## Abstract

It is fairly typical for most cancer patients to undergo radiation therapy at some point during their cancer treatment and recovery process. Radiation therapy targets malignant tumor sites with a high energy concentration in the form of photon or ion radiation. Although conventional methods of radiation therapy are beneficial to the treatment of most tumors, they are not as sufficient for the treatment of high-risk brain tumors such as Glioblastomas and Medulloblastomas. Conventional photon radiation therapy introduces the added risk of healthy tissue exposure to radiation, which is an impediment that a complex organ, like the brain, cannot afford. This paper aims to answer the question: could there be a type of radiation treatment that provides a satisfactory treatment for these brain tumors, concentrating the most dose at the tumor site while concurrently sparing the maximum amount of surrounding healthy tissue? Several studies focusing on a novel method of radiation treatment, proton minibeam therapy (pmbRT), have attempted to answer this question, all to a successful degree. On a cellular level, $\gamma$-H2Ax serves as a biomarker for signaling DNA damage done by radiation, enabling the analysis of the tissue-sparing properties of proton minibeams on a molecular level. The depth at which proton minibeams begin to merge back to a single homogenous beam was determined in another study, giving a concrete value in terms of target depth of how much healthy proximal tissue would be spared at the entrance site of the patient. Each study concluded that proton minibeams do possess even more tissue sparing properties compared to conventional open beams.

# The Biological Effects of Proton Minibeam Therapy on High-Risk Tumors

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## Introduction

Primary brain tumors—tumors originating and developing entirely inside the brain initially—are the most high risk and the most precarious cancers to treat. Not only do they instigate malignant cell proliferation in the brain architecture, but they also often result in other secondary metastases to other parts of the head and neck area, which in turn leads to the spreading of abnormal cell growth through cerebrospinal fluid and attacking the Central Nervous System (1).

The brain is debatably the most enigmatic organ in the human body, and there is still much that is uncharted and many things yet to be investigated about it, making cancer treatment in the intracranial region that much more abstract and particular. Although there are currently no satisfactory or permanent curative treatments for many brain cancers, there are different palliative treatments that have proven to extend survival rates and improve the quality of life for such patients. These include resective surgery, chemotherapy, and radiation therapy (1).

This paper will focus on the radiation therapy aspect of brain tumor treatment. For this particular study of malignant brain tumors, there materializes the following question: is there a method of radiation treatment that could meet the demands of abstruse behavior of the brain, while simultaneously preserving the condition of the remaining healthy the brain tissue, reducing the adverse side effects of radiation and ameliorating the long term wellbeing of patients? A resolution to this issue could introduce a satisfactory outcome for the treatment of high-risk brain tumors, especially in pediatric cases such as Medulloblastomas, where the pediatric patient's

brain development and overall neurocognitive vulnerability especially complicate radiation therapy management.

## I. The Brain

There are four major divisions of the brain architecture: the Cerebellum, the Basal ganglia, the Cerebellum and the Brainstem. Each compartment has different controls over the wide range of brain functions (1).

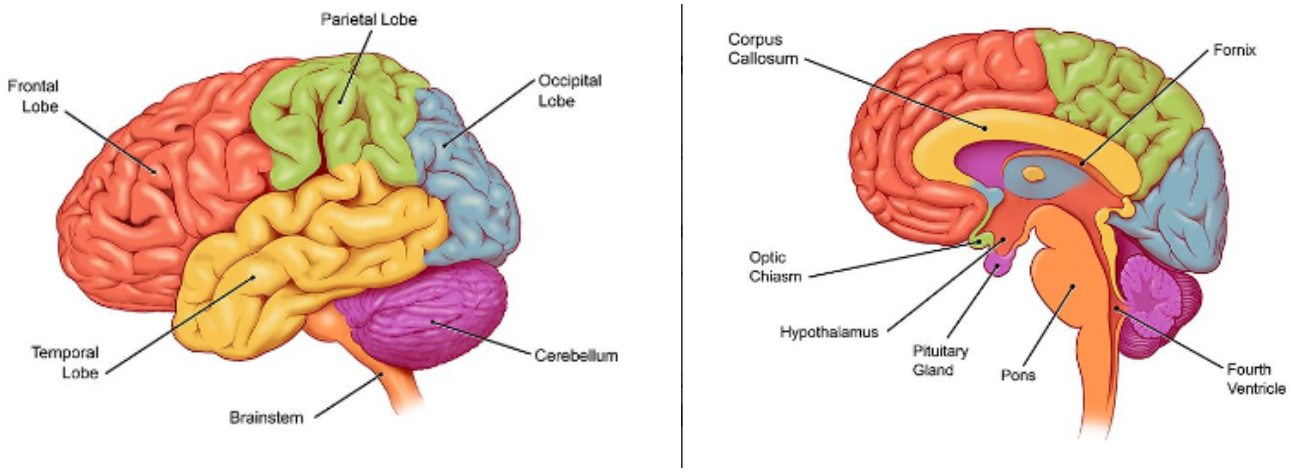


**Figure 1:** Regions of brain architecture color-coded. Sections of the Cerebrum and the Cerebellum indicated left, and the components of the Basal ganglia shown right[(1)]

The Cerebrum makes up the majority of the brain volume and is separated into two hemispheres longitudinally, each consisting of four lobes marking the different regions of the Cerebrum: the Occipital, Parietal, Frontal, and Temporal (Figure 1). Because the Cerebrum is the most substantial part of the brain, it has agency over much of the brain's functions, such as control over emotion, the ability to perceive external sensations such as pain, control over all of the sensory organs, muscle stimulation, and the ability to speak and comprehend language (1).

The Cerebellum is the largest part of the hindbrain that is located occipitally (Figure 1), near the back of the head, and situated underneath the rest of the brain architecture, the Cerebrum (2). The Cerebellum is responsible for balance, coordination, and other fine motor skills, all of which are critical to brain development (2).

The Basal ganglia is located towards the center of the brain, encapsulated by much of the Cerebrum (Figure 1). The Hypothalamus, the Optic Chiasm, the Corpus Callosum, the Fornix, and the Pituitary gland all make up the composition of the Basal ganglia (1). This part of the brain is responsible for some motor movements, but primarily experiential learning, behavior, and the capability to develop emotions (3).

Finally, the Brainstem is located below the Cerebellum and penetrates the Basal ganglia. The Brainstem is made up of an extensive amount of nerve fibers that transmit messages to the Cerebrum from the rest of the body and vice versa (1).

It is evident even in just the responsibilities of the brain alone that if any damage were to be inflicted, it would be detrimental to its overall function. Brain tumors breach healthy tissue such as white and grey matter, cause intracranial pressure, create blockages in cerebrospinal fluid, and in some cases, even intracranial bleeding (1). These invasive tumors severely alter the nature of the different compartments of the brain, consequently causing damage to the rest of the human body depending on what part of the brain architecture the tumor originates in or spreads to. Symptoms of such tissue destruction include headaches due to intracranial pressure, loss of fine motor skills and balance, loss of muscle coordination, deterioration of sensations such as sight or feeling, and varying degrees of personality alteration. Because the symptoms and the effects of brain tumors are so consequential, they are considered to be very high-risk and incredibly challenging to treat (1).

## II. Pediatric Medulloblastomas

One of the most afflicting and most frequent types of brain cancer in pediatric cases is Medulloblastomas, making up about 20% of all pediatric brain tumor cases (4). Medulloblastomas are often referred to as an embryonal neuroepithelial tumor since the cancerous cells originate in the embryonal cells of the fetus and continue to grow after birth (5). Medulloblastomas are highly malignant tumors that arise in the Cerebellum, the most substantial portion of the hindbrain (1). Figure 2 shows an MRI scan of a transverse section of the brain with the Medulloblastoma in question in the Cerebellum region highlighted on the image.

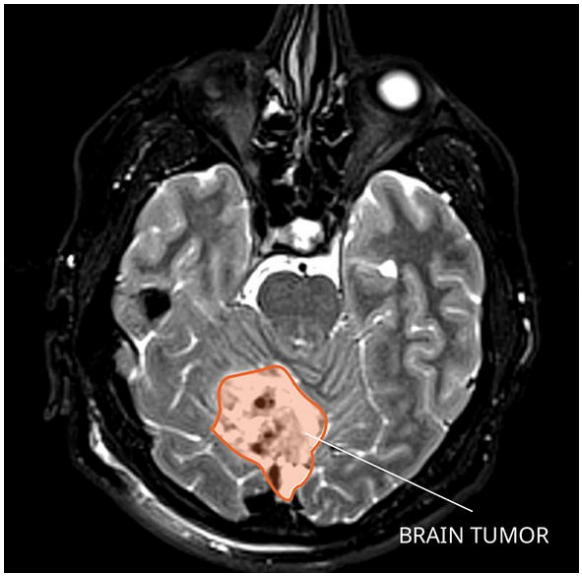


**Figure 1:** A transverse section of a Magnetic Resonance Image (MRI) scan of a pediatric brain. The highlighted portion indicates a Medulloblastoma mass originating from the cerebellum [5]

Because medulloblastomas are a category of brain tumor as discussed earlier, there is the risk of the malignant cells spreading to other parts of the brain and the Central Nervous System (CNS) through cerebrospinal fluid (5).

Patients generally begin to develop and experience symptoms over a few weeks to months. Cerebellar dysfunction and Intracranial Pressure (ICP) are two of the most common symptoms of Medulloblastomas. These symptoms are characterized by irritability, nausea, lethargy, anorexia, vomiting, and a range of other behavioral shifts (6). Because Medulloblastomas primarily originate in the Cerebellum, other symptoms may include a deterioration of fine motor skills, balance, and muscle coordination (5).

Currently, there is no form of satisfactory curative treatment for pediatric Medulloblastomas. There are, however, several methods for treating Medulloblastomas that have proven to extend the survival rate of patients, such as craniospinal radiation therapy, surgery, and chemotherapy (5). Although these different types of treatments allow for increased survival rates, more often than not, the quality of life for such patients is less than optimal.

Because Medulloblastomas are strictly pediatric cases, it is crucial to realize the fact that these patients are highly vulnerable to neuro-cognitive effects due to ongoing brain development. Conventional photon radiation therapy used in most clinics, although being the less invasive approach to treating the tumor mass, is not a sufficient form of treatment due to a Medulloblastoma's high index of photon radiation resistance. In addition, Gliomas, in particular, have a high threshold of radioresistance, and the reasons behind this are still unknown (7).

## III. Radiation Therapy

It is common for most cancer patients to be treated with some type of external radiation therapy at some stage in their treatment and recovery process (8). Radiation therapy is a method of cancer treatment that takes high doses of energy in the form of photons to cause damage to

malignant tumor cells by exogenously causing DNA (deoxyribonucleic acid) double-strand breaks. Because tumor cells are mutant, the rate of recovery from any sort of DNA damage, especially double-strand breaks, is extremely low, which sends the tumor cells into apoptosis—cell death, inhibiting the cells' ability to heal and repopulate. The result of apoptosis means any cells the radiation comes into contact with are killed and subsequently the entire tumor mass along with it, which stops the entire growth altogether. Radiation therapy can shrink the tumor mass in most cases, making it easier to be removed surgically if need be (7).

The most common and widely used type of radiation therapy in most clinics implements the use of photon beams. Radiation therapy is performed in conjunction with chemotherapy and/or surgery, primarily to reduce the tumor mass and volume for ease of resective surgery or to destroy any residual cancerous cells immediately following a tumor resection (5). Radiation therapy can be administered to a wide range of cancers, the most common treatment areas being breast and prostate. However, there are some cases in which radiation therapy can be used to treat cases in more high-risk treatment areas such as the head and neck (7). This type of radiation therapy is called "craniospinal irradiation."

There are some adverse side effects of radiation therapy, which is why the treatment areas are somewhat limited. A significant factor to consider during radiation treatment is the Therapeutic Gain Factor of the patient's treatment plan. The Therapeutic Gain Factor (TGF) is the ratio of healthy tissue spared to cancer tissue damaged during radiation treatment (7).

After the prognosis is made and a dose prescribed, the treatment timeline for most patients usually spans five to six weeks, with clinical visits for treatments happening once or twice weekly with the dose prescription divided into even fractions until the total dose prescribed

is met. This break in treatment periods allows for the healthy cells affected by the radiation treatment to recover, while still damaging the cancerous cells simultaneously (7).

## Theory

### I. Proton Minibeam Radiation Therapy

This is where we arrive at a possible solution to the question of whether or not there is a possible method of radiation therapy that could be advantageous to the treatment of high-risk brain tumors. The answer is found in a novel approach to radiation therapy called proton minibeam radiation therapy (pmbRT), which has the potential to correct shortcomings present in currently utilized methods, such as conventional photon radiation therapy. Specifically, it promises to affect complex brain functions minimally, preserve remaining and otherwise healthy brain tissue, reduce the adverse side effects of radiation exposure, and improve the long term wellbeing of patients. As a result, this treatment potentially meets the demands of even the most high-risk tumors, such as pediatric Medulloblastomas and high-grade Glioblastomas in adults. This method of treatment utilizes radiation energy in the form of heavy ions, in this case, protons, compared to photon (X-ray) radiation commonly used in most cancer treatment clinics. Photons release the highest amount of energy and dose deposition early on in the beam's path through a biological target, with the energy exponentially decreasing the further and deeper the beam travels into the patient. Photon beams continue to travel through the entire width of the patient's body, even after interacting with the tumor mass, as shown in Figure 3. This causes a lot of radiation interaction with healthy tissue at the entrance and exit sites, which subsequently causes damage to the surrounding healthy tissue (7).

This paper, however, is interested in a relatively new method of radiation treatment, proton beams. Unlike photons, protons deposit the most amount of energy at a deeper depth in the target in the form of a sharp dose and energy spike called the Bragg Peak, as shown in Figure 3, before entirely ceasing to irradiate. The Bragg Peak represents the increased amount of ions released in the form of energy in a radiation particle's path through interacting with matter (7). This is a property only evident in heavy ions interacting with matter, such as the protons in question. Photons do not experience the same sharp energy release.

Because of these properties of the proton beam and the Bragg Peak, it is much easier to localize the highest and most concentrated dose deposition to the tumor site, reducing radiation interaction with the surrounding healthy tissue and adjacent vital organs. An ideal treatment scenario would put the Bragg Peak right at the tumor site, allowing for the maximum amount of radiation dose and energy to be deposited at the tumor mass. At the same time, the TGF of healthy tissue and surrounding organs would be increased at both the entrance and exit sites of the patient.

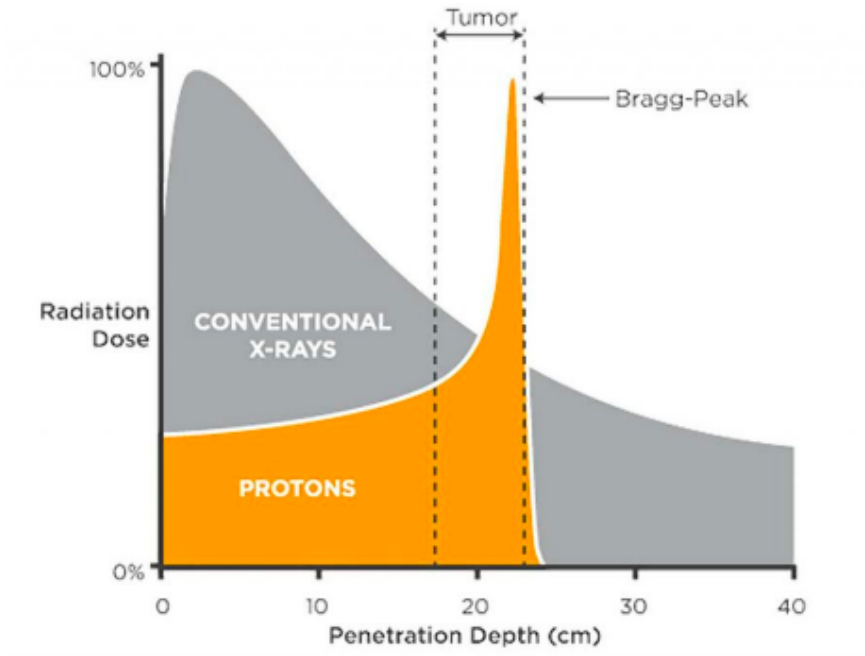


**Figure 3:** A graphical representation of dose percentage relative to depth inside a target for photon beams (X-rays), and proton beams via ionized tracks [(9)]

An experimental adaptation to the already advantageous proton beam method of radiation therapy is the concept of using spatially modulated pencil beams even further to spare healthy tissue from radiation exposure and increasing the TGF. A brass collimator creates these pencil beams, a block made up of submillimeter slits, placed in front of the radiation source. Breaking up the radiation in such a way creates “peaks” and “valleys” of radiation at the entrance site. Due to Coulomb Scattering or electromagnetic interaction, an observed property of protons when interacting with matter in which energy is transferred from the charged proton to other charged particles in the biological tissue releasing energy in the form of radiation (7), these spatially modulated beams eventually broaden and merge back to a single beam at the Bragg Peak, still converging at the ideal tumor site. This approach, currently referred to as proton minibeam therapy (pmbRT), spares the proximal tissue at the beam entrance at an even more significant

degree and improves the already prominent efficacy of proton beams compared to conventional photon beams (10).

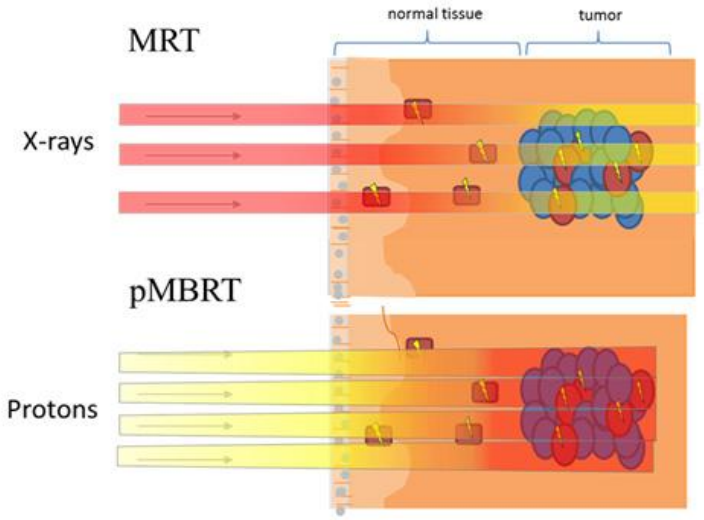


**Figure 4:** Collimated beam trajectory and dose concentration of photon microbeams, X-rays, (top) and proton minibeams (bottom) relative to normal tissue and tumor mass.[10]

Figure 4 shows a comparison between dose concentration and beam trajectory of proton minibeam therapy compared to X-ray microbeam therapy. The most evident difference between the two is the presence of the Bragg Peak region, where the minibeams converge and deliver the maximum dose. The modulated X-ray beams do not undergo the same scattering effect that would allow the beams to broaden and merge; instead, the beams stay evenly sectioned and spaced out, losing energy and dose deposition as the beams travel further (10).

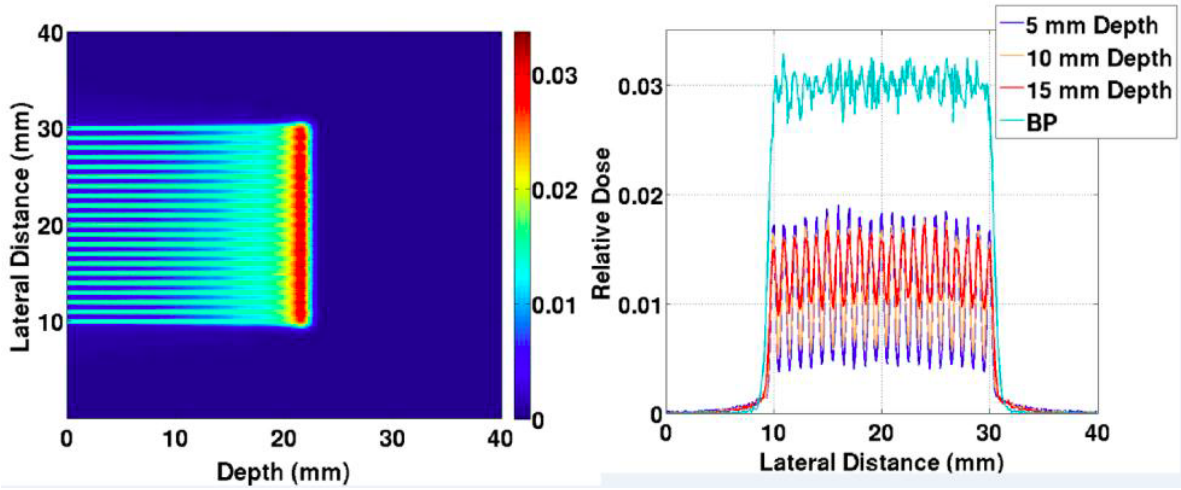


**Figure 5:** Monte Carlo simulation of proton minibeams at a uniform depth (a) and at multiple different depths (b) [11]

Figure 5 demonstrates the properties of the Bragg Peak region relative to the dose percentage via Monte Carlo simulation. The warmer the color, the higher the dose deposition in the target for Figure 5a. Coulomb Scattering is also evident in Figure 5a, with the minibeams converging at about a depth of 22 mm. Figure 5b provides another visualization of the dose deposition percentage relative to particle trajectory and depth. Depths of 5, 10, and 15mm are indicated, and the relative dose percentage at each depth is shown, and as expected, the highest dose concentration is at the Bragg Peak region.

Although the comparison of proton beams to conventional photon beams shows the apparent advantages of proton beams, there are only a few clinics that utilize proton beam therapy due to the cost being so high compared to standard photon radiation therapy in most clinics (7).

## Current Research Studies of Proton Minibeams

The main objective of this paper is to look at the advantages of proton minibeam therapy compared to conventional open beam photon radiation and to answer the question of whether or not the method of spatially modulated proton minibeams create even more tissue-sparing effects for high-risk brain tumors. As articulated in the previous sections of this paper, the benefits of taking spatially modulated proton beams have ameliorated the chances of proximal and exit site tissue survival during treatment.

Although proton minibeam radiation therapy presents results that could potentially have advantageous applications to the treatment of more high-risk tumors such as Glioblastomas and Medulloblastomas, the fruition of this treatment is still relatively new and has not yet reached clinical trials. Because of this, further studies on the behavior of proton minibeams have been performed in order to bring this treatment strategy closer to patient treatment trials and to determine if this type of treatment could provide an acceptable solution to the treatment of brain tumors.

### I. $\gamma$-H2Ax as an *In vitro* Study

A current study at the University of Washington Medical Center focuses on developing a method for quantifying the radiation damage done to biological tissue further to understand the behavior and properties of proton beam irradiation. This study utilized the concept of proton minibeams with hopes of furthering the process of getting them closer to clinical trials. Measuring radiation damage done on a cellular level in order to understand the properties of proton minibeams and how they interact with biological tissue is essential to understanding what happens to the tissue on a molecular level when exposed to radiation.

As discussed earlier, radiation alters the structure of DNA by breaking the double-helix exogenously. A cell's ability to heal and reproliferate after radiation toxicity determines the overall health and survival of biological tissue (7). When healthy cells are damaged by radiation, a variant of the H2A histone—a protein located in the nucleosome in which the DNA double helix wraps around—called $\gamma$-H2Ax gets phosphorylated. This means that a new phosphoryl group attaches to $\gamma$-H2Ax, creating a biomarker to the surrounding healthy cells signaling that there has been damage done to the sugar-phosphate backbone. The $\gamma$-H2Ax signal is the primary step to alerting the DNA repair proteins for damage control (12).

The objective of this study was to obtain an accurate measurement method of DNA damage done by radiation, specifically proton minibeam radiation. Because $\gamma$-H2Ax is so closely related to the impairment of DNA structure as a result of radiation, determining and measuring the number of $\gamma$-H2Ax signals, can provide accurate quantification and a visual assay of DNA damage caused by the proton minibeams, particularly at the entrance site where breaking up the beam initially has the potential to spare healthy surface tissue from radiation damage (13).

Because proton beams have the potential to be advantageous in treating high-risk tumors, especially intracranial tumors, cell cultures of Glioblastoma multiforme were used in this experiment. Glioblastoma multiforme is a grade IV astrocytoma tumor most commonly found in adults (1).

To begin the process, these Glioblastoma cell cultures were placed in glass slides, which were then placed in a water tank downstream from the proton beam radiation source. A brass collimator with submillimeter slits was placed in between the proton radiation source and the cell cultures to create the minibeams at the entrance site. Each slide was irradiated with proton beams

at various depths (0, 5, 10, 15 cm) to account for the gradual energy buildup and the Bragg Peak region (13).

Once the irradiation process was finished, each slide was then returned to incubation and treated with paraformaldehyde in phosphate-buffered saline and an anti-$\gamma$-H2Ax antibody. The nuclei of the irradiated cells were stained with 6-diamidino-2-phenylindole (DAPI), and the $\gamma$-H2Ax foci were stained with fluorescent tags (13).

Microscopy of all the irradiated and suitably stained cell cultures with about a 200-cell count for each image, was completed after the incubation period using a Nikon confocal microscope at the Fred Hutchinson Cancer Research Center. Capturing these images using a confocal microscope allowed for meticulous image analysis of the $\gamma$-H2Ax foci in the irradiated cells (13).

Each image was scanned using MATLAB software to count the average foci for each image, the control, the entrance site of the cells irradiated with the collimated beams creating "peaks" and "valleys," and the entrance site of cells irradiated with one continuous open proton beam.

Figures 6–9 below are examples of images taken by the confocal microscope that show the overlays of the $\gamma$-H2Ax signal on the irradiated Glioblastoma cell culture. Each sample was irradiated with 1 Gy at 50 MeV energy. Gy (Grey) is the unit of radiation absorbed by biological tissue (7).

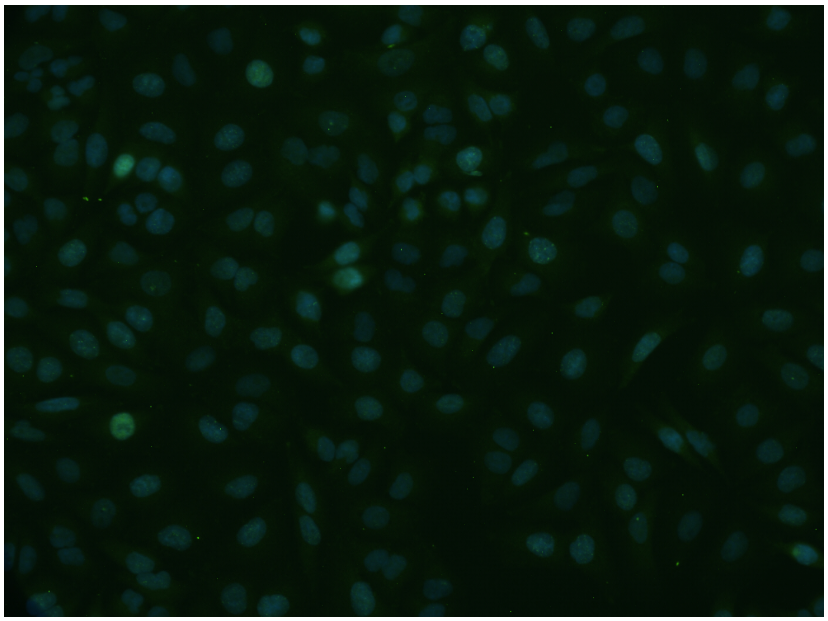

**Figure 6:** Human fibroblast cell culture of Glioblastoma multiforme irradiation control. Blue color indicates DAPI nucleus staining. No exposure to proton irradiation.

The control image for the proton minibeam irradiation of the Glioblastoma cell cultures is shown in Figure 6. The blue DAPI nucleus staining clearly defines the cells, and there are very few fluorescent green spots on these cells. This indicates that there has been no radiation exposure to these cells; therefore, no $\gamma$-H2Ax foci were formed, indicating DNA any double-strand breakage. The cells represented are relatively large, illustrating the healthy cells' ability to form colonies (7).

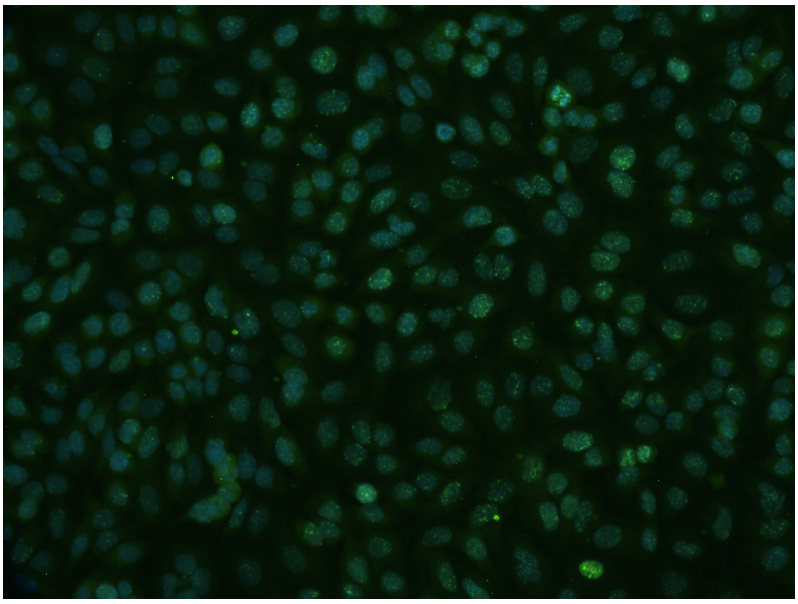

**Figure 7:** Human fibroblast cell culture of Glioblastoma multiforme irradiated with collimated proton beam at 1Gy and 50 MV at entrance site peak. DAPI blue indicates nucleus staining. Fluorescent green indicates $\gamma$-H2Ax signaling DNA double-strand breaks

The cell culture shown in Figure 7 was irradiated with proton minibeams at 1 Gy at an energy of 50 MV 5cm away from the radiation source. Figure 7 represents a snapshot of irradiated cells in the path of the proton minibeam, also called the entrance site "peak." It is exceedingly apparent that there is an increased amount of fluorescent tags in Figure 7 compared to the control cells in Figure 6. These fluorescent tags indicate the $\gamma$-H2Ax signal in response to the radiation damage. It is also noticeable that the size of the cells in Figure 7 are significantly smaller than those of the control cells in Figure 6. This accounts for the radiation inhibiting the cells' ability to colonize, resulting in smaller cells in more significant numbers (7).

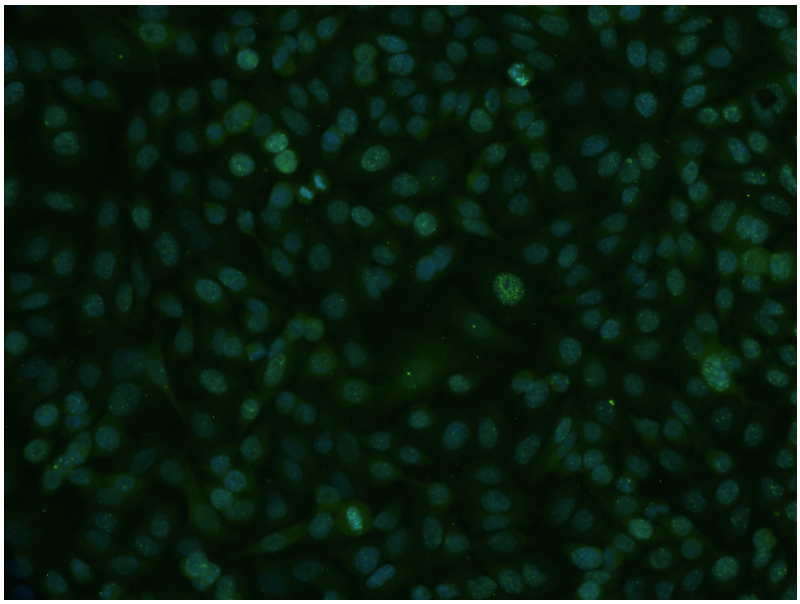

**Figure 8:** Human fibroblast cell culture of Glioblastoma multiforme irradiated with collimated proton beam at 1Gy and 50 MV at entrance valley site. DAPI blue indicates nucleus staining. Fluorescent green indicates $\gamma$-H2Ax signaling DNA double-strand breaks.

Figure 8 shows an image of a cell culture irradiated with proton minibeams at 1 Gy with an energy of 50 MeV at the entrance site 5 cm away from the radiation source. This snapshot exhibits cells that were in one of the "valleys" created by the brass collimator. This means that there was little to no radiation exposure. There are fewer fluorescent $\gamma$-H2Ax tags in Figure 8 compared to Figure 7, indicating that there are fewer DNA double-strand breaks meaning there was less cellular damage done to the cells in the "valley" regions. This supports the theory that the use of minibeams would spare proximal tissue at the entrance site by creating such "peaks" and "valleys." However, because there is still some radiation exposure, even at a marginal scale, the cells represented in Figure 8 are still smaller and in more significant numbers compared to the control cells of Figure 6, indicating that the cells could no longer colonize with each other.

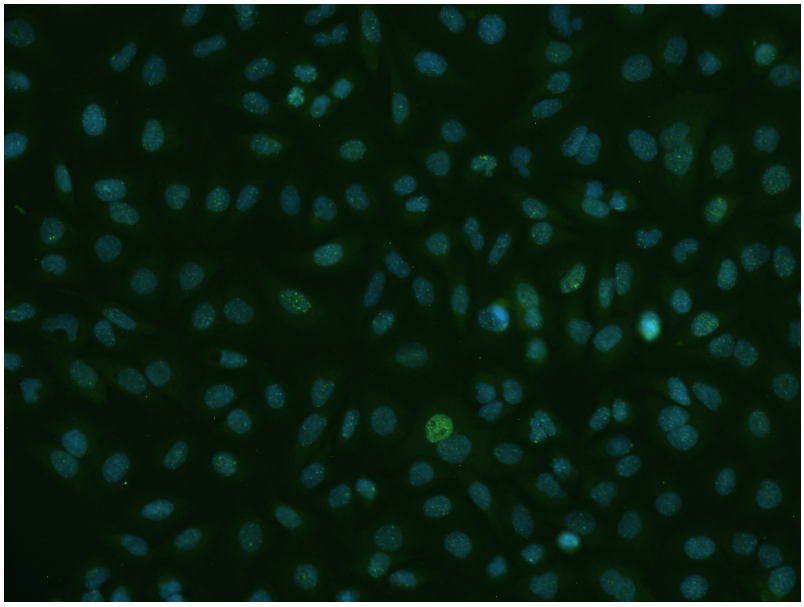

**Figure 9:** Human fibroblast cell culture of Glioblastoma multiforme irradiated with an open proton beam (without the use of a collimator) at 1Gy and 50 MV at the entrance site. DAPI blue indicates nucleus staining. Fluorescent green indicates $\gamma$-H2Ax signaling DNA double-strand breaks.

Figure 9 shows an array of cells irradiated with an open proton beam without the use of a brass collimator. The image represents cells close to the entrance site at 5 cm away from the open beam radiation source at 1 Gy with an energy of 50 MeV. There is radiation damage done to the cell culture by the presence of $\gamma$-H2Ax fluorescent signals, indicating DNA double-strand breakage. The images were then scanned using MATLAB software to count the average $\gamma$-H2Ax foci for each cell culture.

The phosphorylation of $\gamma$-H2Ax served as a visualization for the properties of proton beams interacting with a biological target, specifically for the human Glioblastoma cell cultures. Ultimately, the staining of the foci and the image analysis that followed allowed for a quantification of $\gamma$-H2Ax signals. This quantification led to the following determination that there is a 1:1 ratio of $\gamma$-H2Ax foci signal to DNA damage done by radiation, proving to be

analogous and providing a method to determine and quantify the amount of radiation damage done to a biological target, specifically in the case of proton minibeams.

## II. Radiochromic Film and Monte Carlo Simulation Study

Another current study published by Dilmanian et al. looked at the already advantageous properties of proton beams interacting with a biological target and took it one step further. There is no doubt that proton beams have incredible tissue sparing qualities, but what Dilmanian et al. (2015) were interested in was the idea of possible further improvements upon proton beams and proximal tissue sparing, especially at the entrance site of a patient. Because protons cease to irradiate and deposit energy immediately following the Bragg Peak region (Figure 3), there is hardly much concern for harmful radiation effects to the healthy tissue of the exit site of the patient. However, because protons slowly build up in energy deposition (Figure 3), there is still some risk to healthy tissue and vital organs near the surface of the patient at the entrance site of the beam even though the damage done is significantly marginal compared to other methods of radiation (14).

The types of cancer that are most sensitive to radiation exposure and are most susceptible to its harmful effects are those afflicting the craniospinal region, mainly head and neck tumors (14). Dilmanian et al. (2015) were primarily concerned with cancers affecting the brain, especially pediatric cases. These tumors, such as Medulloblastomas, are considered to be high-risk tumors, as discussed in the introduction section of this paper. Radiation damage done to the white and grey matter of the brain surrounding the tumor site is consequently more harmful compared to the damage done by low energy radiation at the entrance site of other more low-risk tumors and tumor locations.

In this particular study, Dilmanian et al. (2015) proposed the idea of breaking up the beam before the entrance site into small, planar minibeams, thus reducing the amount of radiation interaction with healthy proximal tissue at the entrance site. As a result of this further tissue sparing, treatment hypofractionation could be a potential consideration. Hypofractionation allows for a higher dose concentration for each treatment session, shortening the overall treatment period to only a few weeks or even days (7). The combination of applying increased dose to the tumor site and the shortened time frame for hypofractionated treatments intensify the rate of DNA damage via cell hypoxia and double-strand breaks and reinforces apoptosis to the cancerous tumor cells. The shortened time frame between treatment does not allow the tumor cells hardly any time to try to reproliferate. Escalating the dose concentration while concurrently decreasing the tissue toxicity because of the tissue-sparing effects of proton minibeams is precisely the type of radiation treatment suitable for the complex nature of the brain (14).

Proton minibeams, in theory, would create peaks and valleys of submillimeter beams of radiation by way of a brass collimator made up of 0.3 mm slits (in the case of this particular study) situated between the radiation source and the target. Due to the Coulomb Scattering discussed previously, these beams would broaden and merge at the Bragg Peak region, depositing the most energy (14).

In this study, Dilmanian et al. (2015) devised two methods to observe these proposed minibeams in order to determine the specific depth that the minimbeams began to converge to a single beam once more by determining the full width at half maximum (FWHM), a measurement of the beam width at half of its height (depth in this case), of the minibeams compared to the depth of the target. Ideally, the FWHM should increase as the beams travel to a farther depth, ultimately converging to a larger singular beam at the Bragg Peak.

The first technique to measure the FWHM of the proton minbeams implemented the use of radiochromic film, another method of measuring radiation dose. Radiochromic film is made up of two polyester bases with an active layer of crystalline elements that, when exposed to ionizing radiation, the color of the crystalline elements darkens, which becomes visible on the film (Figure 10). The darker the film, the higher concentration of radiation (15).

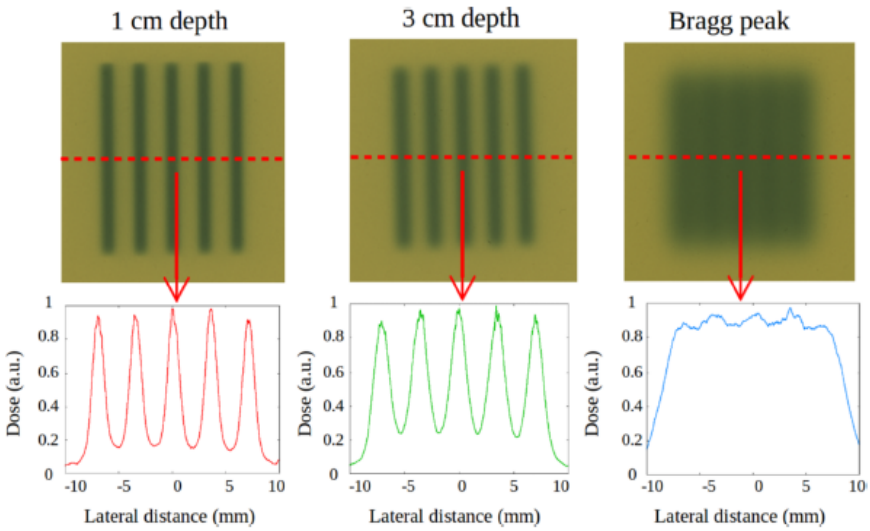


**Figure 10:** Measurement and visualization of the properties of proton minibeams using radiochromic film at different depths. The dark blue color indicates radiation exposure and monitors the transition of the minibeams merging back to a homogeneous single beam at the Bragg Peak region [16].

A proton beam at 109 MeV with a 10 Gy height dose split up into minibeams by a brass collimator with slits of 0.3 mm width was set between the source and the film targets (14). The radiochromic film was spaced out at different distances to measure the FWHM of the beams and visually determined the distance at which they started to converge. Figure 10 gives a representation of a similar study done by Peucelle et al. (2015), in order to visualize the properties of spatially modulated proton beams.

The second technique of measuring the FWHM of the proton minibeams used a Monte Carlo simulation called the Monte Carlo N-Particle System (MCNPX) (14) which simulated a proton minibeams at an initial 0.3mm width with the same dose and energy as used in the radiochromic film study; however, the "target" in this simulation is water, which is more comparable to biological tissue than radiochromic film (14).

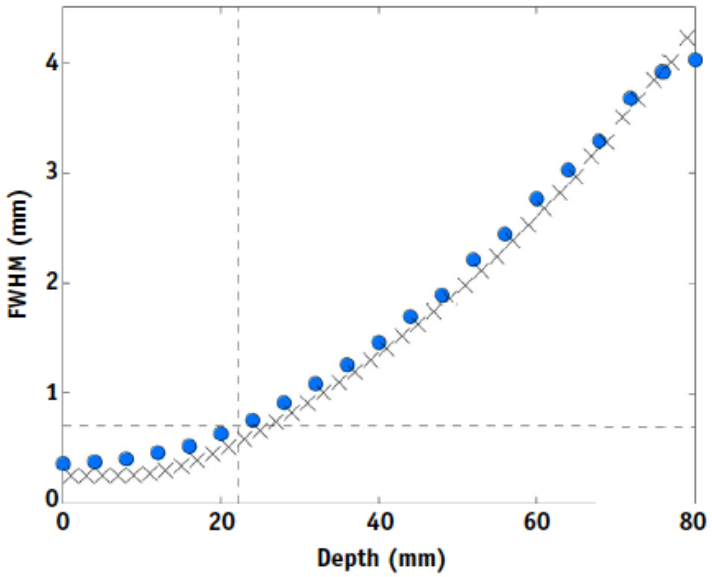


**Figure 11:** The full width half-maximum (FWHM) rate of change in relation to target depth. The proton beam broadening increases exponentially as depth increases. Proton beam at 109 MeV for both studies. The radiochromic film represented by the blue circles and the Monte Carlo simulation represented by the grey x's [(14)].

Figure 11 shows the results of the radiochromic film and the Monte Carlo simulation studies, measuring the FWHM as a function of the target depth in each. The chosen FWHM measurement to indicate the start of the minibeam merging by Dilmanian et al. (2015) was 0.7 mm. This is the depth at which the beams began to converge, therefore reducing the tissue-sparing effect if tested on a biological target.

Both studies indicate that the proton minibeams began to merge at a significant rate around a depth of 22 mm for the radiochromic film data and 23.5 mm for the Monte Carlo simulation data (14), indicated by the dotted line in Figure 11. These depths represent the depth of the target that would be affected by the tissue-sparing properties of the proton minibeams. Any depth after about 22 mm increases the probability of healthy tissue damage in a biological specimen.

Dilmanian et al. (2015) also investigated the properties of other ions in comparison to protons, such as Helium (He) and Lithium (Li), and their FWHM concerning the target depth using the same Monte Carlo simulation in water (MCNPX) (14) as shown in Figure 12. The protons represented in Figure 12 are denoted as Hydrogen (H) because an ionized Hydrogen (H+) atom is just a proton.

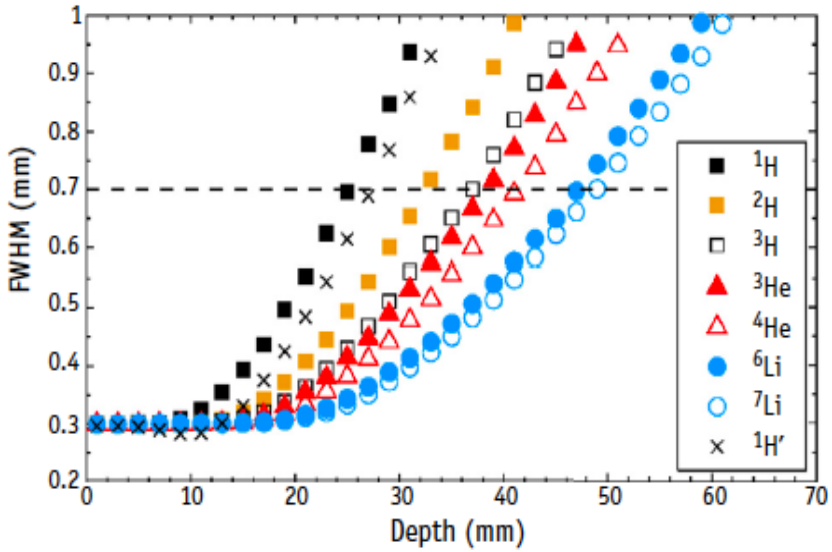


**Figure 12:** The full width half maximum (FWHM) rate of change in relation to target depth. The proton and ion beams broadening increases exponentially as depth increases. The scattering threshold still at a value of 0.7 mm [(14)]

Just as the previous study using radiochromic film and MCNPX dosimetry with proton minibeams demonstrated that at a FWHM of 0.7 mm was reached at a target depth of around 22 mm, so too do the simulated proton minibeams in the study illustrated in Figure 12. It is also quite noticeable that the FWHM measurements relative to the target depth increase in regard to the He and Li. These ions were able to travel much further before the modulated minibeams began converging with a FWHM of 0.7mm, with depth values in the 30 mm range for He and in the 40 mm range for Li ions. The deeper depth of beam convergence is a result of the inverse relationship between these ions and the particle momentum of each (17).

**Conclusions and Future Work**

The human brain is the most complex organ in the human body, serving as the driving force of neurocognitive functions. There is still much to be discovered about the behavior and elusive nature of the brain, so complications emanate when presented with illnesses such as intracranial tumors. Intracranial tumors are extremely high-risk because the brain is so intricate and because it harbors the control over all bodily functions, so any damage done to the brain is damage done to the body. Any form of treatment—whether it be surgery, chemotherapy, or radiation therapy—is incredibly invasive with the added risk of damaging surrounding healthy brain matter.

However, with the proposition of a novel method of radiation treatment, proton minibeam radiation therapy (pmbRT), there affords the opportunity of radiation treatment for high-risk intracranial tumors such as Medulloblastomas and Glioblastomas. Because protons exhibit properties such as the Bragg Peak and a gradual increase in dose concentration when deposited in a target, there lies the potential for healthy tissue sparing at the beam entrance and exit sites of

the patient. Furthermore, the method of proton minibeams could prove to further increase the sparing of healthy tissue, especially at the entrance site, in tissue closest to the surface. Due to Coulomb Scattering, another property of proton beams, these minibeams would begin to merge as the beam travels further in-depth, eventually returning to a homogenous beam at the Bragg Peak region.

Although proton minibeam therapy has not yet made it to clinical trials to this day, several studies are being performed to analyze and quantify the behavior of such proton minibeams all aiming to answer the question of whether or not this new type of radiation could be beneficial to brain tumor treatment and management, correcting the shortcomings present in currently utilized methods of radiation therapy such as X-rays.

A study executed by a clinical research team at the University of Washington Medical Center determined that the phosphorylation of the biomarker $\gamma$-H2Ax in Glioblastoma cell cultures was analogous to DNA damage done by the breakage of the double-helix strand via radiation on a 1:1 ratio. By fluorescently marking the $\gamma$-H2Ax foci, the amount of proton minibeam radiation done was able to be visualized, and the average quantified on the images, and the "peaks" and "valleys" of radiation created by the minibeams at the entrance site were very distinct. The average $\gamma$-H2Ax signal at the entrance region for the minibeams was less than the average signal at the entrance region for an open proton beam. This *in vitro* study of $\gamma$-H2Ax as a biomarker for DNA damage proved that proton minibeams afflict less damage to proximal tissue than even a homogenous proton beam.

Dilmanian et al. (2015) also studied the effects of proton minibeams via radiochromic film and a Monte Carlo simulation. The overall objective was to determine the depth at which the proton minibeams began to converge, due to Coulomb Scattering, and return to a single

uniform beam at the Bragg Peak region. Proton minibeam's tissue sparing properties are determined by the depth at which the minibeams remain under 0.7 mm full width at half maximum (FWHM). According to Dilmanian et al. (2015), the tissue survival rate began to decrease at any width larger than that. Both the data from the radiochromic film study and the Monte Carlo simulation yielded a depth of about 22 mm before the minibeams began to merge. Augmenting the survival rate of about 22 mm of healthy tissue in a biological target is exceptionally advantageous for the treatment of any tumor type, especially brain tumors. Dilmanian et al. (2015) confirmed that proton minibeams have the potential to provide a beneficial treatment to brain tumors.

Even though proton minibeam therapy has not yet been introduced to clinical trials just yet, there is more than enough evidence to show the undeniable fact that proton minibeams have exceptional healthy tissue sparing properties. Specifically, this method of treatment promises to affect even poorly understood brain functions, preserve remaining minimally and otherwise, healthy brain tissue, reduce the adverse side effects of radiation exposure, and improve the long-term wellbeing of patients. As a result, this treatment potentially meets the demands of even the most high-risk tumors, such as pediatric Medulloblastomas, where the patient's limited brain development and overall neurocognitive vulnerability have historically led to less-than-favorable outcomes.

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