume five times greater than that measured at the start of treatment. Tumor regression is defined as a decrease in tumor volume over two or more successive measurements.

Statistical Analysis

Statistical analysis was performed using a personalized SAS statistical analysis program, the Wilcoxon rank order test for growth delay, and Fisher's exact test for tumor regression as previously described (Friedman et al., 1988).

Dose Route and Schedule

Treated mice were dosed with masitinib at a concentration of 25 mg/kg twice daily (Monday-Friday) and/or TMZ at a concentration of 25 mg/kg once weekly via intraperitoneal injection.

Results

TMZ and masitinib as single agents

An initial experiment was conducted to determine the dose response of Temozolomide (TMZ) as a single agent. TMZ demonstrated minimal to modest activity at a dose of 20 mg/kg once weekly with a T-C value of 1.48 days and no drug related deaths. The dose response of masitinib as a single treatment agent was then evaluated on xenograft line 212 (figure 1).

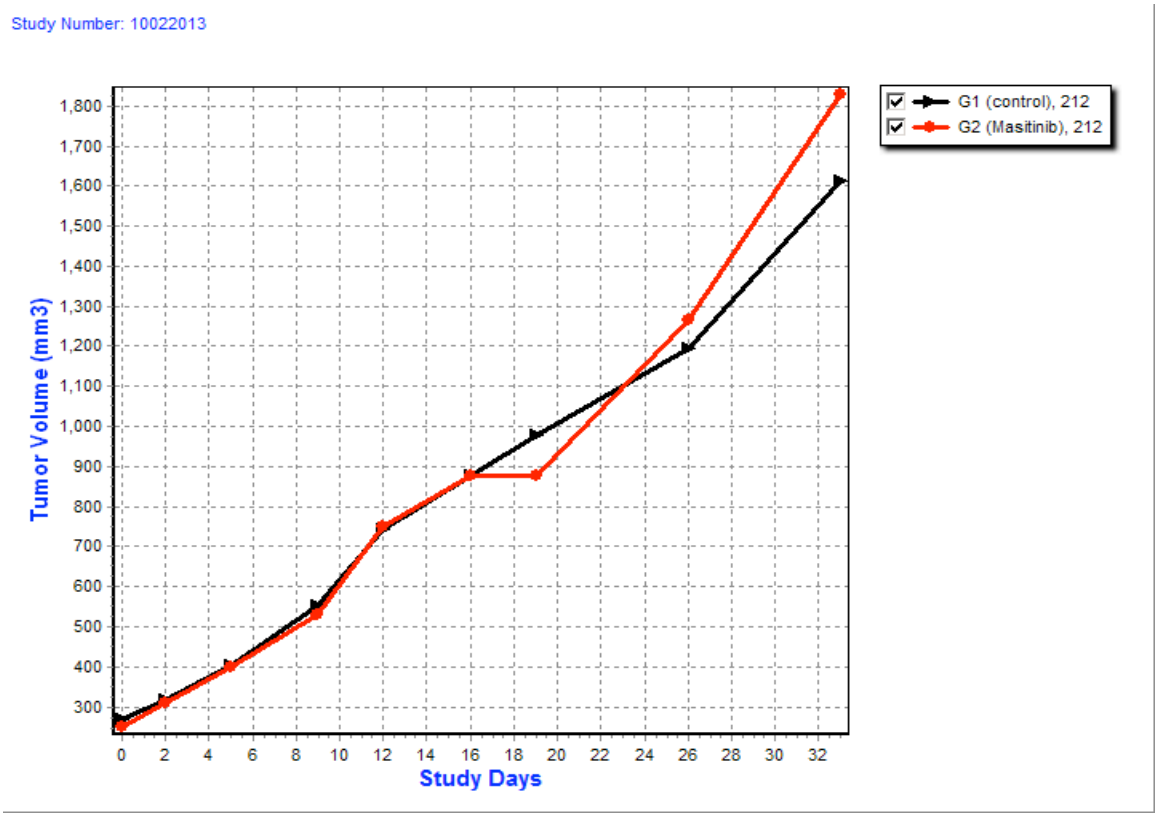

Figure 1: **The effect of masitinib on xeongraft line 212.** Each point on the graph represents the mean tumor volume for the group on that day. Masitinib showed a negative effect when compared to the control group with a T-C* value of -6.06 days. This value was not significant, however, owing to a p value of 0.212.

*T-C value is defined as the difference in days between the median time required for tumors in the treated (T) and the control (C) animals to reach a volume five times greater than that measured at the start of treatment.

In conjunction with previous studies conducted on masitinib tolerability, a dose of 25 mg/kg elicited no observed drug toxicities; furthering our belief that masitinb can be tolerated at a dose of 25 mg/kg (Vermersch et al, 2012). As expected, however, masitinb did not work well against GBM as a single agent and actually caused an increased tumor growth relative to the control

Masitinib + TMZ

Experiment 1

With a proper dose having been determined for both TMZ and masitinib, a classic four-arm study was conducted evaluating the monotherapy of masitinib and TMZ (G2 and G3 respectively) and a combination of the agents (G4) compared to an untreated control group (G1) (figure 2).

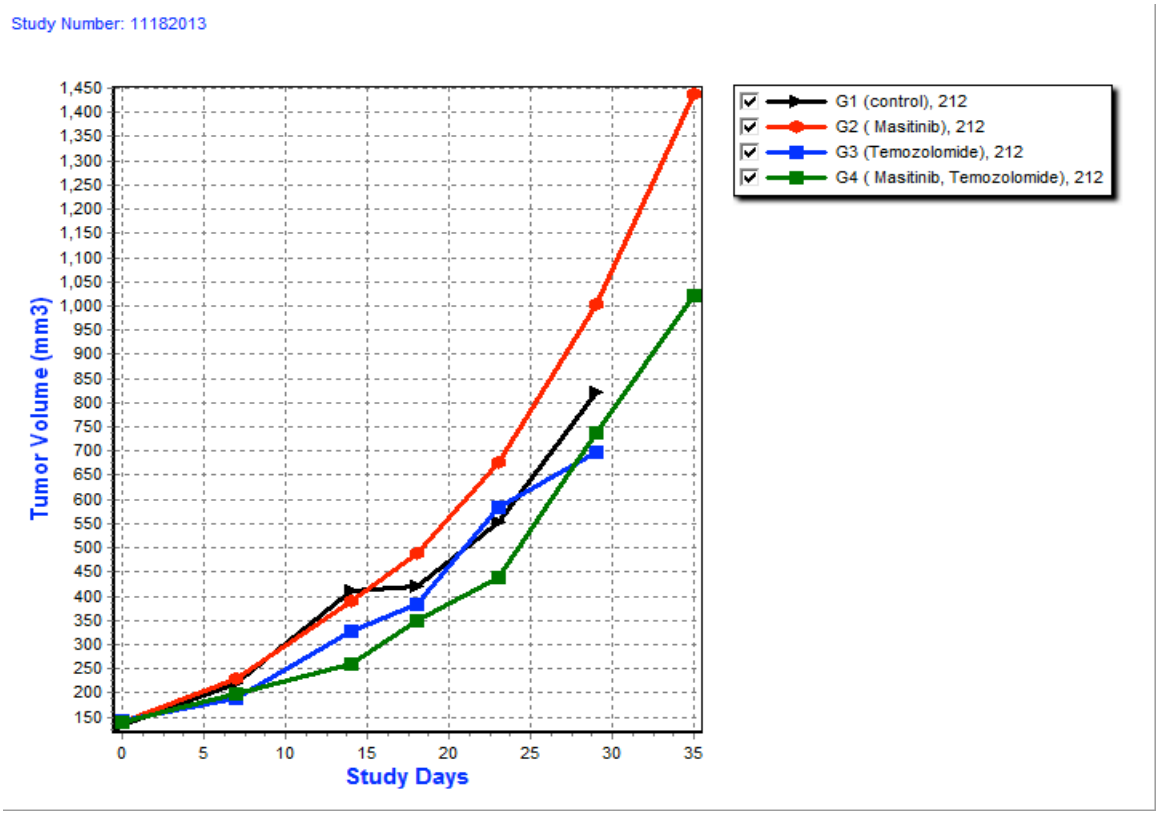

Figure 2: **The effect of masitinib in combination with temozolomide on xenograft line 212.** Each point on the graph represents the mean tumor volume for the group on that day. Masitinib alone showed a positive effect (T-C value = 2.20 days), while temozolomide alone showed a negative effect (T-C value = -1.56 days) when compared to the control group. The combination of masitinib and temozolomide caused an increased positive T-C value of 6.20 days when

compared to the control group. The result of the combination arm suggests significance due to a p value of 0.076.
The results of this study indicated that tumors of the combination group took the longest to reach a volume five times that of the original volume when compared to any of the other three groups. This showed that the combination of TMZ and masitinib working together inhibited tumor growth better than either single agent.

Experiment 2

In an attempt to verify these results, the four-arm study conducted in experiment 1 was replicated in xenograft line 717 (figure 3).

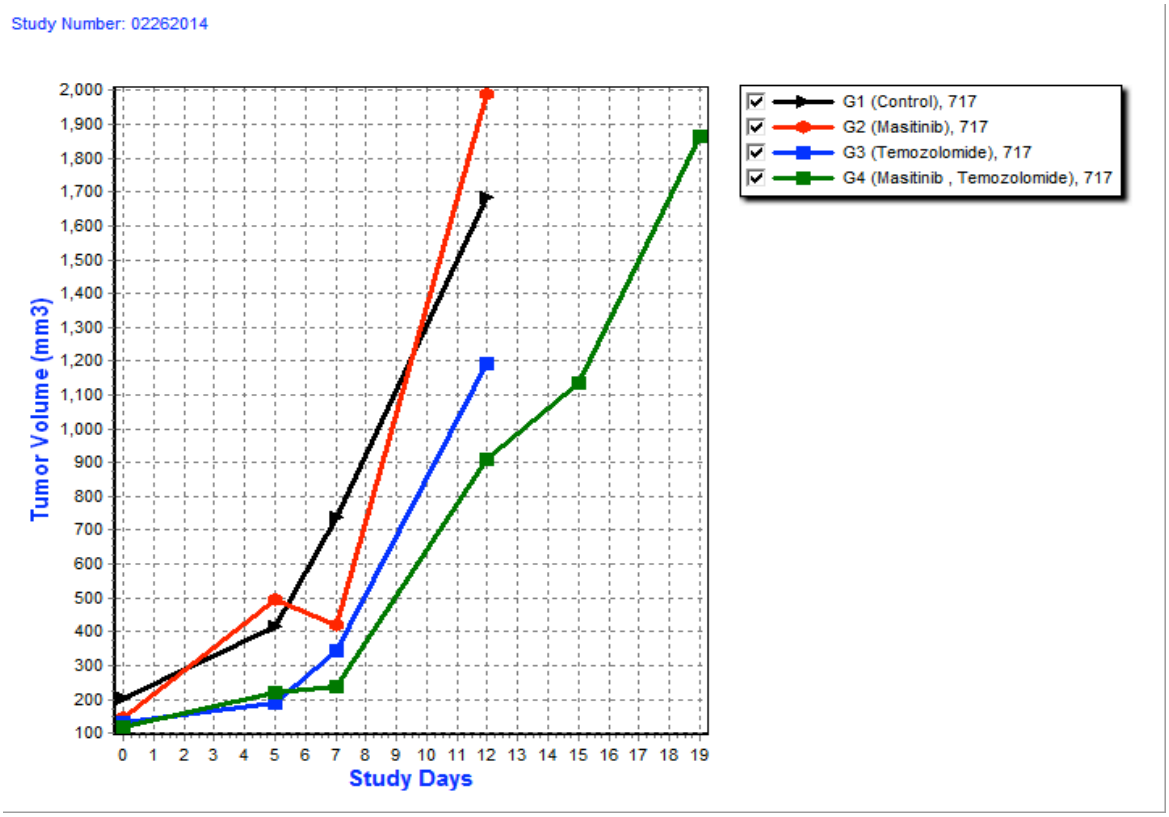

Figure 3: **The effect of masitinib in combination with temozolomide on xenograft line 717.** Each point on the graph represents the mean tumor volume for the group on that day. Masitinib alone showed a negative effect (T-C value = -1.39 days) while temozolomide alone showed a positive effect (T-C value = 1.28 days) when compared to the control group. The combination of masitinib and temozolomide, as previously found, caused an increased positive T-C value of 2.57 days when compared to the control group ($p<0.350$).

Here, as previously observed in experiment 1, tumors of the combination group (group 4) took the longest to reach a volume five times that of the original volume when compared to any of the other three groups.

Discussion

While temozolomide (TMZ) is currently used for treatment of pediatric glioblastoma, not only are the effects of the drug minimal (Barone et al, 2006), but also it is often used in combination with brain radiation (Chamberlain, 2006). Despite unfavorable results, this combination is the best treatment option for patients. In many cases, due to the fragility of the developing brain, this radiation can lead to a number of harmful side effects on the patient, which may sometimes not present themselves until years after treatment (Simon and Harvey, 2012). In order to decrease the amount of radiation needed, I have evaluated the effects of TMZ in combination with masitinib, hypothesizing that masitinib will act as a chemosensitizer to increase the effects of TMZ.

Masitinib as a single agent is not effective against pediatric glioblastoma in xenograft line 212.

The results from the initial study did not show much promise for the use of masitinib as a single agent, with tumors of the treated group growing faster than the control group (figure 1). Although statistical analysis using the Wilcoxon rank order test for growth delay showed this finding was insignificant ($p<0.212$), it is important to note that between days 16 and 22 masitinib decreased tumor volume relative to the control, suggesting that there may be a window of opportunity that could be exploited. In addition, none of the mice died due to drug toxicities, indicating that masitinib can be tolerated at a dose of 25 mg/kg. Thus, while the reasons for its

effects during this time period are unclear, these results indicate that masitinib can impact the proliferation of GBM tumor cells.

Knowing from experimental findings that masitinib does not work well against pediatric GBM as a single agent, the focus of this study turned to the effects of masitinib in combination with TMZ. The relative positive findings seen when using TMZ alone show that it does affect pediatric brain tumors to some degree. Masitinib, however, may amplify these intrinsic effects by acting as a chemosensitizer and allowing TMZ to better attack the brain tumor. The hope, and hypothesis, was that combination therapy would induce a slower tumor growth rate than either masitinib or TMZ alone.

Masitinib + TMZ inhibited tumor growth better than either single agent.

In order to test my hypothesis, two classic four-arm studies were conducted (figure 2 and 3). Group 1 was the control group, acting as a baseline and receiving no treatment whatsoever. Group 2 was treated with masitinib alone at a dose of 25 mg/kg BID. Group 3 was treated with TMZ alone at a dose of 25 mg/kg once weekly**.** The selection of a 25mg/kg dose of TMZ was decided due to the desire to ramp up the mere modest effect seen when using TMZ at 20mg/kg. Group 4 was treated with both masitinib and TMZ at their respective doses and dose schedules.

Results from these experiments were positive, showing an increase in the T-C value to 6.20 days in experiment 1 and 2.57 days in experiment 2 when comparing the combination group to the control group. This effect is greater than a simple additive one that would have yielded a T-C value of 0.64 (2.20 + -1.56 = 0.64) in experiment 1, indicating an almost 500% increase in effectiveness when using TMZ with masitinib than when using TMZ alone. This was also seen in experiment two, as an additive effect would have produced a T-C value of -0.10 (-1.39 + 1.28 = -0.10), indicating a 100% increase in effectiveness when adding masitinib to a TMZ regiment.

The Wilcoxon rank order test for growth delay provided statistical analysis of experiment 1 and 2. Analysis of experiment 1 suggested the results were not due to chance events as a $p<0.076$ was found. Performing the same analysis of experiment 2, however, yielded a $p<0.350$, an insignificant value. This high p value could be due to the small sample size, as the experiment was conducted using only three mice per group as a result of difficulties in initial tumor growth to a volume greater than $100mm^3$.

I believe the experimental finding that masitinib can increase the efficacy of TMZ can be attributed to masitinib's targeting of stem cell factors c-Kit, Platelet Derived Growth Factor Receptors (PDGFR) and Lyn (Humbert et al, 2010). C-Kit is a proto-oncogene that encodes for a tyrosine kinase expressed on mast cells, the main target of masitinib, which have been found to secrete proinflammatory cytokines and other molecules that benefit the tumor (Published Library of Science, 2009). Masitinib has been shown to decrease the effects of other diseases associated with mast cell proliferation including Alzheimer's, mast cell leukemia, and rheumatoid arthritis (Vermersch et al, 2012). This could be due to the fact that mast cells have been found on both sides of the BBB (Vermersch et al, 2012). PDGFR is a cell surface tyrosine receptor present in GBM whose α and β subunits are important in cell proliferation, differentiation, and growth (Lokker et at, 2002). Finally, Lyn is a member of the Src family of protein kinases whose activity is elevated in GBM (Stettner et al, 2005).

By targeting these critical stem cell factors, masitinib seems to slow the ability of the stem cells to replicate. Once the tumor has been damaged by TMZ, the repair mechanism is then slowed by masitinib, resulting in prolonged tumor recession. As a result, by confronting the tumor in two distinct ways, the combination of masitinib and TMZ seems to be more effective than either single agent.

Future Directions

Although the results from the evaluation of masitinib as a single agent and from experiment 2 were insignificant, experiment 1 showed a positive T-C value for the combination arm relative to the control in a way that suggests significance. This line of research is highly experimental and these results are being used to help guide the future research direction of this drug combination. As such, I recommend:

- Increasing the dose of masitinib and/or TMZ
    - No drug toxicities were seen at a dose of 25 mg/kg indicating that masitinib and TMZ may be tolerated at a higher dose, which may elicit a better response.
- Altering the dose schedule of masitinib and/or TMZ
    - Altering the dose schedule of masitinib to once daily at a higher dose and/or TMZ five days in a row, rather than once weekly for five weeks, may increase the effectiveness of the drug combination.
- The addition of masitinb to other chemotherapy drugs that have the ability to cross the BBB
    - Although TMZ is known as the gold standard chemotherapeutic agent for treatment of pediatric glioblastoma, various other chemotherapeutic drugs are known to cross the BBB. Masitinib may also act as a chemosensitizer towards these drugs, possibly to a greater degree than seen in TMZ.

- In vitro screening of xenograft lines for stem cell factors c-Kit, PDGFR, and Lyn, prior to injection of tumor into the mice
    - Identification and use of tumors with specific sites that masitinib has been designed to target may increase the response seen in both trials of masitinib alone and in combination with TMZ.

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References

Albright (1993). Pediatric brain tumors. *CA: a cancer journal for clinicians*, *43*(5), 272-288.

American brain tumor association - glioblastoma. (2014). Retrieved from: http://www.abta.org/brain-tumor-information/types-of-tumors/glioblastoma.html

Barone, Maurizi, Tamburrini, Riccardi (2006). Role of temozolomide in pediatric brain tumors. *Child's Nervous System*, *22*(7), 652-661.

Bullard and Bigner (1979). Heterotransplantation of human craniopharyngiomas in athymic "nude" mice. *Neurosurgery*, *4*(4), 308-314.

Butler, Rapp, Shaw (2006). Managing the cognitive effects of brain tumor radiation therapy. *Current treatment options in oncology*, *7*(6), 517-523.

Chamberlain (2006). Treatment options for glioblastoma. *Neurosurgical focus*, *20*(4), E19.

Connell, Chatain, Cornelissen, Vallis, Hamilton, Seymour, Anthony, Sibson (2013). Selective Permeabilization of the Blood–Brain Barrier at Sites of Metastasis. *Journal of the National Cancer Institute*, *105*(21), 1634-1643.

Fangusaro (2012). Pediatric high grade glioma: a review and update on tumor clinical characteristics and biology. *Frontiers in oncology*, *2*.

Fraley, Arrington, Bilodeau, Hartman, Hoffman, Kim, Hungate (2001). *U.S. Patent No. 6,306,874*. Washington, DC: U.S. Patent and Trademark Office.

Friedman, Houghton, Schold, Keir, Bigner (1994). Activity of 9-dimethylaminomethyl-10-hydroxycamptothecin against pediatric and adult central nervous system tumor xenografts. *Cancer chemotherapy and pharmacology*, *34*(2), 171-174.

Friedman, Colvin, Skapek, Ludeman, Elion, Schold, Jacobsen, Muhlbaier, Bigner (1988). Experimental chemotherapy of human medulloblastoma cell lines and transplantable xenografts with bifunctional alkylating agents. *Cancer research*, *48*(15), 4189-4195.

Furnari, Fenton, Bachoo, Mukasa, Stommel, Stegh, Hahn, Ligon, Louis, Brennan, Chin, DePinho, Cavenee (2007). Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes & development*, *21*(21), 2683-2710.

Genetics home reference A - cdkn2a. (2014). Retrieved from: http://ghr.nlm.nih.gov/gene/CDKN2A

Genetics home reference B - rb1. (2014). Retrieved from: http://ghr.nlm.nih.gov/gene/RB1

Genetics home reference C - tp53. (2014) Retrieved from: http://ghr.nlm.nih.gov/gene/TP53

Gerstner and Fine (2007). Increased permeability of the blood-brain barrier to chemotherapy in metastatic brain tumors: establishing a treatment paradigm. *Journal of clinical oncology*, *25*(16), 2306-2312.

Humbert, Castéran, Letard, Hanssens, Iovanna, Finetti, Bertucci, Barder, Mansfield, Moussy, Hermine, Dubreuil (2010). Masitinib combined with standard gemcitabine chemotherapy: in vitro and in vivo studies in human pancreatic tumour cell lines and ectopic mouse model. *PLoS One*, *5*(3), e9430.

King, Anthony (2011). "Breaking Through the Barrier." *RSC*. Retrieved from: http://www.rsc.org/chemistryworld/Issues/2011/June/BreakingThroughTheBarrier.asp

Lannering, Marky, Lundberg, Olsson (1990). Long-term sequelae after pediatric brain tumors: Their effect on disability and quality of life. *Medical and pediatric oncology*, *18*(4), 304-310.

Lokker, Sullivan, Hollenbach, Israel, Giese (2002). Platelet-derived Growth Factor (PDGF) Autocrine Signaling Regulates Survival and Mitogenic Pathways in Glioblastoma Cells Evidence That the Novel PDGF-C and PDGF-D Ligands May Play a Role in the Development of Brain Tumors. *Cancer research*, *62*(13), 3729-3735.

Minniti, Muni, Lanzetta, Marchetti, Enrici, (2009). Chemotherapy for glioblastoma: current treatment and future perspectives for cytotoxic and targeted agents. *Anticancer research*, *29*(12), 5171-5184.

Mitry, Hammel, Deplanque, Mornex, Levy, Seitz, Moussy, Kinet, Hermine, Rougier, Raymond (2010). Safety and activity of masitinib in combination with gemcitabine in patients with advanced pancreatic cancer. *Cancer chemotherapy and pharmacology*, *66*(2), 395-403.

Mrugala, Adair, Kiem (2010). Temozolomide: Expanding its role in brain cancer. *Drugs of today (Barcelona, Spain: 1998)*, *46*(11), 833-846.

Pollack (2011). Multidisciplinary management of childhood brain tumors: a review of outcomes, recent advances, and challenges: A review. *Journal of Neurosurgery: Pediatrics*, *8*(2), 135-148.

Published Library of Science (2009). Masitinib: Targeted Therapy for Cancers, Inflammatory Diseases and Neurological Indications. *Science Daily*. Retrieved from: http://www.sciencedaily.com/releases/2009/09/09092920329.htm

Simon, Harvey (2012). "Brain Tumors - Primary." *University of Maryland Medical Center*. Retrieved from: http://umm.edu/health/medical/reports/articles/brain-tumors-primary

Stettner, Wang, Nabors, Bharara, Flynn, Grammer, Gillespie, Gladson (2005). Lyn kinase activity is the predominant cellular SRC kinase activity in glioblastoma tumor cells. *Cancer research*, *65*(13), 5535-5543.

Thamm, Rose, Kow, Humbert, Mansfield, Moussy, Hermine, Dubreuil (2012). Masitinib as a chemosensitizer of canine tumor cell lines: A proof of concept study. *The Veterinary Journal*, *191*(1), 131-134.

Vermersch, Benrabah, Schmidt, Zéphir, Clavelou, Vongsouthi, Debreuil, Mussy, Hermine (2012). Masitinib treatment in patients with progressive multiple sclerosis: a randomized pilot study. *BMC neurology*, *12*(1), 36.

Verhaak, Hoadley, Purdom, Wang, Qi, Wilkerson, Miller, Ding, Golub, Mesirov, Alexe, Lawrence, Kelly, Tamayo, Weir, Gabriel, Winckler, Gupta, Jakkula, Feiler, Hodgson, James, Sarkaria, Brennan, Kahn, Spellman, Wilson, Speed, Grey, Meyerson, Getz, Perou, Hayes (2010). Integrated Genomic Analysis Identifies Clinically Relevant Subtypes of Glioblastoma Characterized by Abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer cell*, *17*(1), 98-110.

Zhang, Stevens, Bradshaw (2012). Temozolomide: mechanisms of action, repair and resistance. *Current molecular pharmacology*, *5*(1), 102-114.