UNIVERSITY OF CALIFORNIA,
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The Effects of Sequential Treatments on Hippocampal Volumes in Malignant Glioma Patients

THESIS

Submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical and Translational Science

by

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DEDICATION

To

My family and friends who have continued to support my goals. Thank you for your love and laughter, which keeps me grounded and pushes me forward. Moreover thank you for instilling in me that no mountain is too high to climb, and no dream is too far out of reach. A very special thank you to my mother who has been the light in my life and to my father who actively invests in everything I do.

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LIST OF ABBREVIATIONS

MG = Malignant gliomas

CRCI = Cancer-related Cognitive Impairment

QOL = Quality of Life

UCIMC = University of California, Irvine Medical Center

AA = Anaplastic Astrocytoma

GBM = Glioblastoma

VEGF-A = Vascular Endothelial Growth Factor A

MCI = Mild Cognitive Impairment

AD = Alzheimer's Disease

MRI = Magnetic Resonance Imaging

MIPAV = Medical Image Processing, Analysis and Visualization

SEM = Standard Error Mean

APC = Annual Percent Change

WBRT = whole-brain radiation

FBRT = focal brain radiation

RR = Relative Risk

WHO = World Health Organization

FDA = U.S. Food and Drug Administration

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The text of this thesis/dissertation is a reprint of the material as it appears in the Journal of Neuro-oncology. The co-authors listed in this publication directed and supervised research which forms the basis for the thesis/dissertation.

ABSTRACT OF THE THESIS

The Effects of Sequential Treatments on Hippocampal Volumes in Malignant Glioma Patients

By

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Master of Science in Biomedical and Translational Science

University of California, Irvine, 2016

Associate Professor Daniela Bota, MD, PhD, Chair

Objectives: Malignant gliomas (MG) are very aggressive tumors. Patients receive multi-modal therapies such as surgery, radiation and chemotherapy [temozolomide (Temodar or TMZ) followed in many cases by bevacizumab (Avastin)] to improve their likelihood of survival. The survivors are affected by multiple learning and memory deficits referred to as cancer-related cognitive impairment (CRCI). Greater deterioration over time in hippocampal specific cognitive tasks was shown in patients receiving bevacizumab in addition to radiation and temozolomide for a longer period of time (RTOG 0825). However the rate of hippocampal atrophy following treatment using these multi-modal therapies has not yet been determined. The goal of this study is to determine the rate of hippocampal atrophy in patients treated with radiation and temozolomide followed by bevacizumab.

Methods: We used the serial MRIs obtained as parts of standard clinical care in patients with MG. Measurements were done using the Medical Image Processing, Analysis and Visualization (MIPAV) software. The hippocampus in the contralateral hemisphere was manually traced and

measured, to avoid morphological structure changes induced by the tumor, radiation fields or surgical markers.

Results: Retrospective analysis of 13 patients being treated for recurrent brain tumor revealed a longitudinal progression of hippocampal atrophy, with a maximum volume loss of 33.3% for patients on treatment for up to 5 years. There was no detectable hippocampal atrophy during the chemo-radiation followed by adjuvant temozolomide. A significant decrease in the absolute hippocampus volume was noted after 6 months of continuous bevacizumab treatment ($p<0.05$) and progressed over the next three years. Our overall rate of hippocampal atrophy is higher than the one previously reported in Alzheimer disease patients.

Conclusions: The loss of hippocampal volume is minimal during the first months after diagnosis, when the patients receive chemo-radiation and adjuvant temozolomide. However, prolonged treatment and bevacizumab is associated with a significant rate of hippocampal volume loss.

Chapter 1

INTRODUCTION: Why Study Brain Cancer?

Cancer is the fifth leading cause of death worldwide and second in the US. Brain cancer accounts for 1.8% of newly diagnosed cancers and is the 22nd most common cancer in the world. Approximately 700,000 people in the US are living with a primary brain tumor, with almost 78,000 new cases reported annually.[25] Furthermore another 20 – 40% of other primary cancers will metastasize to the brain.[26] The incidence of brain tumors is most frequent in whites, males, developed industrial countries, metropolitan counties, and older age groups.[27]

Brain cancer is a rare, but virulent disease. It is the second leading cause of death in children and ranks 12th overall in mortality for all cancers. The percentage of people who will survive 5 years after diagnosis, also referred to as the 5-year survival rate, is lower for brain cancer (37%)[29] as compared to other more common cancers such as breast (89%)[28], prostate (99%)[28], lung (54%)[29], ovarian (46%)[28] and colorectal (90%).[28] However, survival rates will vary per patient because the prognosis for brain cancer is largely contingent on the grade and type of tumor. Newly diagnosed cases of lower-grade tumors have a median survival of 6 to 8 years.[25] High-grade tumors have a median survival of 14.6 months to 3 years.[25] In the last decade, rates of survival have increased an average of 0.2% per year[29] **(see Table 1.1)** with the development of new surgical instruments and targeted chemotherapy drugs.

Table 1.1 Brain Cancer 5-Year Relative Survival by Year

Year	1975	1980	1985	1990	1995	2000	2004	2008
5-Year Relative Survival	22.8%	22.9%	24.7%	28.4%	33.3%	34.7%	35.0%	35.7%

*This table is from the SEER website. It shows age-adjusted rates of Seer 9 Incidence and Mortality (1975-2013). Rates include all Races and both sexes.

There are more than 120 different types of brain tumors. The US Drug and Food Administration (FDA) have approved only 4 drugs and one medical instrument for the treatment of specific types of tumors. However, for most brain tumors the standard of care will be surgery followed by radiation. Many patients experience adverse physical, psychological, and sociological effects during and after treatment as a result of their medical care.[30] The likelihood of experiencing these negative outcomes is largely dependent on the length of survival after diagnosis and the type of therapy and medications they undergo. Patients who survive longer may experience greater adverse effects because they are exposed to cancer treatments for substantially longer periods of time. For most patients these adverse drug effects can have significant negative influences on quality of life (QOL).[30]

QOL is a subjective evaluation of an individual's life satisfaction. It can be measured in a few different ways depending on the population. In neurodegenerative populations, measures of cognitive function are a strong indicator of QOL.[31] It is common for patients with brain tumors to suffer from cognitive impairment.[32] Decreased mental performance has been shown to lower QOL. Therefore brain cancer patients may experience lower quality of life as a result of their reduced cognitive function. Cognitive impairment can stem from deleterious changes to

areas of the brain that affect its functionality. The literature has shown varied anatomical changes in the brain of patients with brain tumors after exposure to multimodal treatments.[3,9,14,21] This structural brain damage if sustained over long periods can cause cognitive impairment and severely decrease QOL. The long-term impact of cancer drugs and their potential for neurotoxicity and brain injury need to be further studied.

The threat of adverse drug effects and brain injury to cognitive performance and QOL is growing as people continue to live longer after brain cancer diagnosis. A patient's cognitive function, specifically in areas of memory and learning, is at risk for deficits. Life-saving cancer care and increased lifespan shouldn't create further disability, nor should it stimulate the loss of brain function or lessen a patient's freedom to enjoy simple everyday activities. Our study will examine changes in the brain caused by cancer treatments and its relationship to cognitive impairment. We concentrate specifically on the hippocampus, which is the primary area of the brain responsible for memory and learning.

We conducted a retrospective cohort study of patients with recurrent malignant gliomas from the University of California, Irvine Medical Center's (UCIMC) brain tumor clinic. Our goal was to determine whether hippocampal atrophy occurred in patients being treated for a primary brain tumor for at least 18 months. We hypothesized that hippocampal atrophy occurs in patients being treated with long-term chemotherapy, which might be linked to cognitive impairment in this population. We performed manual tracing of the contralateral side of the hippocampus, from the start of diagnosis to the end of life or end of the study period, using magnetic resonance imaging (MRI) of the patients brain. Statistical analysis was used to determine the rate of hippocampal atrophy by percent and absolute volume.

Chapter 2

BACKGROUND: Brain Cancer At a Glance

2.1. Global Burden of Brain Cancer

The incidence of brain cancer is low compared to most other cancers. Brain tumors are less than 2% of the global cancer burden. Rates are unevenly distributed throughout different geographical regions across the globe. The majority of cases are located in developed countries like the USA, Canada, Australia, and regions of western and northern Europe.[27, 34] There are more cases of men with brain tumors than women and whites have higher incidence rates compared to African Americans and Asians.[27, 34] Moreover, people aged 55 and older are more likely to be diagnosed with a primary brain tumor.[27, 34] The etiology of why certain groups seem to be more susceptible to brain tumors is not yet understood.

Despite low brain tumor occurrence, mortality in this population is higher compared to more common cancers. In developed countries like the UK and Australia where incidence rates are high, brain cancer will kill more people under age 40 than any other cancer.[35, 37] In the last 30 years survival rates in Australia from brain cancer have gone up only 2% as compared to 19% for all cancers.[36] Moreover in the UK brain tumors account for 20 years of potential life lost (YPLL), which by definition is the amount of time a person could have survived if they did not die prematurely.[35] Additionally, brain tumors will kill more men under 45 and woman under 35 than any other cancer, including breast and prostate cancers.[35] Brain cancer prognosis is met with severe difficulties. The complexity and virulence of brain tumors deserve further study.

There is no known discernible cause of brain cancer. Risk factors include age, compromised immune system, chemical exposure, radiation, and genetics.[33] However the

extent of one's risk is largely dependent on the individual and difficult to distinguish by group. As a result there are no protective measures or screening tools for early detection and prevention of brain cancer. Brain tumors are difficult to diagnose. Patients may present with headaches, impaired speech or vision, personality changes, nausea or vomiting, and fatigue prior to diagnosis before seeing a physician. Improvements in neuroimaging have helped improve the identification of brain tumors. Neuroimaging is also advantageous when tracking brain tumor progression over the course of a patient's treatment.

Treatments for brain cancer and the brain tumors themselves can result in negative outcomes. These outcomes include fatigue, nausea, pain ad many others. A more specific outcome is cancer-related cognitive impairment (CRCI) also referred to as chemo brain. CRCI describes cognitive problems in executive functioning, which result from cancer treatment. This includes but is not limited to problems in organization, attention, inhibition, learning and memory. The burden of cognitive impairment in brain cancer patients is a global problem.

2.2. Types of Brain Tumors

For every category of cancer, tumor types are specific to location in the body and the type of cell from which the tumor is derived. There are three main types of cells that can become cancerous. Cancers from epithelial cells are called carcinomas and make up 80-90% of cancers. Leukemias and lymphomas are derived from cells in the blood and lymphatic system. They make up 7% of all cancers. Lastly, 1% of cancers will be sarcomas, derived from connective tissue cells. The majority of brain tumors with come from cells in the latter category. These cells

are called glial cells, which make up the surrounding nervous connective tissue in the central nervous system (CNS).

Glial cells are the most abundant cell type in the CNS. They function as support for the nervous system and aid in the preservation and nutrition of neurons. There are multiple subtypes of glial cells. Tumors derived from any of these subtypes are called gliomas. Gliomas are the most common types of brain tumors, making up 55% of brain tumor cases. Within this group are astrocytomas, derived from the glial cell type astroctyes. Astrocytomas make up 80% of diagnosed gliomas. The World Health Organization (WHO) has classified astrocytomas into four separate tumor grades based on parameters such as malignancy, histology, growth rate, and location. **Figure 2.1** shows the WHO classification of brain tumors for four astrocytomas. Grades I and II are low-grade gliomas (LGG) and grades III and IV are high-grade gliomas (HGG). In some cases LGG can evolve to become HGG over time. In this study we look at two specific types of high-grade astrocytic gliomas (anaplastic astrocytoma (AA) and glioblastoma (GBM)). AA is a rare tumor type whereas GBM is the most common and most aggressive type of brain tumor.

Table 2.1 WHO Classifications of Brain Tumors

Tumor Grade	WHO Classification	Description	Median Survival
Grade I	Pilocytic Astrocytoma	Benign, common in children and young adults, long term survival	
Grade II	Diffuse Astrocytoma	Slow growing, common in children and young adults, can recur as higher-grade	6 to 8 years
Grade III	Anaplastic Astrocytoma (AA)	Malignant, grows quickly and spreads to nearby tissue, high rate of recurrence	2 to 3 years
Grade IV	Glioblastoma (GBM)	Most common glioma, grows fast, easily spreads, poor prognosis, tumors have necrosis, vascular proliferation	14 months to 2 years

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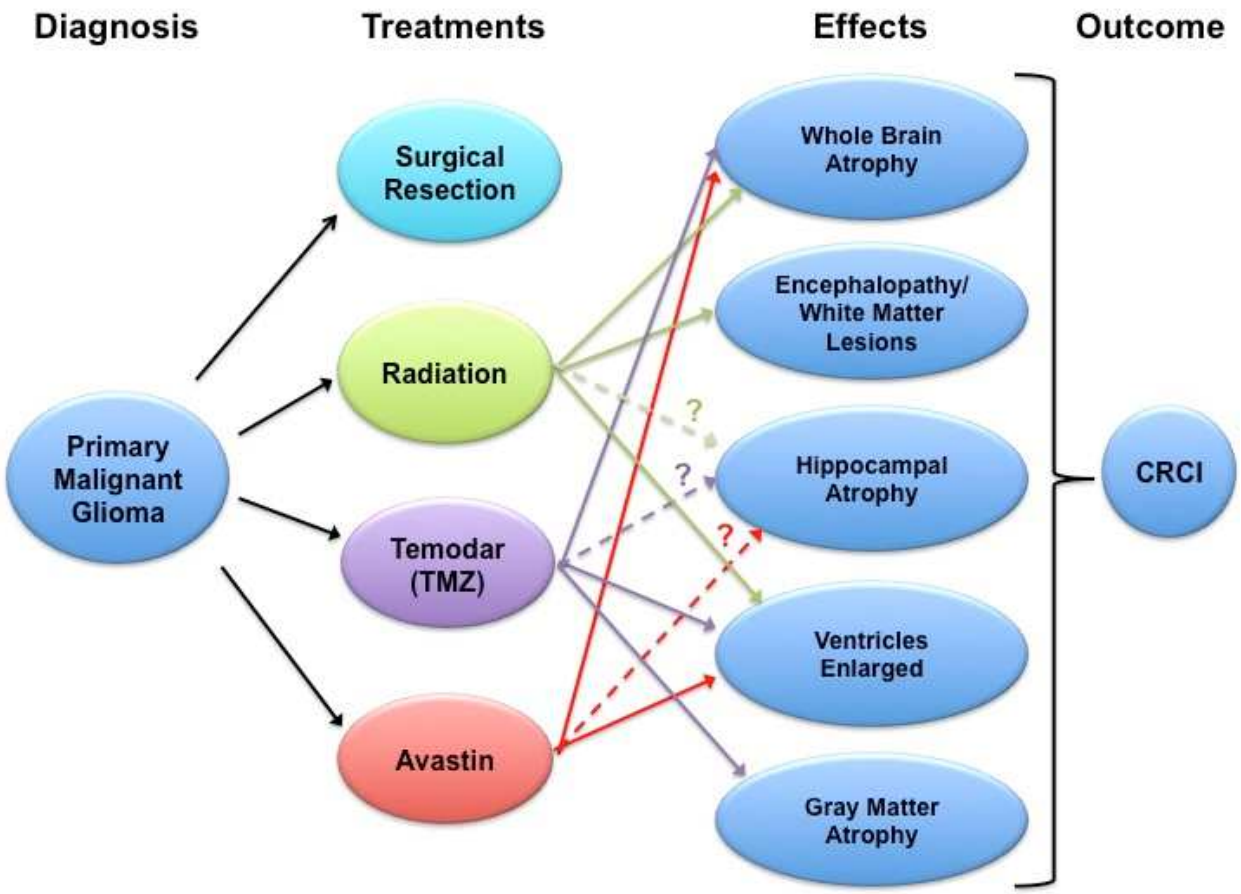
2.3. Glioma Treatment and Cancer-related Cognitive Impairment

Treatment advances have led to prolonged survival for patients with high-grade astrocytic gliomas. Long-term survivors will be exposed to more harmful drugs over longer periods of time. Therefore it has become progressively more important to study long-term treatment effects on the brain, which could reduce cognitive function in long-term survivors. **Figure 2.1** is a conceptual model illustrating how the diagnosis of a malignant glioma can result in CRCI. An explanation of the model separated into 3 steps can be found below:

Figure 2.1 Conceptual Model of Brain Cancer to CRCI



Conceptual model explanation:

(1) Following the diagnosis of a primary malignant glioma, the patient undergoes multimodal treatments. The first treatment is surgery to remove the tumor or reduce its' size without causing considerable damage to normal brain tissue. The second treatment is radiation to slow or prevent tumor growth and to kill the excess cancer cells leftover from surgery. Radiation is often given concurrently with chemotherapy. The third treatment temozolomide is a chemotherapy drug, FDA approved in 2005, for the treatment of primary malignant gliomas. Chemotherapy when used simultaneously with radiation or alone after radiation is complete is used to destroy cancer cells and reduce tumor growth and cancer cell division. The fourth

treatment is bevacizumab, a targeted drug therapy, FDA approved in 2009 for the treatment of recurrent malignant glioma. This means that bevacizumab is only used after other first line therapies have failed and the tumor has progressed. Bevacizumab slows tumor growth by targeting the blood vessels that cancer cells need to grow and invade nearby tissues.

(2) The model lists four potential areas of the brain (whole brain not included) at risk for adverse effects following brain tumor treatment: Grey matter, white matter, ventricles and hippocampus. Each area plays a part in brain communication and the control of physical, emotional, and mental functioning. Radiation, temozolomide and bevacizumab have been shown in the literature to cause damaging effects to the whole brain, gray matter, white matter, and ventricles. The evidence to support which treatment contributes more to adverse brain changes is mixed. The information is summarized in the conceptual model. The solid arrow lines from treatments to effects indicate that the effect was caused by the treatment. For example, bevacizumab causes whole brain atrophy and enlarged ventricles.[14] The dotted arrow lines indicate there has been no proof of a causal relationship between the treatment and the effect in the literature. For example, bevacizumab has not been shown in the literature to cause hippocampal atrophy. More detailed information on brain damage caused by radiation, temozolomide, and bevacizumab is in subsequent paragraphs.

(3) Lastly, the effects listed on the model are all potential contributors leading to the CRCI outcome, although it has not yet been proven in brain cancer populations. The loss of brain volume in essential locations used for cognitive functioning interferes with patient mental performance, by changing the composition and framework of their brain.

Radiotherapy

Radiotherapy can induce cognitive decline[2] and in extreme cases can cause dementia[3]. Early studies on radiotherapy in patients with gliomas or metastatic brain tumors found significant whole brain atrophy, white matter abnormalities and enlargement of the ventricles 3-8 months post radiation[3]. Cognitive impairment presented prior to discovering changes in brain pathology. Any adverse effects to the brain induced by radiation did not progress past 8 months[3]. More recent articles have reported similar reports of delayed toxicity induced by radiation. M.H.J. Swennen et al describes an increased risk for brain atrophy, white matter abnormalities and clinical encephalopathy after whole-brain radiation (WBRT) for low-grade gliomas.[21] Age and the amount of radiation are listed as additional risk factors. With the risk being greater in older adults compared to younger adults and in patients who had WBRT versus focal brain radiation (FBRT). Although there is substantial evidence to conclude radiation induces brain atrophy and causes cognitive deficits, there are also competing studies that suggest radiation does not cause cognitive impairment[20]. Alternatively the use of radiation with adjuvant chemotherapy drugs could be the source of brain atrophy and cognitive impairment.

Temozolomide (Temodar or TMZ)

The chemotherapy drug temozolomide is used as a first line treatment against malignant gliomas. It acts as an alkylating agent to damage DNA and prevents the growth or division of cancer cells. However, because of the cytotoxic nature of the drug, the use of temozolomide with radiotherapy enhances neurotoxicity and contributes to further cognitive deficits[4]. A recent study followed 14 patients with a newly diagnosed glioblastoma who were

being treated with TMZ alone for 6 months post-radiation. They found significant changes to whole brain, gray matter, and ventricle volume[14]. No significant changes were observed for white matter or hippocampal volume[14]. The onset of brain atrophy and ventricular enlargement was a delayed response to treatment and continued to progress until 6 months[14]. Although the study did not examine the association between structural brain changes and cognitive function, we can infer from their results that the toxicity induced from temozolomide plays a role in the observed cognitive impairment in glioma survivors. The results of this study presented evidence to show that the treatments for brain tumors have damaging effects to the brain. However, 6 months is not a sufficient amount of time to examine the effects of radiation and temozolomide on long-term survivors, surpassing the median survival time. Further studies are needed on the subject of long-term survival.

Bevacizumab (Avastin)

Bevacizumab is commonly used in patients with recurrent malignant gliomas that have failed temozolomide[5]. Bevacizumab is a monoclonal antibody against vascular endothelial growth factor A (VEGF-A)[6]. VEGF-A stimulates tumor angiogenesis, but it also promotes neurogenesis in the brain, particularly in the hippocampus, effecting fundamental processes needed for learning and memory[7]. Therefore, bevacizumab might contribute to cognitive impairment, especially in patients receiving this agent for prolonged periods of time[8]. Previous research (Bag A.K. *et al*.) suggested that high-grade glioma patients using bevacizumab experience a significant increase in ventricle volume over time as well as a significant decrease in whole brain volume and grey matter volume[9]. However, the authors were unable to segment

grey matter, white matter, or hippocampus due to the poor contrast resolution of their images[9]. Further exploration of treatment effects from bevacizumab is needed to understand how the functionality of bevacizumab impacts the hippocampus. This may help explain why survivors of brain cancer experience cognitive impairment in learning and memory.

Treatment Conclusion

The conceptual model demonstrates that although multiple changes to the brain have been found following treatments for malignant glioma, none of the previous literature has been able to detect hippocampal atrophy. However, multiple deficits in memory and learning are observed in brain tumor patients. The hippocampus role in memory and learning suggests the brain should suffer hippocampal atrophy after exposure to brain cancer drugs. The biggest limitations to measuring hippocampal atrophy in previous research studies have been the quality of their images and the length of the study. Our study will examine the hippocampus over a longer treatment period using high quality brain imaging, with the hypothesis that hippocampal atrophy occurs later in treatment.

2.4. Hippocampal Atrophy and Cognitive Impairment in Other Populations

Cognitive impairments in brain tumor patients resemble clinically those seen in other neurodegenerative conditions such as mild cognitive impairment (MCI) and Alzheimer's disease (AD). Hippocampal atrophy is also observed in patients with neurodegenerative conditions. The process of hippocampal atrophy (both expressed as an absolute loss of hippocampal volume, as well as the percentage of volume loss each year) is more severe in the MCI and AD patients

compared to the normal population.[10, 11] Assuming that the mechanisms behind hippocampal atrophy and cognitive impairment are associated with one another, suggests hippocampal atrophy might be present in brain tumor patients with cognitive impairment. The rate of which is higher than what is observed in normal aging.

There is a normal rate of hippocampal atrophy associated with normal aging. **Table 2.2** lists the rates of hippocampal atrophy in neurodegenerative populations and normal aging. Jack C.R. et al (2011) showed that annual hippocampal atrophy in MCI (3.0%) and AD (3.5%) higher compared to normal aging (1.9%). For every the patients with more cognitive impairment had greater hippocampal atrophy. If long-term patients exhibit more cognitive impairment than patients treated for shorter periods of time, the question of whether or not similar findings of hippocampal atrophy can be identified in long-term survivors of malignant gliomas as opposed to other survivors from previous studies is the focus of the present research study.

Table 2.2. Published Rates of Hippocampal Atrophy in Memory Disorders.

Pathology	Study	Methodology	Imaging	% Annual atrophy		
Normal				Total	Stable	Decliner/Converter
	Jack,C.R., et al[16]	Cohort	Manual Tracing	1.55		
	Jack,C.R., et al[11]	Cohort	Manual Tracing	1.9	1.7	2.8
	Jack,C.R., et al[17]	Cohort	Manual Tracing		1.4	3.3
	Schuff, N., et al[18]	Cohort	High-dimensional fluid transformation algorithm	0.3-0.97		
Mild cognitive impairment (MCI)				Total	Stable	Decliner/Converter
	Jack,C.R., et al[11]	Cohort	Manual Tracing	3.0	2.5	3.7
	Jack,C.R., et al[17]	Cohort	Manual Tracing		1.8	3.3
	Schuff, N., et al.[18]	Cohort	High-dimensional fluid transformation algorithm	2.0-3.07		
Alzheimer's disease (AD)				Total	Slow P	Fast P I
	Jack,C.R., et al[16]	Cohort	Manual Tracing	3.98		
	Jack,C.R., et al[11]	Cohort	Manual Tracing	3.5		
	Jack,C.R., et al[17]	Cohort	Manual Tracing		3.0	3.6
	Ridha, Basil H., et al[19]	Randomized Case Control	Manual Tracing	3.43		
	Schuff, N., et al.[18]	Cohort	High-dimensional fluid transformation algorithm	3.3-5.77		

* ***"****Total"* represents the reported overall % atrophy for all the patients in study. *"Stable"* are patients whose health status did not change. *"Decliner/Converter"* are patients whose health status changed. *"Slow P"* stands for slow progressor and are patients whose disease progressed slowly. *"Fast P"* stands for fast progressor and represents patients whose disease progressed quickly.

Chapter 3

METHODS

3.1. Study Aims

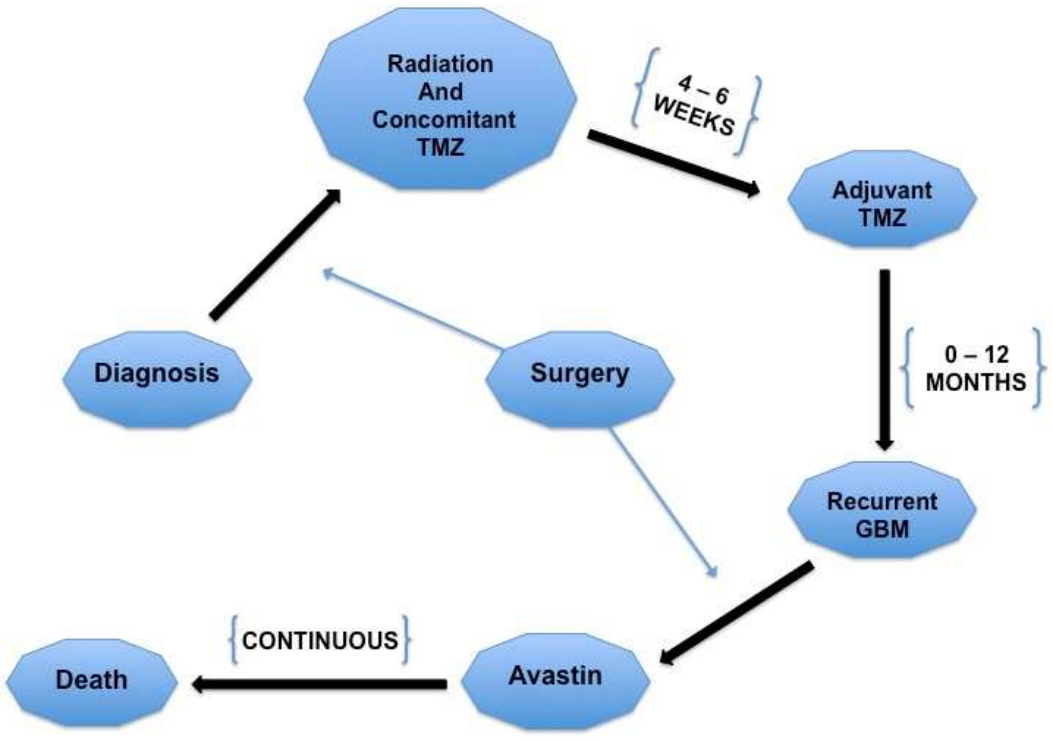
Previous research has identified multiple adverse effects in whole brain, gray matter, white matter and ventricle volume as a result of cancer treatments such as radiation, temozolomide and bevacizumab. However, previous studies have yet to identify changes in hippocampal volume. An indicator of hippocampal volume loss in brain cancer patients is largely attributed to deficits in learning and memory. This study has 2 aims. The primary objective is to identify and measure hippocampal volume loss in patients on treatment for a malignant glioma. Quantitative measures of hippocampal atrophy will be separated into two primary outcome measures: annual absolute hippocampal volume loss and annual percent hippocampal volume loss. The decision to measure hippocampal atrophy in two ways is based on the data reported previously in the MCI and AD studies[10, 11] The secondary objective is to identify any potential confounding or covariate variables that influence rates of hippocampal atrophy. As well as determine if the amount of a specific treatment given, greater influences hippocampal atrophy compared to the length of overall time a patient is treated.

Two limitations in the current literature are the quality of brain imaging and the length of the study period. Because patients on treatment for longer periods of time experience more cognitive deficits, this study will examine better quality high definition images of long-term survivors of malignant gliomas and determine when hippocampal atrophy starts to occur in this study population.

3.2. Study Setting

The study was conducted at the University of California, Irvine Medical Center's (UCIMC) Comprehensive Brain Tumor Clinic in Orange, CA. The current standard of care **(Figure 3.1)** at UCIMC to treat malignant gliomas is surgery to resect the tumor followed by 4 to 6 weeks of radiation with concurrent Temozolomide. Then patients receive adjuvant temozolomide for 12 months or until tumor progression. After recurrent tumor patients are treated with Bevacizumab. UCIMC services a racially and ethically diverse patient population. All patients are 18 and older with health insurance and varying socioeconomic status.

Figure 3.1. UCIMC Standard of Care for Primary Malignant Glioma



3.3. Study Design

This was a retrospective cohort study. Approval was obtained from Institutional Review Board (IRB). Upon approval patient medical records were searched for study participants who met the criteria for enrollment. After identifying the study participants, all available MRI images from the day of diagnosis were collected for each patient. MRI images were manually traced and the hippocampal volume was calculated using MIPAV. Statistical analysis was used to detect rates of hippocampal atrophy.

3.4. Patient Selection

To be eligible for inclusion in this retrospective study, the patients needed to meet the following criteria: newly-diagnosed, supratentorial malignant glioma (GBM or AA), treated at UCIMC Comprehensive Brain Tumor Clinic, on active treatment for at least 18 months, and on bevacizumab for at least 6 months of their clinical course, with sagittal, fine cuts 3D contrast enhanced MPRAGE T1 weighted images available at the key analysis points. Every patient had the same standard of care as previously mentioned above. Clinical Variables for each patient are outlined in **Table 3.1.** Brain MRI's were obtained before and after surgery, two weeks after completion of radiotherapy, and every 4 to 8 weeks during the chemotherapy treatment, as clinically indicated.

Table 3.1 Patient Characteristics and Clinical Variables.

Case	Age	Sex	Dx	Tumor Location (Brain Region)	Adjuvant Temozolomide cycles	Bevacizumab Duration (Months)	Disease Duration (Years)	Status
1	40	F	AA (R)	Temporal	2	38	3.65	Dead
2	54	F	GBM (R)	Frontal Temporal	0	33	3.05	Dead
3	50	F	GBM (L)	Frontal	3	55	6.32	Dead
4	61	F	GBM (R)	Temporal	7	8	1.75	Dead
5	63	F	GBM (L)	Frontal	12	43	4.51	Alive
6	68	M	GBM (R)	Frontal	1	24	2.54	Alive
7	52	M	AA (L)	Temporal	0	47	6.81	Alive
8	59	M	GBM (R)	Frontal	1	27	2.54	Alive
9	19	M	AA (L)	Frontal	2	40	5.85	Alive
10	55	M	GBM (R)	Parietal	7	22	3.26	Alive
11	74	M	GBM (L)	Temporal	3	37	3.76	Alive
12	64	F	GBM (R)	Frontal	12	15	3.23	Alive
13	43	F	GBM (L)	Occipital	3	30	3.07	Alive

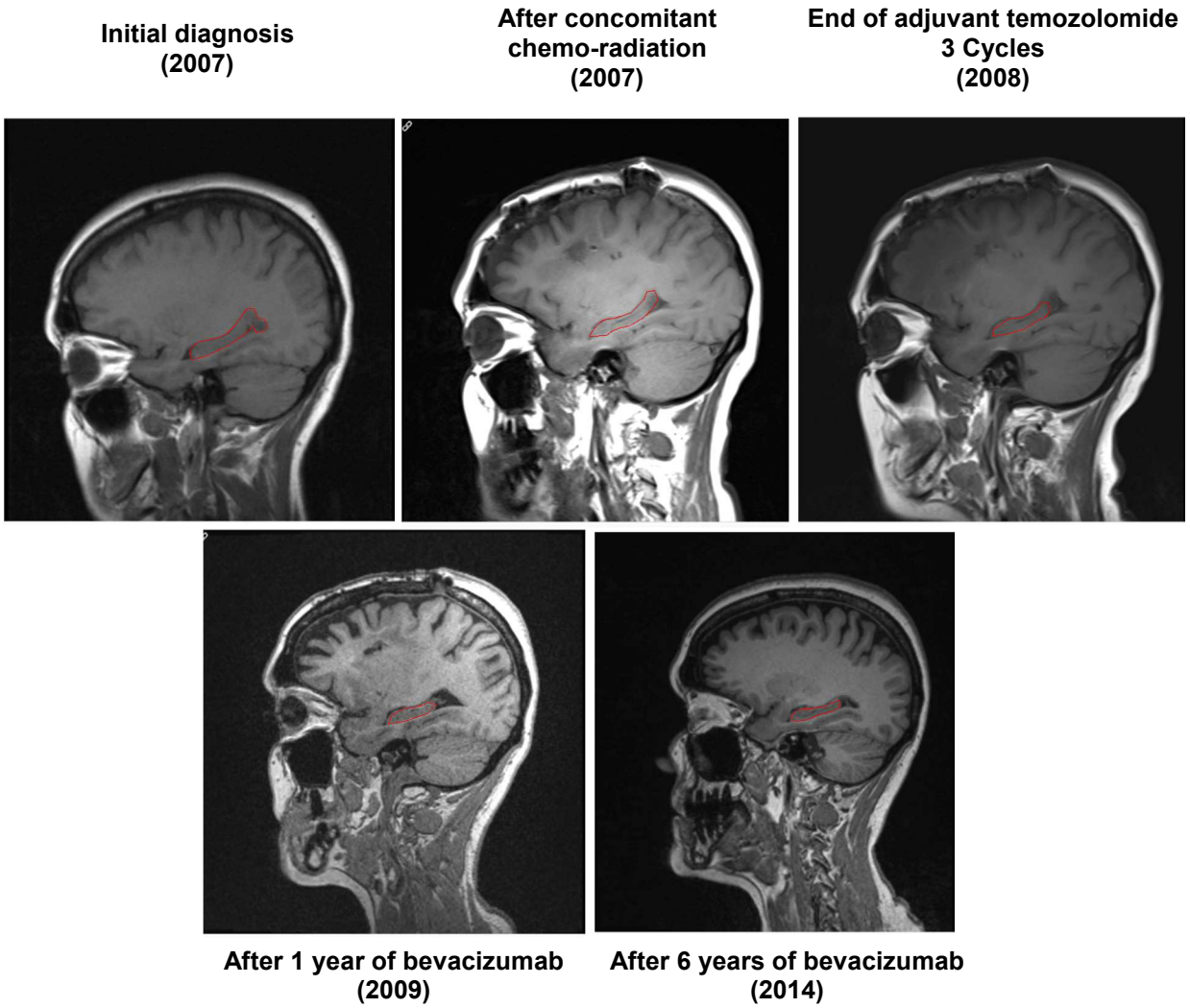
* R and L indicate right or left side location of the tumor in the brain.

3.5. MRI Methods and Image Analysis

MRI imaging was conducted concurrently with clinical appointments every 4 to 8 weeks or earlier if a patient displayed evidence of progressive disease. 1.5 Tesla and 3.0 Tesla MRIs were used to generate sagittal 3D contrast enhanced MPRAGE T1 weighted images. Of the 289 MRIs collected from eligible patients, 243 were suitable for volumetric measurement.

Measurements were done using the MIPAV software by a research associate blinded to the patient treatment history, and then validated by another research associate, who was independent from the first. The principal investigator also verified the manual segmenting. The manual tracing of hippocampal boundaries were done consecutively from the rostral to the caudal side of brain for each image. The hippocampus in the contralateral hemisphere was traced and measured, to avoid morphological structure changes induced by a tumor, shunt, radiation fields or surgical markers. MIPAV software calculates the absolute volume automatically, determined by the number of voxels in each delineated image, and a value is given in mm^3. (See **Figure 3.2**, representative patient).

Figure 3.2. Representative Imaging Changes.



* T1 Magnetic resonance images of sagittal cross-sections of hippocampus for patient 3 during seven years of treatment.

3.6. Statistical Analysis

Patient parameters and clinical variables were analyzed using descriptive statistics. The two primary outcome measures for this study were annual rates of percent of hippocampal atrophy (%) and annual rates of absolute hippocampal atrophy (mm^3). Each measure determined the longitudinal progression of atrophy over time. Linear regression was used to

evaluate significant ($p<0.05$) differences in hippocampal volume overtime. The first primary outcome assessed hippocampal atrophy as a percent of total volume loss as compared with the hippocampal volume at the time of diagnosis. The second primary outcome calculated the rate of hippocampal atrophy using the difference in absolute volume (mm^3) at each time interval. Other statistical methods were used to solve for the study's secondary aim regarding covariates and confounding variables. Pearson's correlation coefficients were computed to examine the association between rates of volumetric change and patient characteristics (age, tumor grade, progression time, chemotherapy duration). Patients on bevacizumab for a minimum of 3 years were evaluated separately using paired sample T-Tests to determine whether the mean rates of atrophy were significant between different time periods. The criteria for this analysis eliminated to the potential for missing data. Every included patient had a value for each time point. Multiple regression models assessed the effect of covariates (age, gender, tumor location, tumor grade, progression time, length of time on temozolomide, temozolomide cycles, bevacizumab duration, disease duration and survival status) on absolute and percent of hippocampal atrophy. Statistical analysis was performed using the SPSS version 23 (IBM Corp, Armonk, NY).

Chapter 4

RESULTS

4.1. Patient Demographics

A total of 13 patients, 6 males and 7 females were identified with a mean age of 54 ± 14.2 (median= 55). Of the 10 GBM and 3 AA tumors in study, 7 were on the right hemisphere and 6 were on the left. All patients received radiotherapy with concomitant temozolomide, but 2 patients did not have post-radiation adjuvant temozolomide due to unequivocal tumor progression and were started directly on bevacizumab. For the 11 patients that received adjuvant temozolomide, the average time on adjuvant temozolomide was 8.7 months. At the first tumor progression all the patients received bevacizumab. The total duration of bevacizumab treatment for a single patient in our study ranged from 8 months to 55 months, with the overall average of 32.2 ± (median= 33.5) months. Median follow up time from diagnosis was 3.26 years, with 9 of the 13 patients still alive at the conclusion of the study. Median time from diagnosis to start of bevacizumab treatment was 7.86 months (34.1 weeks). The average number of MRIs measured per patient was 19.7 ± 5.7 (median= 20).

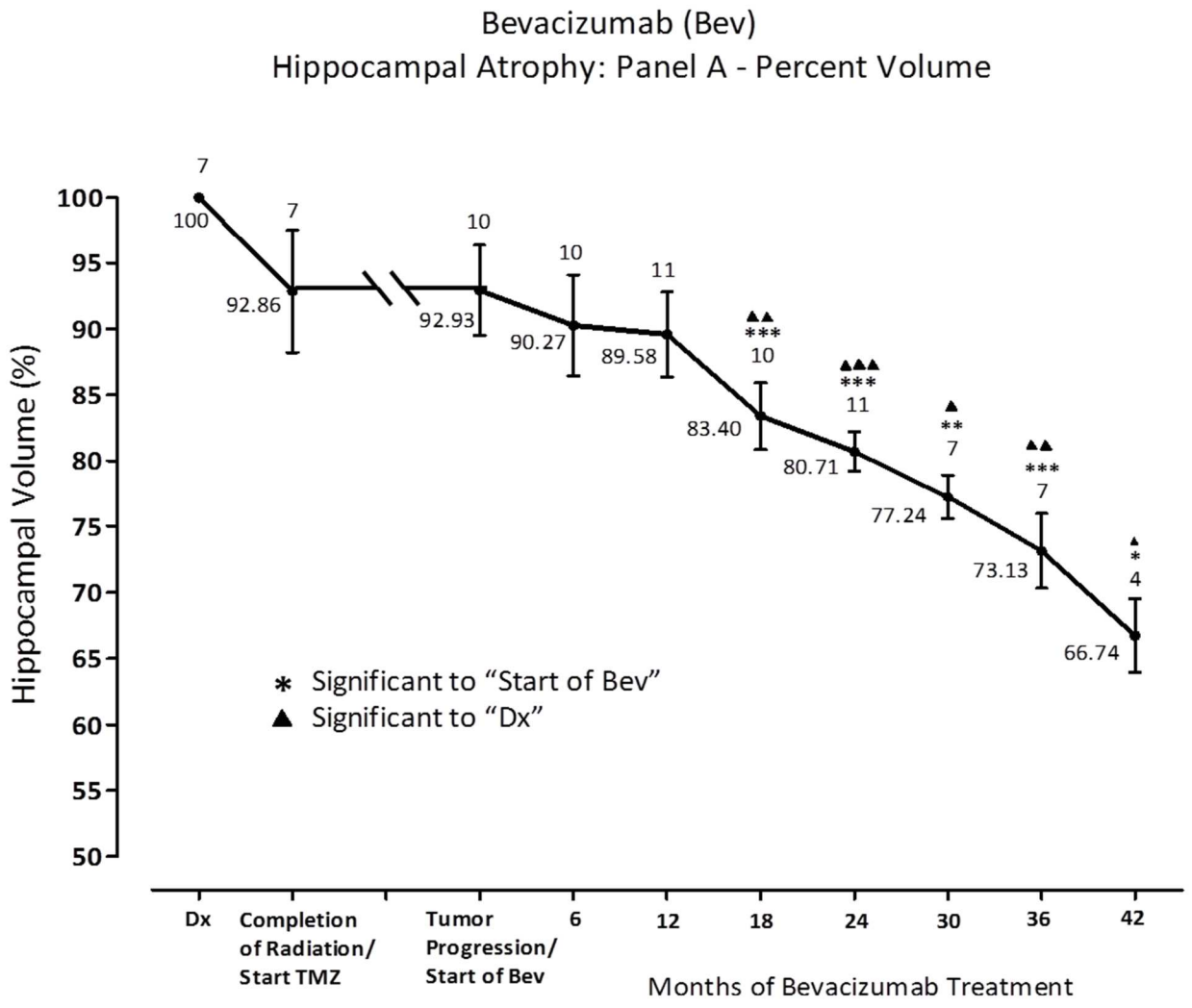
4.2. Hippocampal Atrophy

We determined that the volume of hippocampus declined in all our patients over the duration of their treatment. **Figure 4.1 Panel A** shows the longitudinal progression of hippocampal atrophy, with a maximal level of volume loss of 33.3% being reached at almost 5 years after the initial diagnosis. **Figure 4.1 Panel B** shows identical data as a function of absolute volume loss.

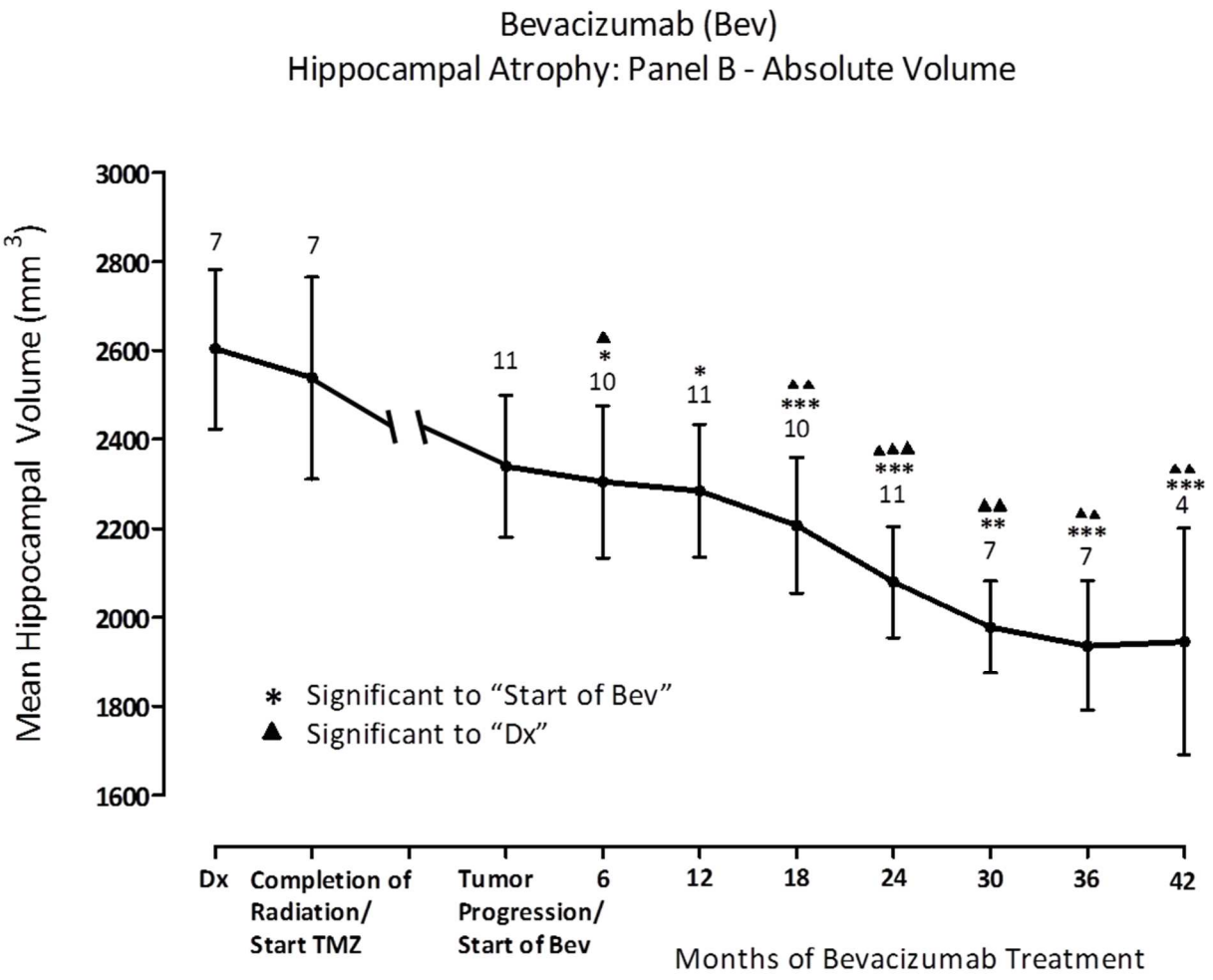
Hippocampal atrophy rates were also calculated separately for each step in the treatment (radiation and concomitant temozolomide, adjuvant temozolomide and bevacizumab). We did not find a significant loss of hippocampal volume from the time of diagnosis until the end of radiation and the completion of the first 5 months of adjuvant temozolomide. **Figure 4.1 Panel C** shows the longitudinal progression of hippocampal atrophy, with a modest level of volume loss of 7.07% being reached at the time of tumor progression. **Figure 4.1 Panel B** shows identical data as a function of absolute volume loss. A significant ($p<0.05$) percent volume was detected only at one single time point early in the treatment course (after 6 months of temozolomide treatment), but was not confirmed by the absolute volume analysis.

A significant decrease in the absolute hippocampus volume was noted after 6 months of continuous bevacizumab treatment ($p<0.05$). A similar trend was noted also for the rate of hippocampal volume atrophy, with the statistically significant difference being detected 18 months after the start of bevacizumab ($p<0.01$). The volume loss continued for as long as the patients received bevacizumab (**Figure 4.1, Panel A and B**). Note: There was no significance between adjacent time points of 6-month time intervals, during any of the individual treatment periods. In **Figure 4.1 Panel E** we compared hippocampal volume changes at the start of bevacizumab and after 1 year, 2 years, and 3 years of bevacizumab treatment. For the patients who received three years of continuous bevacizumab treatment (n=6), the hippocampal volume continued to decline every year. Although linear regression of all 13 patients did not find significance when measuring adjacent time points in 6-month increments, the paired sample t-test did find significance between adjacent points measured in per year increments.

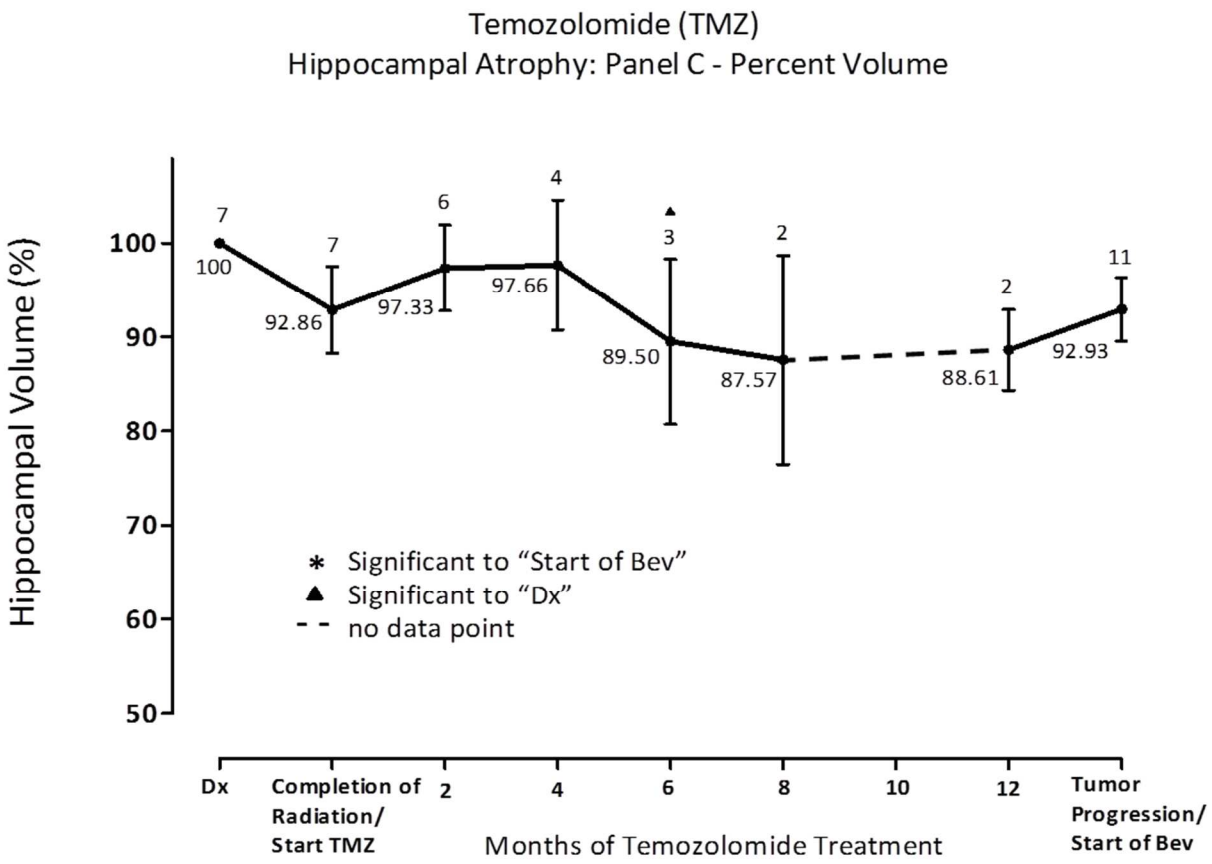
Figure 4.1 Hippocampal Atrophy



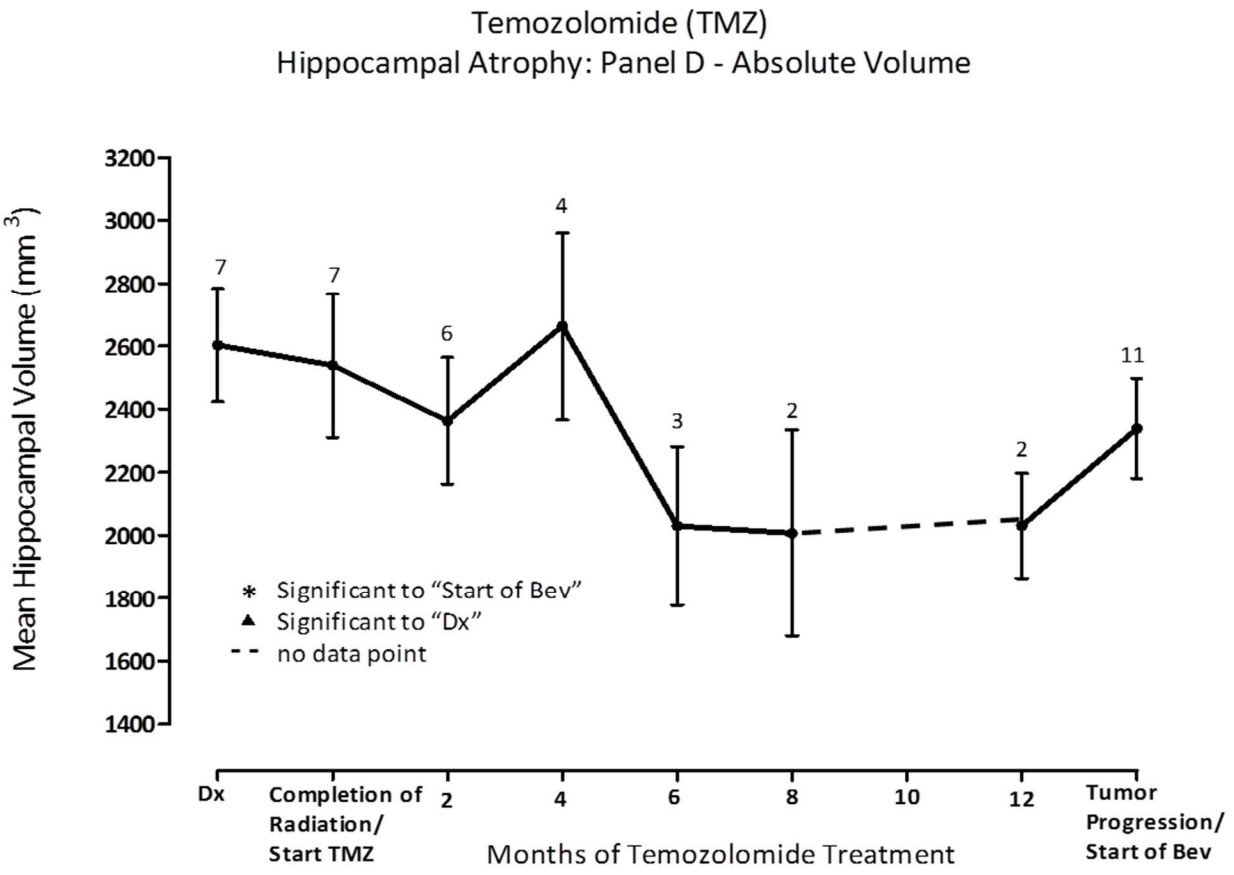
*** (A)** The longitudinal progression of hippocampal volume after start of bevacizumab presented ***as percent of hippocampal atrophy*** from baseline volume at diagnosis. The error bars represent standard error mean (SEM) for each time point. The numbers are (n) patients included at the exact time point. For diagnosis, all patients had a recorded value at diagnosis but some were 1 to 2 weeks off the exact time point and were therefore not included in (n), but are represented in the figure. The "stars" show a significant mean difference from start of bevacizumab. The "triangles" show a significant mean difference from diagnosis. Linear regression was used to compute p-values ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$; $^{▲}p \leq 0.05$, $^{▲▲}p \leq 0.01$, $^{▲▲▲}p \leq 0.001$).



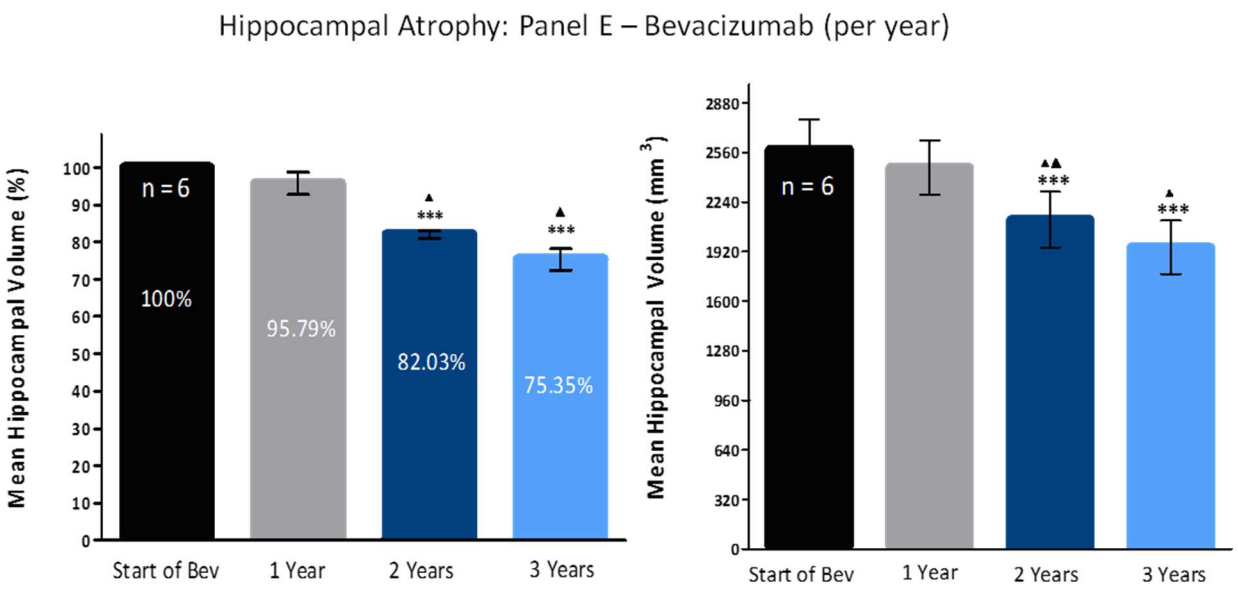
*** (B)** The longitudinal progression of hippocampal volume after start of bevacizumab presented as ***the mean volume (mm^3) of hippocampus*** at specific time point. The error bars represent standard error mean (SEM) for each time point. The numbers are (n) patients included at the exact time point. For diagnosis, all patients had a recorded value at diagnosis but some were 1 to 2 weeks off the exact time point and were therefore not included in (n), but are represented in the figure. The "stars" show a significant mean difference from start of bevacizumab. The "triangles" show a significant mean difference from diagnosis. Linear regression was used to compute p-values ($^{*}p\leq 0.05$, $^{**}p\leq 0.01$, $^{***}p\leq 0.001$; $^{▲}p\leq 0.05$, $^{▲▲}p\leq 0.01$, $^{▲▲▲}p\leq 0.001$).



*** (C)** The longitudinal progression of hippocampal volume after start of adjuvant temozolomide presented ***as percent of hippocampal atrophy*** from baseline volume at diagnosis. The error bars represent standard error mean (SEM) for each time point. The numbers are (n) patients included at the exact time point. The "stars" show a significant mean difference from start of temozolomide. The "triangles" show a significant mean difference from diagnosis. Linear regression was used to compute p-values ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$; $^{▲}p \leq 0.05$, $^{▲▲}p \leq 0.01$, $^{▲▲▲}p \leq 0.001$).



*** (D)** The longitudinal progression of hippocampal volume after start of adjuvant temozolomide presented as ***the mean volume (mm^3) of hippocampus*** at specific time point. The error bars represent standard error mean (SEM) for each time point. The numbers are (n) patients included at that time point. The "stars" show a significant mean difference from start of temozolomide. ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$; $^{▲}p \leq 0.05$, $^{▲▲}p \leq 0.01$, $^{▲▲▲}p \leq 0.001$).



*** (E)** Baseline (100%) is start of bevacizumab. The *"stars"* show a significant mean difference from start of bevacizumab. The *"triangles"* show a significant mean difference compared to the previous year. The error bars represent standard error mean (SEM) Paired t-tests were used to determine p-values ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$; $^{▲}p \leq 0.05$, $^{▲▲}p \leq 0.01$, $^{▲▲▲}p \leq 0.001$).

4.3. Potential Covariates and Group Mean Differences

Multiple statistical methods were used to assess the secondary aim of this study. The Pearson correlation matrix showed that "age" and "tumor grade" were significantly associated with each other (0.683, p=.010), as well as hippocampal atrophy **(See Table 4.1).** No correlation was detected for any measures of hippocampal atrophy during treatment of radiation or temozolomide alone. When age and tumor grade were individually compared against hippocampal atrophy in a linear regression model, higher tumor grade and older age were a significant predictor of greater total atrophy ($p<0.05$). When adjusting for both variables, ANOVA showed $F=4.155$, $p=0.049$ for the regression model.

We further explored the potential for confounders and covariates using independent samples t-Tests to determine whether group mean differences were significant. Study patients were separated into multiple sets of groups organized by patient characteristics. Analysis showed that GBM patients had higher monthly and annual rates of total and bevacizumab atrophy as compared with the AA patients ($p<0.05$). This is in concurs with our results from the regression because GBM is a high-grade tumor more common in older patients. Again age and tumor grade are the conflicting variables. No detectable significance for gender, temozolomide duration, bevacizumab duration, or disease duration was found.

Table 4.1 Variables Associated With Hippocampal Atrophy

VARIABLE	Total Monthly Atrophy	Total Yearly Atrophy	Bevacizumab Monthly Atrophy	Bevacizumab Yearly Atrophy
Age	0.657, p=0.015	0.653, p=0.016	0.634, p=0.020	0.727, p=0.005
Tumor Grade	0.571, p=0.042	0.567, p=0.043	0.643, p=0.018	0.580, p=0.038

Chapter 5

DISCUSSION

5.1. Summary of Study Aims

This study revealed that hippocampal atrophy is present in patients being treated for primary malignant gliomas. Significant volume loss is delayed after the start of treatment. Though we cannot separate the effects of radiation and the temozolomide from the effects of bevacizumab our pilot study suggests that the hippocampal atrophy might be accelerated by long-term bevacizumab use - as our patients reached a statistically significant level of hippocampal atrophy as expressed as absolute volume loss only after 6 months of bevacizumab treatment. We also did not find any measurable hippocampal atrophy when we compared the values obtained at the time of diagnosis with the post-radiation MRIs and the MRIs obtained after 6 months of temozolomide treatment (approximately also 35 weeks after the initial diagnosis), similar with Prust's study[14].

Hippocampal atrophy was adjusted for age, similar to results in other studies. Additionally, the variables age and tumor grade were associated with higher hippocampal atrophy in patients. The small sample size raises uncertainty on the validity of these results to hold true in the general population. Statisticians recommend 10 observations per IV for multiple regressions (Bland, 2000). Nonetheless the results are interesting to explore in further research and could suggest older age and virulence of tumor make a patient susceptible to faster rates of hippocampal atrophy. This does not however suggest that age or tumor grade is a discernable cause for hippocampal volume loss. Moreover, no results were found to suggest

that the amount given of any treatment has a stronger influence over rates of hippocampal atrophy then the length of time on treatment. Neither was found to have any significance at all.

5.2. Drugs and Hippocampal Atrophy

Chemotherapeutic drugs such as temozolomide and bevacizumab have led to improved progression free survival and overall survival in patients with malignant gliomas. However, many patients exhibit patterns of cognitive impairment involving hippocampal related learning and memory paradigms – which potentially associates with prolonged bevacizumab use (such as the GBM patients who received bevacizumab from the initial diagnosis in the RTOG 0825 study)[13]. No changes were seen in the hippocampal volumes for the GBM patients receiving standard radiation and temozolomide treatment[14]. Nevertheless, the Prust *et al* study followed the patients for only a short period of time (35 weeks after the initial diagnosis – less than nine months)[14], while our study followed our patients for up to 6 years – which supports the hypothesis that hippocampal atrophy is a delayed effect of malignant glioma treatment.

Bevacizumab is a VEGF inhibitor with anti-angiogenic properties. Animal models have shown that VEGF expression is required for hippocampal neurogenesis involved in learning and memory[18]. It has been suggested that VEGF contributes to neuroprotection and neuronal repair in the central nervous system via its role in neurogenesis, long-term potentiation and cerebral blood flow following focal brain ischemia[19]. A recent study showed that prolonged treatment with bevacizumab is potentially associated with brain atrophy in malignant glioma patients[20]. The same study proposes that restricting VEGF may decrease the amount of neuronal repair, neurogenesis, and learning[20]. We propose that bevacizumab could contribute

specifically to hippocampal atrophy by impairing hippocampal neurogenesis and healing of normal brain from surgical trauma, radiation and chemotherapy.

5.3. Cognitive Impairment and Neurological Deficits

In patients with MCI and AD, the rate of hippocampal atrophy correlates with disease progression and with the severity of cognitive loss[10, 11] The annual percent change (APC) of hippocampal atrophy in normal (1.4-1.73), MCI (1.8-3.3), and AD (3.43-3.98) also correlates with the disease severity (See **table 2.2.**).[10, 11] As the annualized hippocampal volume loss measured in our study is higher than the one reported in Alzheimer disease patients, it is possible that treatment-induced hippocampal atrophy might directly explain the very high rate of memory deficits seen in long-term GBM survivors[15]. Data from clinical studies has identified severe treatment-induced dementia in a high number of long-term GBM survivors and cancer patients with brain metastasis[15, 16]. The evidence of such cognitive impairment has encouraged the use of particular AD drugs in brain cancer patients, to combat damaging neurological deficits resulting from the treatment of primary and metastatic brain tumors[17].

5.4. Study Limitations

Previously published papers report limitations due to resection, hemispheric tumor burden, and length of study, which may have impacted their ability to detect significant changes in the hippocampus over time[14]. Our data are limited by the absence of control groups – patients that received only radiation and temozolomide, and did not require any other treatments for the next one to three years. We have tried to identify control MG patients with

similar pathology that have survived similarly long periods of time (four or more years) without tumor progression and without receiving bevacizumab – but were able to identify only two such patients in our large practice. To mitigate the argument that hippocampal atrophy was induced by a latent response to radiation and TMZ, we measured the hippocampal volumes on the opposite side of the brain then the tumor, and we made sure that the contralateral hippocampus was not affected by surgery and not included in the radiation fields. Although, it is suggested that the investigation into radiation and chemotherapy separately, in addition to novel targeted therapies over a longer period of time with stringent surgical parameters and a larger sample size, may be sufficient enough to determine further brain changes[14].

5.5. Future Directions

The retrospective nature of this study did not allow for concurrent investigation of cognitive impairment in our patients. Future studies should prospectively evaluate brain atrophy and cognitive function simultaneously. Cognitive testing should examine all upper level executive functions in addition to learning and memory. Structural volume changes in the hippocampus with corresponding loss of cognitive function could be an early indicator of tumor recurrence. Moreover, cognitive impairment could act as an independent projection of disease progression. An accurate family and medical history should be assessed for each patient to determine any confounding variables that may increase the risk of cognitive impairment in this population.

5.6. Intervention Programs and Cognitive Rehabilitation Services

If in fact treatment brain cancer is causing cognitive impairment, efforts and strategies to protect or delay the course of cognitive decline should be implemented for all at risk patients. Strategies to regain function should also be considered. Known intervention methods include psychostimulants, restructuring a patients environment, new aids and technology, coping strategies, and repetitive stimulation by practicing cognitive exercises[22].

The current literature on the effectiveness of cognitive rehabilitation in brain cancer populations is limited. Research on this topic has been met with difficulty due to methodology[22]. Many studies have struggled to recruit and retain patients for a significant sample size, others have started data collection but then stopped before completion and most have found results that have been inconclusive. Thus far pharmacological interventions have seen some improvements in psychomotor processing[22], attention and mood[24]. While neuropsychological treatment programs have had some success in improving QOL, attention, mental fatigue, working memory[22], and daily activities[24]. Some found no improvement at all[22].

5.7. Conclusions

Our study analyzed sagittal cross sections of the hippocampus, imaged by magnetic resonance, over a span of several years beginning at diagnosis. The study detected concerning atrophy rates in glioma patients (8.903%/year) over the course of their entire treatment -- twice the reported rate of AD populations, three times the rate of MCI, and nearly four times the rate of normal aging (see Table 2). Therefore hippocampal atrophy could be an essential cause of cognitive impairment in these populations. More research to explore the mechanisms and potential causes for the decline in brain volume is needed. Moreover, neuropsychological

rehabilitation programs aimed at improving their quality of life should treat patients who experience cognitive impairment.

In conclusion, this study suggests that hippocampal atrophy is a relatively late phenomenon in the treatment of malignant glioma patients. The overall survival of malignant glioma patients is on the rise, and reached over two years in recent studies[21] – which potentially exposes the patients to prolonged use of chemotherapy drugs, including bevacizumab. We are currently planning a prospective study to examine the association between the rate of hippocampal atrophy, cognitive impairments and decreased quality of life in malignant glioma patients on active chemotherapy for long periods of time.

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