2021

Isocitrate Dehydrogenase-1 Mutation as a Prognostic Factor in Recurrent Glioblastoma

Haroon R. Hashmi
Walden University

Walden University

College of Health Professions

This is to certify that the doctoral dissertation by

Haroon R. Hashmi

has been found to be complete and satisfactory in all respects,
and that any and all revisions required by
the review committee have been made.

Review Committee
Dr. Harold Griffin, Committee Chairperson, Public Health Faculty
Dr. James Rohrer, Committee Member, Public Health Faculty
Dr. Chinaro Kennedy, University Reviewer, Public Health Faculty

Chief Academic Officer and Provost
Sue Subocz, Ph.D.

Walden University
2021

Abstract

Isocitrate Dehydrogenase-1 Mutation as a Prognostic Factor in Recurrent Glioblastoma

by

Haroon R. Hashmi

MS, University of Alberta, 1996

MPH, Walden University, 2014

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health - Epidemiology

Walden University

August 2021

Abstract

Glioblastoma (GBM) is an aggressive form of brain cancer that has a high recurrence rate and very poor prognosis. The prognostic value of various molecular markers (e.g., IDH-1 mutation, MGMT promoter methylation, etc.) and clinical factors (e.g., age, KPS, surgery and chemotherapy) has been studied in GBM after initial diagnosis but not as extensively in the recurrent GBM. Utilizing a retrospective cohort design, based on quantitative data collected through medical chart reviews, and the conceptual framework of outcomes research in oncology, this study evaluated the prognostic value of IDH-1 mutation in recurrent GBM in the context of key predictor variables of age, MGMT promoter methylation, KPS, and surgery and chemotherapy at recurrence. The study specifically evaluated if there was a significant difference in overall survival and progression free survival between rGBM patients with and without IDH-1 mutation and if selected molecular and clinical covariates affected these outcomes. The results of this study indicated, albeit with its limitations, that IDH-1 mutation was not a prognostic factor in recurrent GBM. The prognostic value of IDH-1 mutation from initial diagnosis in this study was inconclusive, consistent with previous reports. The results of this study also indicated that although methylated MGMT promoter was a strong prognostic factor from initial diagnosis as previously reported, it was not a prognostic factor in recurrent GBM. Overall, the results of this study suggest that the prognosis and treatment of GBM may need to be considered differently at initial diagnosis and following disease recurrence. It is anticipated that the results of this study will bring about a positive social change by affecting both patient treatment and health care practice in recurrent GBM.

Isocitrate Dehydrogenase-1 Mutation as a Prognostic Factor in Recurrent Glioblastoma

by

Haroon R. Hashmi

MS, University of Alberta, 1996

MPH, Walden University, 2014

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health - Epidemiology

Walden University

August 2021

Acknowledgments

I would like to express the deepest appreciation to my dissertation committee chair, **Dr. Harold R. Griffin**, for without his patience, guidance, and understanding I would not have made it this far with my dissertation. I would also like to sincerely thank my committee member, **Dr. James E. Rohrer**, for his valuable input on the study proposal and encouragement during the dissertation process. I am grateful for all the support I received from the faculty and administrative staff at Walden University during the entire Ph.D. program.

My sincere gratitude to my collaborating investigators **Dr. Timothy Smith**, Director, Computational Neuroscience Outcomes Center and Assistant Professor of Neurosurgery, Harvard Medical School, Boston, MA, and **Dr. Daniyal Siddiqui**, Chief, Division of Hematology and Medical Oncology, St. Vincent Hospital, Worcester, MA. This research project would not have been possible without their collaboration and support from their team particularly Jack McNulty and Syed Masood Pasha.

Finally, I am grateful to my family members for their sacrifices and understanding as I pursued this degree program and appreciate the encouragement I received from my friends and colleagues during my studies.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction to the Study

Glioblastoma (GBM) is by far the most frequent malignant glioma. It is associated with a particularly aggressive course and a dismal prognosis (Ostrom et al., 2014). Glioblastoma is characterized by symptoms including slow progressive neurological deficit, weakness in motor skills, headache, increased intracranial pressure, and seizures (Ostrom et al., 2014). The tumor location may be indicated by these neurological symptoms as well as by focal signs, including hemiparesis, sensory loss, visual loss, and aphasia (Ostrom et al., 2014). Extremely rapid cell infiltration is a key biological feature of glioblastoma; tumor cells travel to other sites within the brain, which makes it very difficult to completely remove tumors through surgery (Olar & Aldape, 2014). Therefore, in conjunction with inadequate response to treatment, the recurrence rate is very high with GBM, resulting in poor overall prognosis (Li et al., 2015). Newly diagnosed GBM subjects have a median overall survival (mOS) of 12 to 15 months and a 2-year-overall survival (OS) rate of up to 27% (Omuro et al., 2013). Subjects who have experienced multiple recurrences, referred to as recurrent GBM (rGBM), have a particularly poor prognosis, with a mOS of 6 to 7 months. The OS in subjects who have failed temozolomide (TMZ) and bevacizumab, or equivalent salvage chemotherapy, is as short as 3 to 5 months (Iwamoto et al., 2009; Omuro & DeAngelis, 2013).

According to the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) database, there are an estimated 166,039 people living with brain and other nervous system cancers in the United States in 2015 (*Cancer of the Brain and Other Nervous System - Cancer Stat Facts,* n.d.). The National Brain Tumor Society

indicates that there are currently 700,000 Americans living with a brain tumor, of which 560,000 are benign and 140,000 are malignant (*Quick Brain Tumor Facts*, n.d.). Given that gliomas comprise approximately 80% of all malignant brain tumors (*Quick Brain Tumor Facts*, n.d.), the prevalence of malignant gliomas in the United States is approximately 112,000. The 5-year survival rate for GBM is estimated at 5.6%; survival decreases if the disease is diagnosed at an older age (Ostrom et al., 2018).

Glioblastoma is more common in older adults. The median age at diagnosis is 65 years and the incidence rate is highest in adults aged 75-84 years (Ostrom et al., 2018). While the incidence rate is 1.6 times higher in males, the frequency of secondary GBM is higher in females, with a male-to-female ratio of 0.65 (Ostrom et al., 2018; Thakkar et al., 2014). The incidence rates of malignant brain and other central nervous system (CNS) tumors are higher in Whites (7.62 per 100,000 persons) than Blacks (4.52 per 100,000 persons) and the incidence rate of GBM is approximately two times greater in Whites than Blacks (Ostrom et al., 2018). There are no well-established environmental or behavioral risk factors associated with brain or CNS tumors except exposure to ionizing radiation (Ostrom et al., 2018); however, the risk of developing a brain cancer is twice as high in individuals who have a parent, child, or full sibling diagnosed with brain cancer (Ostrom et al., 2018).

Isocitrate dehydrogenase-1 (IDH-1) mutation status is now used in the classification of gliomas, based on an understanding that IDH-1 mutant and wild-type gliomas have different underlying tumor biology and therefore need to be treated differently (Ostrom et al., 2018). A combination of radiotherapy (RT), chemotherapy, and

surgical resection is typically used in the treatment of glioma patients. Clinical data have showed benefits from the aggressive treatment of glioma patients with IDH-1 mutation, making upfront and initial treatment with RT and chemotherapy a standard of care for patients with IDH-1 mutant Grade II and III gliomas (Miller et al., 2017). Because the role of IDH-1 mutation in rGBM is not well understood, it is difficult to determine whether similar upfront aggressive treatment would confer any significant benefit to the patients, given the risks associated with RT and chemotherapy. Hence, this study specifically addressed this gap in research on the potential role of IDH-1 mutation in rGBM, with the aim to improve disease prognosis and survival outcome in GBM and rGBM patients. It is anticipated that the results of this study will help GBM patients and their treating physicians make a more informed decision about the most appropriate treatment regimen for managing the disease. After general background on GBM, this chapter addresses the problem statement and purpose of the study along with specific research questions. The conceptual framework of the study is briefly described followed by study limitations, expected significance of the study, and overall chapter summary.

Background

Glioblastoma is an aggressive form of brain cancer that has very poor prognosis (Ostrom et al., 2018). It is difficult to completely remove tumors through surgery, and the cancer cells rapidly infiltrate other parts of the brain (Olar & Aldape, 2014); therefore, GBM has a very high recurrence rate, which, in conjunction with inadequate response to existing treatment, results in poor prognosis. The mOS for rGBM is 6-7 months; the mOS for patients who have failed standard of care treatments is as short as 3 to 5 months

(Iwamoto et al., 2009; Omuro & DeAngelis, 2013). IDH-1 mutation status is now used to classify gliomas, based on the understanding that IDH-1 mutant and wild-type gliomas have different underlying tumor biology and therefore need to be treated differently (Miller et al., 2017).

Waitkus et al. (2016) provided a review of IDH mutations in gliomas, including information about the biochemistry and effects of IDH mutations. They also highlighted the utilization of IDH mutations as putative biomarkers for glioma, including its potential role in disease prognosis and treatment outcomes (Waitkus et al., 2016).

Calvert et al. (2017) and Labussiere et al. (2010) underlined the role of IDH mutation in GBM (Calvert et al., 2017; Labussiere et al., 2010). Calvert et al. highlighted the upregulation of wild-type IDH-1/2 in GBM, while Labussiere et al. showed a four-fold longer survival among GBM patients with an IDH-1 mutation than among those with wild-type IDH-1.

Amelot et al. (2015), Mukasa et al. (2012), and Zou et al. (2013) provided different views of the controversy over the prognostic value of IDH mutation in GBM (Amelot et al., 2015; Mukasa et al., 2012; Zou et al., 2013). For example, while Amelot et al. identified IDH mutation as a weak prognostic factor for survival, Mukasa et al. indicated that, at least in Grade III gliomas, IDH mutation was associated with long-term survival. The meta-analysis conducted by Zou et al. also supported the prognostic value of IDH mutation in GBM.

Taal, et al. (2014) and Mandel et al (2016) noted the paucity of knowledge about the prognostic value of IDH-1 mutation in rGBM because limited studies have been

conducted on this topic (Mandel et al., 2016; Taal et al., 2014). Moreover, the studies conducted on rGBM included a total of only 10 patients with IDH-1 mutation, so they indicated that the results of these studies must be interpreted with caution (Mandel et al., 2016; Taal et al., 2014). Considering that the role of IDH-1 mutation as a prognostic factor remains controversial, even at initial diagnosis, more studies are needed, particularly in rGBM, to understand the role of IDH mutations in this disease.

Problem Statement

Glioblastoma is one of the most common types of malignant gliomas and has an aggressive disease course and very poor prognosis (Ostrom et al., 2018). GBM may manifest at any age, but it typically affects adults at age 45-84 (Ostrom et al., 2017; Ostrom et al., 2014). IDH-1 gene mutation has been extensively studied as a prognostic factor in GBM following initial diagnosis, but it has not been studied as much in rGBM (Amelot et al., 2015; Mukasa et al., 2012; Ostrom et al., 2018). The prognostic value of IDH-1 mutation is debated even following initial diagnosis of GBM: studies have demonstrated both weak and strong association between IDH-1 mutation and overall survival (Amelot et al., 2015; Mukasa et al., 2012; Zou et al., 2013). A few studies have examined the role of IDH-1 mutation in rGBM, but only in the clinical trial setting and with inconclusive results (Mandel et al., 2016; Taal et al., 2014). These studies suggest that patients with IDH-1 mutated tumors show an improved trend in overall survival at first recurrence; however, in rGBM trials, IDH-1 mutation did not result in prolonged progression-free survival or overall survival compared to IDH-1 wild-type tumors (Mandel et al., 2016). Moreover, studies conducted by Mandel et al. and Taal et al.

included only a total of 10 patients (five patients in each study) with IDH-1 mutation. The authors indicated that the results of these studies must be interpreted with caution, given the very small sample size, and suggested additional studies be conducted to better understand the role of IDH-1 mutation in rGBM (Mandel et al., 2016; Taal et al., 2014).

IDH-1 mutation status is now used in the classification of gliomas, given the understanding that IDH-1 mutant and wild-type gliomas have different underlying tumor biology and therefore need to be treated differently (Miller et al., 2017). A combination of RT, chemotherapy, and surgical resection is typically used in the treatment of glioma patients (Wick et al., 2018). Even though the long-term side effects of RT and chemotherapy were initially questioned, particularly in low grade gliomas, over the years clinical data has shown benefits from aggressive treatment of glioma patients with IDH-1 mutation (Czapski et al., 2018; Kazda et al., 2018; Paolillo et al., 2018; Zang et al., 2018). This has made initial upfront treatment with RT and chemotherapy a standard of care for patients with IDH-1 mutant Grade II and III gliomas (Miller et al., 2017). Because the role of IDH-1 mutation in rGBM is not well understood, it is difficult to determine whether similar upfront aggressive treatment would confer any significant benefit to patients, considering the risks associated with RT and chemotherapy. This study addressed this gap in research and evaluated the potential role of IDH-1 mutation in rGBM, with an aim to improve disease prognosis and survival outcomes in rGBM patients. It is anticipated that the results of this study will help rGBM patients and their treating physicians make more informed decisions about the most appropriate treatment regimen for managing the disease.

Purpose of the Study

The purpose of this study was to determine whether IDH-1 mutation is a prognostic factor in rGBM considering other molecular and clinical prognostic factors as covariates. In a retrospective cohort study, time to first recurrence from initial diagnosis and time to disease progression or death from first recurrence was evaluated in GBM patients with IDH-1 mutated and wild-type tumors. The effect of key variables (i.e., O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, age, Karnofsky performance score (KPS), surgery for resection, and chemotherapy at progression) on correlation between IDH-1 mutation status and disease progression and survival was also evaluated. The study also estimated the overall prevalence of GBM as a type of nervous system cancer at the participating hospitals in Massachusetts.

Research Questions and Hypotheses

RQ1: Is there a significant difference in time to disease progression and overall survival after first recurrence between rGBM patients with IDH-1 mutation and those without IDH-1 mutation?

$H_0$1: Based on IDH-1 mutation status, there is no statistically significant difference in the time to disease progression and/or overall survival of rGBM patients.

$H_1$1: Based on IDH-1 mutation status, there is a statistically significant difference in the time to disease progression and/or overall survival of rGBM patients.

RQ2: Is the correlation between IDH-1 mutation status and disease progression and survival after first recurrence affected by the covariates of MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression?

$H_0$2 – The correlation between IDH-1 mutation status and disease progression and survival is not affected by covariates MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression.

$H_1$2 - The correlation between IDH-1 mutation status and disease progression and survival is affected by covariates MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression.

Conceptual Framework for the Study

The aims and scope of the study were congruent with the conceptual framework of outcomes research. The goal of outcomes research in oncology is to improve medical practice in order to achieve better outcomes in patients (Lee et al., 2000). Outcomes research draws on multiple specialties and subspecialties of clinical science to better understand the effectiveness of treatments and to enable clinicians to make more informed decisions (Lee et al., 2000). In oncology, outcome research addresses a broad range of questions and oncology-related endpoints, such as overall survival (OS) and progression-free survival (PFS), which are studied utilizing administrative databases and cohort or case-control study designs (Lee et al., 2000). This conceptual framework is also supported by other researchers, who are increasingly advocating that outcomes research in oncology is more than health services research *per se* and that outcomes research requires an integrated multidisciplinary approach in order to understand the complexity of tumorigenesis and factors that impact patient outcomes (Apolone, 2003; Fay et al., 2015; Kovvali, 2014; Melamed et al., 2017; Roberts et al., 2019). This study, from the perspective of its research scope, methodology, and potential application, was within the

parameters of research and applications of outcomes research (Lee et al., 2000). First, the purpose of this study, to determine whether IDH-1 mutation is a prognostic factor in rGBM, broadly fits the treatment options and prediction rules of the outcomes research framework because IDH-1 mutation is an important prognostic factor that informs treatment options. Second, the study utilized clinical outcomes like OS and PFS that are suggested in the outcomes research framework (Lee et al., 2000). Furthermore, the application aspect of the outcomes research conceptual framework suggests that the research should lead to clinical or policy decisions (Lee et al., 2000). It is anticipated that the results of this study will inform clinical decisions in terms of treatment recommendations and clinical practice guidelines for the management of rGBM patients, using IDH-1 mutation as a prognostic factor. The results of this study may also provide some future directions for policy. For example, considering that the results of the study indicate that aggressive treatments may not be necessary in rGBM, policy changes may be made over time that could lead to substantial savings in the overall health care costs associated with the management of this disease. Therefore, the overall scope of this study – its research inquiry, methodology, and potential applicability – was contextualized within the conceptual framework of outcomes research in oncology.

Nature of the Study

This study was a retrospective cohort study based on quantitative data collected through retrospective chart reviews of adult patients diagnosed with GBM at select hospitals in Massachusetts. An observational study design, rather than an experimental design, was selected considering the scope of the study that aimed to evaluate the

association between exposure (i.e., IDH-1 mutation) and disease outcome (i.e., time to disease progression and overall survival) (Euser et al., 2009; Song & Chung, 2010). Retrospective cohort design was selected mainly for efficiency because a prospective cohort study would have been costly and time-consuming making it impractical for this dissertation project. Individual chart reviews for patients provided data on initial diagnosis and IDH-1 mutation status as well time to first recurrence, time to disease progression or subsequent recurrence, and death. Disease outcomes – time to recurrence from initial diagnosis, time to disease progression following first recurrence, and overall survival – was evaluated to determine whether IDH-1 mutation is a prognostic factor for adult GBM or rGBM patients. The Cox proportional-hazards model was used to assess the effect (hazard ratio) of IDH-1 mutation status on time to disease progression and survival. In addition, a Kaplan-Meir curve were generated to obtain the survival rate for patients with and without the IDH-1 mutation. A Cox regression analysis was also conducted to test the effects of other key covariates, including Karnofsky performance score (KPS), surgery at the time of recurrence, and O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status on progression and survival. The prevalence of adult GBM was estimated based on the total number of adult brain cancers diagnosed at the select hospitals during the same period as the study.

The study covered a duration of 12 years, from 2008 to 2020. To get an estimate of the incidence and prevalence of GBM, the medical records at select hospitals were searched for the total number of adult brain cancers and GBM patients admitted and diagnosed at those centers during the specified time-period. The

prevalence of GBM was estimated as a proportion of overall adult brain cancers reported at the same centers during the specified time-period.

Retrospective medical chart reviews of patients with GBM or rGBM were conducted to determine whether there is a significant difference in time to disease progression or median overall survival. The key data that were collected from the chart reviews included but not limited to: (a) patient demographics (e.g., age and gender), (b) date of initial diagnosis of GBM, (c) standard treatment received at disease onset, (d) IDH-1 phenotype and other genetic markers, (e) date of disease recurrence, (f) treatment following disease recurrence, (g) time to disease progression following first recurrence, and (h) date of death. Time to disease progression and overall survival for each patient was calculated based on the date of disease onset or diagnosis and the date of disease progression and death.

Definitions

Glioblastoma: A fast-growing central nervous system tumor that forms from glial (supportive) tissue of the brain and spinal cord (Ostrom et al., 2014; Ostrom et al., 2018).

Recurrent glioblastoma: Glioblastoma typically returns or recurs after initial treatment that may include chemotherapy and surgical removal (Apolone, 2003; Li et al., 2015).

Overall survival: The duration or length of time that a patient is alive after initial date of diagnosis of the disease or the start of treatment (Iwamoto et al., 2009; Omuro & DeAngelis, 2013). In this study overall survival was assessed from the initial date of diagnosis of the disease to death and date of first recurrence to death.

Median overall survival: The duration or length of time that half of the patients in a group of patients are alive after initial date of diagnosis of disease or the start of treatment (Omuro & DeAngelis, 2013; Ostrom et al., 2014).

Time to disease progression or progression free survival: Time to disease progression or progression free survival (PFS) is the duration or length of time after initial treatment when patient goes in remission and recurrence or relapse of cancer (Lamborn et al., 2008). In this study time to disease progression was considered as duration of time to first recurrence from initial treatment and duration of time to second recurrence from first recurrence.

Resection: Surgery performed to remove the tumor mass, which can be total or partial resection depending on tumor location and access (Brown et al., 2016; Wilson et al., 2014).

Prognostic factors: Patient characteristics or conditions that can provide some estimation about the chance of recovery or recurrence of a disease in patients (Audureau et al., 2018; Czapski et al., 2018; Goldman et al., 2018; Ostrom et al., 2018; Thakkar et al., 2014). In this study molecular markers that is, IDH-1 mutation status and MGMT promoter methylation status, and clinical factors like KPS, surgery for resection, and chemotherapy at progression were evaluated as prognostic factors.

Assumptions

The study was a retrospective cohort study based on quantitative data collected from electronic medical records (EMR) of adult patients diagnosed with GBM at select hospitals in Massachusetts; therefore, the major assumption of this study was that the

patient data in the EMR was collected and entered in a reliable and accurate manner, particularly for the key variables. This assumption was supported by the robust processes and standard operating procedures that each participating hospital have in place to ensure data integrity. It would not have been feasible for the researcher to conduct an independent audit of the data due to hospital policies, magnitude of the database, and patient privacy concerns under the Health Insurance Portability and Accountability Act (HIPAA) of 1996 (Rights (OCR), 2009). The other key assumption was the clinical assessment of recurrence and disease progression in GBM patients. While radiographic imaging was used as an objective measure to determine recurrence and progression of disease, the assessment was nonetheless made by a clinician. GBM patients are under the care of trained neurooncologists and such radiographical assessments are part of regular clinical practice, including oncology.

Scope and Delimitations

The study specifically evaluated the role of IDH-1 mutation in recurrent GBM because IDH-1 gene mutation has been extensively studied as a prognostic factor in GBM following initial diagnosis, but it has not been studied as much in rGBM (Amelot et al., 2015; Mandel et al., 2016; Ostrom et al., 2014; Ostrom et al., 2018; Thakkar et al., 2014); therefore, only those GBM patients that have at least one confirmed diagnosis of recurrence following initial diagnosis were included in the study whereas GBM patients that do not have a confirmed diagnosis of recurrence were excluded from the study. The study was designed as a retrospective cohort study based on quantitative data collected on adult GBM patients at select hospitals in Massachusetts, including Brigham and

Women's Hospital and St. Vincent Hospital. These hospitals were selected to make the research feasible through ease of collaboration with oncologists at these centers and better access to EMR, which required the researcher to go through training at these hospitals. This approach would not have been possible if too many cancer centers had been selected, particularly ones in other states.

The study was conceptualized within the research component of the outcomes research conceptual framework (Lee et al., 2000). It could be argued, however, that Determinants of Health model (*WHO | The Determinants of Health*, n.d.) could have been considered as the conceptual framework for this study. According to this model, the health of an individual is affected by a combination of multiple factors or determinants, and these determinants can be categorized into socioeconomic and physical environment, and the person's characteristics and behaviors (*WHO | The Determinants of Health*, n.d.). Genetics and epigenetics, as a person's individual characteristics, can play a role in the development of an illness, including cancer, and affect the response to treatment (Notterman & Mitchell, 2015). The model also entails, however, that multiple factors like diet, environment, and biology may impact the genetic and epigenetic profile of an individual (Mohammed et al., 2012). While the study investigated an epigenetic biomarker, IDH-1 mutation, as a "determinant of health" in rGBM patients, it was beyond the scope of this study to identify and account for all the factors that may lead to this epigenetic phenotype and reasonably address the key research questions. Therefore, outcomes research conceptual model was considered more appropriate for the scope of this study and the research questions it aimed to address. The study directly tested the

prediction rule (i.e., the prognostic value of IDH-1 in rGBM) using the outcomes (OS and PFS) within the research component of the conceptual framework (Lee et al., 2000). The results may provide future direction for the application component of the conceptual framework, including both clinical practice and policy aspects, but further research would be needed to validate the results of this study before such changes could be implemented.

Since the data collected for the study was limited to two hospitals in Massachusetts and are not representative of the U.S. population, caution would need to be taken in the generalizability of the results and conclusions. However, there is no evidence to suggest that the pathophysiology and clinical course of GBM would be different across the United States, and the data collected for this study had an appropriate distribution of age, gender, and race/ethnicity variables consistent with the demographic data reported for GBM (Ostrom et al., 2018). Furthermore, EMR provides detailed clinical data that is relevant for outcomes research and is now increasingly used in clinical oncology and epidemiology studies (Lau et al., 2011). Therefore, the risk of external validity was considered minimal for this study.

Limitations

The key anticipated challenge and barrier for the study was access to GBM patient medical records for retrospective chart review; however, collaborations were established at select leading hospitals in Massachusetts, providing access to the data of close to 1500 GBM patients. As anticipated, there were enough GBM patients in the database to have a reasonable sample size for the study, the sample size was reduced once the inclusion criteria of disease recurrence and IDH-1 mutation status, which was the key

variable for the study, was applied. Clinical and molecular prognostic factors (e.g., age, Karnofsky performance status (KPS), surgical resection, chemotherapy at progression, and MGMT promoter methylation status) are considered key prognostic factors for survival in recurrent GBM (Archavlis et al., 2014; Audureau et al., 2018; Chaichana et al., 2013; Cloughesy et al., 2014; D'Amico et al., 2015; Ringel et al., 2016; Stupp et al., 2012; Terasaki et al., 2007). Heterogeneity in these clinical and molecular prognostic factors in the study population was also anticipated. Considering that the prognostic value of the IDH-1 mutation for the rGBM patients in this study was determined by comparing outcomes like time to disease progression and mortality, the confounding variables were also factored in as covariates in the final data analysis. Cox regression analyses were conducted to address this limitation and to evaluate the effect of these confounding variables on the outcomes of interest. Analyses that matched the groups for these covariates were not conducted due to the small sample size. Inability to match cohorts for confounding variables is a general limitation and challenge that researchers face when conducting research in a rare disease space, like GBM. In rare diseases, the sample size is relatively small to begin with and patient heterogeneity makes it difficult to adjust for all confounding variables while still maintaining a reasonable sample size for statistical analysis.

Cohort studies may also be susceptible to selection, information, and comparison bias (Euser et al., 2009). Inherent nature of the study that includes objective assessment of exposure and outcome adequately addresses these potential biases. In terms of selection bias, the inclusion of the GBM subjects in the study with the exposure of

interest (IDH-1) mutation was not dependent on the likelihood of them having the outcome of interest (disease progression and survival). Moreover, the diagnosis of GBM, both initial and recurring, was based on radiographic assessment and therefore it eliminated the selection bias due to differential referral or diagnosis (Euser et al., 2009). Similarly, information bias due to misclassification was unlikely since presence or absence of IDH-1 mutations is made by established laboratory diagnostic test.

Significance

This research study is significant from both epidemiological and patient care perspectives. The results of the study, including demographics, genetic features, clinical characteristics, prognoses, and outcomes, should provide insight to epidemiologists and health professionals regarding the similarities and potential differences of this disease in Massachusetts compared with national trends as reported in the literature. This study may inform how best to identify, diagnose, and treat rGBM patients at the selected centers in Massachusetts.

The presumed role of the IDH-1 mutation as an overall prognostic factor upon initial diagnosis of GBM typically results in the selection of treatment modalities that are relatively aggressive, including a combination of resection, chemotherapy, and adjuvant therapy. In the absence of a clear understanding of the role of IDH-1 mutation status in recurrent GBM, there is a gap in knowledge about whether such an aggressive treatment approach in the recurrent setting would confer any added clinical or survival benefit to patients over standard of care. Considering that there are significant risks associated with aggressive treatments like craniotomy for resection, chemotherapy, and participation in

clinical trials of investigational drugs, patients' quality of life and an overall risk/benefit profile need to be considered when selecting the optimal treatment course. The results of this study are expected to contribute to positive social change by affecting both patient management and health care delivery in rGBM. The results of this study indicate that IDH-1 may not carry the same prognostic value after disease recurrence and treatment decision that are made based on this marker at initial diagnosis may not be relevant or accurate at disease recurrence. Considering that there are significant risks associated with aggressive treatments like combination of chemotherapies that are selected based on prognostic factors like IDH-1 mutation at initial diagnosis, the results of this study may mitigate unnecessary exposure of rGBM patients to the safety risks that are associated with such treatments. Similarly, if such treatments are not found necessary in the recurrent setting, then positive social change may be affected over time as it could lead to substantial savings in the overall health care costs associated with the management of this disease.

Summary

Glioblastoma is by far the most frequent malignant glioma. It is associated with a particularly aggressive course and a dismal prognosis (Ostrom et al., 2014). Extremely rapid cell infiltration is a key biological feature of glioblastoma: Tumor cells travel to other sites within the brain, which makes it very difficult to completely remove tumors through surgery (Olar & Aldape, 2014). Therefore, in conjunction with inadequate response to treatment, the recurrence rate is very high with GBM, resulting in poor overall prognosis (Li et al., 2015). Subjects who have experienced multiple recurrences,

referred to as recurrent GBM (rGBM), have a particularly poor prognosis, with a median OS (mOS) of 6 to 7 months. The OS in subjects who have failed temozolomide (TMZ) and bevacizumab, or equivalent salvage chemotherapy, is reported to be as short as 3 to 5 months (Iwamoto et al., 2009; Omuro & DeAngelis, 2013).

Isocitrate dehydrogenase-1 (IDH-1) mutation status is now used in the classification of gliomas, based on an understanding that IDH-1 mutant and wild-type gliomas have different underlying tumor biology and therefore need to be treated differently (Ostrom et al., 2018). Clinical data have showed benefits from the aggressive treatment of glioma patients with IDH-1 mutation, making upfront and initial treatment with RT and chemotherapy a standard of care for patients with IDH-1 mutant Grade II and III gliomas (Miller et al., 2017). However, the prognostic value of IDH-1 mutation in GBM remains controversial (Amelot et al., 2015; Mukasa et al., 2012; Zou et al., 2013) and limited studies have been conducted to evaluate the prognostic value of IDH-1 in rGBM (Mandel et al., 2016; Taal et al., 2014). Because the role of IDH-1 mutation in rGBM is not well understood, it is difficult to determine whether similar upfront aggressive treatment would confer any significant benefit to the patients, given the risks associated with RT and chemotherapy. Therefore, the purpose of this retrospective cohort study was to determine whether IDH-1 is a prognostic factor in rGBM. The Cox proportional-hazards model was used to assess the effect (hazard ratio) of IDH-1 mutation status on time to disease progression and survival. A multivariate Cox proportional-hazard analysis was also conducted to evaluate if the correlation between IDH-1 mutation status and disease progression and survival is affected by the covariates

of MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression. It is anticipated that the results of this study will help GBM patients, and their treating physicians make more informed decisions about the most appropriate treatment regimen for managing the disease, particularly in the recurrent setting. Chapter 2 provides additional background on the disease and the current understanding on the most relevant molecular and clinical prognostic factors in GBM/rGBM—it therefore provides the foundation for the study hypotheses and rationale for the selection of key variables and covariates of the study.

Chapter 2: Literature Review

Glioblastoma (GBM) is one of the most common types of malignant gliomas. It has an aggressive disease course and very poor prognosis. GBM may manifest at any age, but it typically affects adults at age 45-84 (Ostrom et al., 2018). Isocitrate dehydrogenase-1 (IDH-1) gene mutation has been extensively studied as a prognostic factor in GBM following initial diagnosis, but it has not been thoroughly studied in recurrent GBM (rGBM). Even following initial diagnosis of GBM, the prognostic value of IDH-1 mutation has been debated, as studies have demonstrated both weak and strong association between IDH-1 mutation and overall survival (Amelot et al., 2015; Mukasa et al., 2012; Zou et al., 2013). A few studies have examined the role of IDH-1 mutation in rGBM, but only in the clinical trial setting and with inconclusive results (Mandel et al., 2016; Taal et al., 2014).

The purpose of this study was to determine whether IDH-1 mutation is a prognostic factor in rGBM. In this retrospective cohort study, time to first recurrence from initial diagnosis and time to disease progression or death from first recurrence was evaluated in GBM patients with IDH-1 mutated and wild-type tumors.

This literature review contextualizes the research study by focusing on topics and publications related to the construct and variables of interest. The key variable of interest for the research study is IDH-1 gene mutation status and its value as a prognostic factor in the overall survival of rGBM patients. Therefore, the review first focuses on molecular classification of GBM to highlight some important genetic markers, like IDH-1, that have been identified in GBM and their potential role in the disease pathophysiology. The

review then focuses on the current knowledge base on prognostic factors, both clinical and molecular, in GBM and rGBM. These reviews of the molecular classification of the disease and prognostic factors provide the rationale for selecting IDH-1 mutation status as the key variable of the study, as well as the important molecular and clinical covariates that need to be considered when evaluating the association between IDH-1 mutation and overall survival in rGBM patients. Finally, a review of current treatment provides insight on how prognostic factors can inform treatment decisions. This review is relevant in the context of the social change that this study is anticipated to make in terms of the management of rGBM patients. There is a dearth of studies that have specifically focused on molecular and clinical prognostic factors in rGBM. However, the rationale for hypothesizing a role for various prognostic factors in rGBM can be derived from the existing research on GBM in general and rGBM in particular.

Literature Search Strategy

Pub-Med, Medline, Society of Neuro-Oncology publications, and Walden University Library were the primary sources for the literature search, which mainly focused on peer-reviewed journal articles. Key search terms included *glioblastoma, recurrent glioblastoma, IDH mutation in glioblastoma, IDH mutation in recurrent glioblastoma, prognostic factors in glioblastoma, prognostic factors in recurrent glioblastoma, prognostic factors in glioblastoma meta-analysis, treatment of glioblastoma and recurrent glioblastoma, risk factors in glioblastoma, IDH mutation as prognostic factor in glioblastoma, IDH mutation as prognostic factor in recurrent*

glioblastoma, conceptual framework in cancer and oncology, and *outcomes research in oncology*.

Considering the rapidly evolving research and knowledge about GBM, articles published over the last 3 to 5 years (2014 to 2020) were preferred for information on key variables of interest (e.g., IDH, molecular markers, and prognostic factors in GBM and rGBM). Earlier publications were considered for providing general background on GBM, foundational research, and conceptual framework. The search term *meta-analysis* was included for key variables like IDH mutation in GBM and prognostic factors in GBM.

Conceptual Framework

The aims and scope of the proposed study are congruent with the conceptual framework of outcomes research, particularly outcomes research in oncology, the goal of which is to improve medical practice to achieve better outcomes in patients (Lee et al., 2000). The definition of outcomes research and what it encompasses has evolved since the mid-1960s, particularly in the field of oncology, with the realization that the emergence of tumors and tumorigenesis are complex phenomena involving genetic, epigenetic, metabolic, proteomic, and physiologic pathways (Kovvali, 2014). While the definition of outcomes research will continue to evolve, it is broadly understood as a field that describes, interprets, and predicts the influence of different factors on a final endpoint that may range from survival to patient satisfaction with care (Apolone, 2003).

In 1966 Avidence Donabedian used the term “outcome” in the context of quality of medical care, with a focus on health services and care provided according to the expected standards (Lee et al., 2000). Advances in technology brought an increase in

healthcare costs and the focus of outcomes research shifted to health care costs (Lee et al., 2000). For example, in 1973 John Wennberg and Alan Gittelsohn highlighted different patterns and variations in care in terms of resource utilization and costs (Lee et al., 2000). In the 1990s, however, outcomes research began to include more specialties and subspecialties of clinical science in order to better understand the effectiveness of treatments and to enable clinicians to make more informed decisions (Lee et al., 2000). In the mid-1990s, a distinction between outcomes research and health services research began to emerge. Along with new technologies, therapeutic interventions, and clinical trials, the definition of clinical research also encompassed epidemiologic studies, outcomes research, and health services research (Nathan, 1998). Outcomes research began to address a broad range of questions and oncology-related endpoints, like overall survival (OS) and progression-free survival (PFS), which were studied utilizing administrative databases and cohort or case-control study designs (Lee et al., 2000).

Lee et al. (2000) included a diagrammatic representation of the conceptual framework of outcomes research, which is reproduced as Figure 1.

Figure 1

Conceptual Framework for Outcomes Research

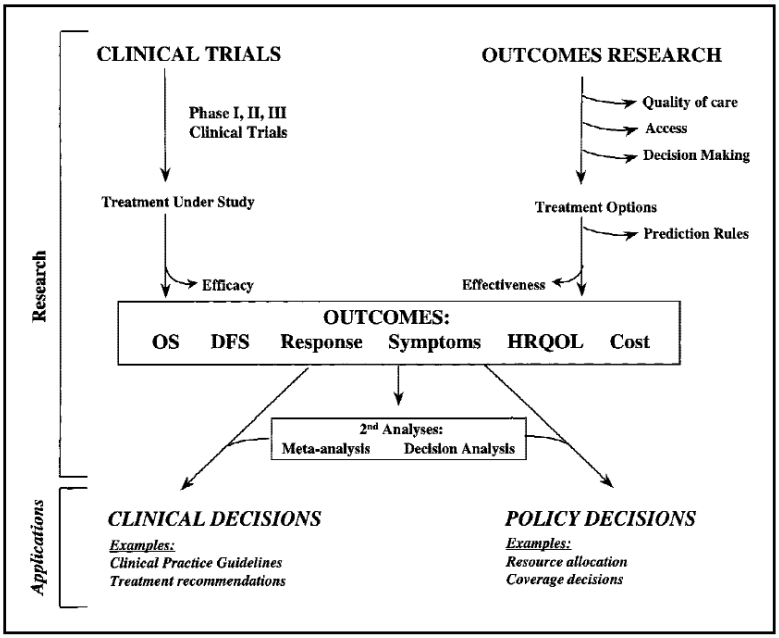

Fig. 1. Conceptual framework. Interaction is shown between research topics, end points, analytic techniques, and applications in defining outcomes research. Depicted in the **upper left corner** are the classic clinical trials and analytic techniques that are *not* outcomes research. In the **upper right corner** are shown the study topics, end points, and analytic techniques that are considered to be outcomes research. Outcomes depicted in the **center box** may or may not constitute outcomes research, depending on the context. For example, overall survival as measured in a phase III trial is not an outcomes study (efficacy), whereas it is if observed in a large community cohort (effectiveness). Symptoms have both efficacy and outcomes influences. Applications are indicated in **italics** and may emanate from either clinical trials or outcomes research. *See* text for further details. OS = overall survival; DFS = disease-free survival; HRQOL = health-related quality-of-life.

Note: Lee et al. (2000, p. 200) (see Appendix for copyright permission).

As indicated in Figure 1, Lee et al. (2000) suggested that clinical trials are not part of outcomes research, whereas quality of care, access, decision making, prediction rules, and effectiveness, along with outcome endpoints, are considered part of outcomes research. Lee et al. suggested that studies that use administrative databases are typically considered outcomes studies regardless of the questions they seek to address. Similarly, while endpoints like OS and PFS are also included in clinical trials, these endpoints are considered part of outcomes research when they are used in cohort studies with administrative databases (Lee et al., 2000). While Lee et al. acknowledged that the term

outcomes research has been liberally used and the nomenclature will continue to evolve, this study adopts their conclusion that "outcomes research is fundamentally concerned with improving the practice of medicine as applied to patients treated outside clinical trials" (Lee et al., 2000 p. 203)

The view of Lee et al. (2006) seems to be corroborated by other researchers, who are increasingly arguing that outcomes research in oncology is more than health services research *per se* and that outcomes research itself requires an integrated multidisciplinary approach in order to understand the complexity of tumorigenesis and the factors that impact patient outcomes (Apolone, 2003; Fay et al., 2015; Kovvali, 2014; Melamed et al., 2017; Roberts et al., 2019). For example, drawing upon systems biology, Kovvali (2014) has proposed the term *systems oncology*, suggesting that the disease should be studied as a phenomenon from the perspective of multiple areas of research like molecular biology and immunology. Melamed et al. (2017) suggested that randomized clinical trials may not be feasible in gynecologic oncology, thus outcomes research based on well-designed observation studies can provide better guidance on clinical decisions. Similar views were expressed by Fay et al. (2015) with respect to GBM. They indicated that an integrated multidisciplinary research approach is needed to better understand this cancer and improve patient outcomes because GBM treatment practices have not significantly changed over the past 10 years.

This study is aligned with the conceptual framework of outcomes research, particularly outcomes research in oncology as proposed by Lee et al. (2000) and generally adopted by other researchers (Apolone, 2003; Fay et al., 2015; Kovvali, 2014;

Melamed et al., 2017; Roberts et al., 2019). The study's research scope, methodology, and potential application fall within the parameters of research and applications of outcomes research that Lee et al. outlined and illustrated in the conceptual framework (see Figure 1). First, the purpose of this study was to determine whether IDH-1 mutation is a prognostic factor in rGBM by evaluating time to first recurrence from initial diagnosis and time to disease progression or death (overall survival) from first recurrence in GBM patients with IDH-1 mutated and wild-type tumors. This broadly fits the treatment options and prediction rules of the outcomes research framework. In GBM, IDH-1 mutation is an important prognostic factor that informs treatment options, and this study evaluated the role of this mutation in the recurrent setting. Second, the study utilized clinical outcomes, OS and PFS, that are suggested in the outcomes research framework. Third, the study was a retrospective cohort study and analyses were based on data obtained from electronic medical records (EMR) at select hospitals. EMR provides detailed clinical data that are relevant for outcomes research and is now increasingly used in clinical oncology and epidemiology studies (Lau et al., 2011). Moreover, Lee et al. (2006) suggested that studies that use administrative databases and study these endpoints are typically considered outcomes studies regardless of the questions they seek to address. Finally, the application aspect of the outcomes research conceptual framework suggests that the research should lead to clinical or policy decisions (Lee et al., 2000). The results of this study may inform clinical decisions in terms of treatment recommendations and clinical practice guidelines for the management of rGBM patients, using IDH-1 mutation as a prognostic factor. The results of this study indicate that IDH-1

mutation is not a prognostic factor in the recurrent setting and an aggressive treatment approach will most likely not confer any clinical or survival advantage over standard of care; therefore, the results of this study may mitigate the unnecessary exposure of patients to the risks associated with such procedures and treatments. Similarly, if such treatments are not found necessary in the recurrent setting, then policy changes may be made over time that could lead to substantial savings in the overall health care costs associated with the management of this disease. Therefore, the overall scope of this study, in terms of its research inquiry, methodology, and potential applicability, was contextualized within the conceptual framework of outcomes research in oncology.

Literature Related to Key Variables

Molecular Characterization of Glioblastoma

A review of the molecular classification of GBM highlights some important genetic markers, like IDH-1, that have been identified in GBM and their potential role in the disease's pathophysiology. Glioblastoma is morphologically or histologically divided into two identical subtypes: primary and secondary glioblastoma (Lieberman, 2017; Olar & Aldape, 2014). Primary glioblastoma occurs de novo without the presence of a precursor lesion and constitutes approximately 90% of GBM (Lieberman, 2017; Olar & Aldape, 2014). Secondary glioblastoma follows the progression of WHO Grades II or III with preexisting low-grade astrocytoma (Lieberman, 2017; Olar & Aldape, 2014).

Recent advances in molecular neuropathology have shown that molecular characterization can be utilized to further classify glioblastomas that are histologically identical (Lieberman, 2017; Olar & Aldape, 2014). Moreover, even though these

molecular alterations are complex, this genetic profiling has suggested there is prognostic value in these molecular variations and therefore a possible association with clinical outcomes of GBM. The following is a brief outline of the key genetic alterations in GBM that are considered clinically relevant (Lieberman, 2017; Olar & Aldape, 2014).

Isocitrate Dehydrogenase (IDH) Mutation

IDH has three isoforms, the most common of which includes mutation in IDH-1 (IDH1-R132H) (Lieberman, 2017; Olar & Aldape, 2014). IDH mutations are noted in approximately 5% of primary GBM and 80% of secondary GBM (Lieberman, 2017; Olar & Aldape, 2014). IDH-1 mutation has been associated with better prognosis, particularly in high-grade gliomas (Lieberman, 2017; Olar & Aldape, 2014). In 2016, IDH mutation status was included in the WHO classification of GBM. Currently, GBM is classified as a WHO Grade IV tumor of the central nervous system and is divided into three subtypes based on histology and molecular parameters: IDH-wildtype, IDH-mutant, and not otherwise specified (Louis et al., 2016). The Cancer Genome Atlas (TCGA) researcher network has suggested that other genetic abnormalities may also be clinically relevant (Verhaak et al., 2010).

Epidermal Growth Factor Receptor (EGFR)

Upregulation of EGFR has been reported in 40-50% of glioblastomas, predominantly in primary glioblastomas but also in secondary glioblastomas (Lieberman, 2017; Olar & Aldape, 2014). EGFR, including its mutant variants like EGFRvIII, has been shown to confer heterogeneity to tumor cells and upon activation leads to angiogenesis, DNA transcription, cell proliferation, and delayed apoptosis (Lieberman,

2017; Olar & Aldape, 2014). The effects of EGFR upregulation in GBM and rGBM are not clearly understood.

O-6-methylguanine-DNA Methyltransferase (MGMT) Promoter Methylation

MGMT encodes a DNA repair protein that is responsible for removing alkyl groups that cause DNA damage (Olar & Aldape, 2014; Yang et al., 2015). The MGMT promoter contains CpG islands in the promoter region, and methylation of these CpG sites suppresses MGMT transcription (this is called MGMT silencing), thereby affecting DNA repair (Olar & Aldape, 2014; Yang et al., 2015). Based on this underlying molecular biology, methylation status of the MGMT promoter is associated with a more favorable response to alkylating chemotherapies, like temozolomide (TMZ). Over 50% of primary and secondary GBM patients have methylated MGMT promoter (Olar & Aldape, 2014; Yang et al., 2015). While MGMT promoter methylation status is now considered a prognostic factor in patients with GBM, its prognostic value in rGBM is not fully established (Olar & Aldape, 2014; Yang et al., 2015).

TP53 Mutation

TP53 mutation has been reported in up to 30% of primary and 70% of secondary GBM patients (Thakkar et al., 2014). The prognostic value of TP53 mutation has not been established and studies conducted to evaluate it as a prognostic marker have been inconclusive (Thakkar et al., 2014).

ATRX Mutation

ATRX mutations result in genomic instability by causing alternative lengthening of telomerases (ALT). They have been noted in multiple tumors (Thakkar et al., 2014).

The ATRX is mutated in 57% of secondary GBM patients and tends to cluster with IDH-1 and TP53 mutations (Thakkar et al., 2014). In astrocytic tumors, better prognosis has been reported for patients with ATRX mutation than in those that expressed unmutated ATRX and had IDH mutation (Thakkar et al., 2014).

TERT Mutation

TERT is important for growing cells and maintains telomeres. TERT mutations are the most frequently occurring genetic mutations in GBM and are significantly higher in primary GBM (Thakkar et al., 2014). While TERT mutations in GBM have been shown to correlate with EGFR upregulation, they have been shown to inversely correlate with TP53 and IDH mutations (Thakkar et al., 2014).

Prognostic Factors

This review of prognostic factors first covers GBM in general and then rGBM in particular. The intent is to provide background information that supports the investigation of IDH-1 mutation status as a potential prognostic factor in rGBM. The review of other clinical and molecular markers provides a rationale for the selection of appropriate covariates in the overall analysis.

Prognostic Factors in GBM

GBM patients have very poor prognosis: a 5-year survival after diagnosis is seen in less than 5% of patients (Ostrom et al., 2018; Thakkar et al., 2014). Research has focused on both clinical and molecular prognostic factors (Ostrom et al., 2018; Thakkar et al., 2014). Age at diagnosis, tumor location, performance status, and tumor resection have been identified as favorable clinical prognostic factors, while the key molecular

markers discussed above (MGMT promoter methylation, IDH-1 mutation, EGFR upregulation, TP53 mutation, ATRX mutation, and TERT mutations) have been studied as potential molecular prognostic factors in GBM (Ostrom et al., 2018; Thakkar et al., 2014).

Clinical Prognostic Factors. Age 50 years is typically used as the cut-off from a prognostic value perspective, with a higher risk of death seen in patients over 70 years. The shorter survival rate for older GBM patients is most likely due to comorbidities and inability to tolerate the effects of the cancer itself and treatments like surgery and chemotherapy (Ostrom et al., 2018; Thakkar et al., 2014). In terms of tumor site, while the difference in the prognosis of cerebellar and supratentorial GBM is not clearly understood, frontal lobe tumors in supratentorial GBMs have better prognosis and survival outcomes (Ostrom et al., 2018; Thakkar et al., 2014).

Surgical treatment includes complete macroscopic tumor removal or gross total resection (GTR) and subtotal resection (Czapski et al., 2018). While resection is an important treatment option in GBM, it does not offer a cure and is not completely effective due to the unclear boundary between tumor and healthy brain tissues and the infiltration of tumor cells into surrounding areas (Czapski et al., 2018). Even with this limitation, GTR has been shown to increase survival to up to 20 months in malignant gliomas, compared to 8.8 months with no GTR (Czapski et al., 2018). Lu et al. (2019) conducted a meta-analysis to look at the survival benefit of maximal resection for glioblastoma and reported that radiographic GTR was the most prognostic in terms of survival (HR 0.52; 95% CI, 0.44-0.61; $p < 0.01$) (Lu et al., 2019). Intraoperative MR

imaging, ultrasonography, and tumor staining are now utilized to better define the tumor boundaries and maximize resection (Czapski et al., 2018). Recent studies, however, have highlighted the timing of resection and its association with PFS and OS in GBM (Goldman et al., 2018; Y.-H. Zhao et al., 2019). For example, a lower risk of death (HR: 0.62) was noted with repeat resection without taking the timing of resection into account, but a higher risk of death was noted (HR: 2.19) after adjustment for the timing of resection (Goldman et al., 2018). An association has also been noted between tumor resection and IDH mutation, as patients with IDH-mutated tumors showed better prognosis following maximal tumor resection (Czapski et al., 2018).

Molecular Markers as Prognostic Factors. Several molecular prognostic markers have been investigated in GBM and their interactions are complex (Xavier-Magalhães et al., 2013). The prior section provided a brief outline of the key molecular markers in GBM. This subsection focuses on the two markers, MGMT promoter methylation and IDH-1 mutation, that are considered the most promising in terms of their prognostic value and that have been extensively studied for this purpose.

MGMT Promoter Methylation. As discussed earlier, silencing of MGMT by promoter methylation suppresses MGMT transcription (MGMT silencing), thereby affecting DNA repair (Olar & Aldape, 2014; Yang et al., 2015). Two landmark studies showed that MGMT silencing leads to increased sensitivity to chemotherapy with temozolomide, thereby improving patient survival (Hegi et al., 2005; Stupp et al., 2009). Hegi et al. (2005) showed that mOS in patients with methylated MGMT was 21.7 months, compared to 12.7 months in patients with unmethylated MGMT. Similarly, in

Stupp et al. (2009), longer survival was noted in patients with MGMT promoter methylation. These patients also responded better to a combination of radiotherapy (RT) and chemotherapy. These two studies provided initial evidence for MGMT promoter methylation as a prognostic molecular marker in GBM.

Several studies have been conducted over the last 10 years to further evaluate the role of MGMT promoter methylation as a prognostic factor in GBM. This body of work has been captured in three meta-analyses (Olson et al., 2011; H. Zhao et al., 2016; Y.-H. Zhao et al., 2018). Olson et al. (2011) was based on 2018 patients in 20 different studies and showed a high association between MGMT promoter methylation and overall survival in patients receiving chemotherapy (Olson, 2011). Similarly, both H. Zhao et al. (2016) and Y.-H. Zhou et al. (2018) indicated that MGMT promoter methylation was associated with improved PFS and OS in GBM patients. For example, Y.-H. Zhao et al. included 64 studies and evaluated the association between OS and MGMT promoter methylation in GBM patients. The meta-analysis showed that the OS was significantly better (HR = 0.52) in patients with methylated MGMT promoter than in patients with unmethylated status (Y.-H. Zhao et al., 2018). Overall, these studies suggest a prognostic value for MGMT promoter methylation in GBM patients (Olson et al., 2011; H. Zhao et al., 2016; Y.-H. Zhao et al., 2018). There are, however, other studies that did not show a statistical significance between MGMT promoter methylation and survival (Costa et al., 2010; van den Bent et al., 2009; Xavier-Magalhães et al., 2013). For example, Costa et al. (2010) included 90 GBM patients who received post-operative TMZ; while a trend was

noted in MGMT promoter methylation and PFS and OS, it was not statistically significant (Costa et al., 2010).

IDH Mutations. IDH-1, IDH-2, and IDH-3 are three different isoforms of IDH. Mutations in IDH-1 and IDH-2 have been identified in both hematologic and solid tumors, including low-grade gliomas and secondary glioblastomas (Golub et al., 2019; Kaminska et al., 2019; Tommasini-Ghelfi et al., 2019). IDH-1 mutation is the most common (> 95%) type of IDH mutation, while an association between tumors and IDH-3 mutation has not been reported (Deng et al., 2018; Golub et al., 2019; Kaminska et al., 2019). IDH-1 and -2 are nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent enzymes that are involved in the decarboxylation of isocitrate to α-ketoglutarate (α-KG) and protect cells and DNA from being damaged by reactive oxygen species (ROS) and other oxidative stress (Kaminska et al., 2019). The mutated IDH enzyme not only loses the aforementioned catalytic function, but also leads to reduction of α-KG to 2-hydroxyglutarate (2-HG), which is an oncometabolite that causes cancer (Kaminska et al., 2019).

Approximately 90% of GBM cases are IDH-wild type and are known as primary GBM, while the 10% of cases that carry IDH mutation, predominantly IDH-1 mutation, are considered secondary GBM (Tateishi et al., 2017; Tommasini-Ghelfi et al., 2019). Over the years, many studies have established IDH-1 mutation as a favorable prognostic factor for both PFS and OS in adult GBM (Chen et al., 2016; Cheng et al., 2013; Juratli et al., 2012; Kaminska et al., 2019; Nobusawa et al., 2009; Sun et al., 2013; Tateishi et al., 2017; Xia et al., 2015; Yan et al., 2009). For example, Chen et al. (2016) conducted a

meta-analysis that included randomized controlled trials and prospective and retrospective studies of patients with GBM; it used PFS and OS to evaluate the association between IDH mutation and prognosis. The pooled hazard ratios of 0.322 (95% CI 0.24200.455, P < .001) and 0.358 (95% CI 0.264-0.487, P < .001) indicated that IDH mutation was associated with PFS and OS, respectively (Chen, 2016). Similarly, Xi et al. (2015) performed a meta-analysis of 55 observational studies, including 9,487 glioma patients, and found that patients with IDH mutation had a better prognosis in terms of both PFS (HR = 0.42, 95% CI: 0.35-0.51; P < .001) and OS (HR = 0.39, 95%CI: 0.34-0.45; P < .001). An earlier meta-analysis by Cheng et al. (2013) that included nine-studies, with a total of 1,669 GBM patients, also confirmed that IDH-1 mutation was associated with improved survival in patients with GBM (HR = 0.45, 95%CI 0.29-0.69, P < .001). Some studies, however, do not support an association between IDH-1 mutation and long-term survival in GBM and indicate that IDH mutation is a weak predictor of overall survival in GBM (Amelot et al., 2015; Mukasa et al., 2012). For example, Amelot et al. (2015) conducted a retrospective analysis of 207 GBM patients and reported that the rate of IDH mutation was not statistically significant between non-long-term survivor and long-term survivor groups (1.16% versus 5.9%, $p = .14$).

Some underlying mechanisms by which IDH-1 mutation may confer survival benefit to GBM patients include: (a) while the IDH-1 mutation plays a role in causing cancer, it also makes cells carrying this mutation susceptible to ROS-based chemotherapies; (b) tumors with IDH-1 mutation seem to be located in less risky parts of the brain and have sharper tumor margins, making them more amenable to complete

resection, which plays an important role in survival; and (c) patients with IDH-1 mutated tumors typically display better neurocognitive function and overall performance score (Tateishi et al., 2017).

Prognostic Factors in Recurrent GBM

Identifying factors that can predict survival outcomes following recurrence of GBM is of interest because these factors can inform treatment modalities for such patients. Age, Karnofsky performance status (KPS), surgical resection, and chemotherapy at progression have been identified as key clinical prognostic factors for survival in recurrent GBM (Archavlis et al., 2014; Audureau et al., 2018; Chaichana et al., 2013; Cloughesy et al., 2014; D'Amico et al., 2015; Ringel et al., 2016; Stupp et al., 2012; Terasaki et al., 2007). However, the complexity of disease pathophysiology and interactions between molecular and clinical markers of prognosis make it challenging to conclusively determine the most appropriate prognostic factors in GBM, including rGBM. For example, while Audureau et al. (2018), in their study of 777 adult patients with recurrent glioblastoma, identified surgical resection at recurrence as an independent predictor of long-term survival (HR, 0.57; 95% CI 0.44-0.73; $p < .001$), their findings are confounded by the exclusion of IDH mutation status and MGMT promoter methylation status, which are independent predictors of overall survival, at least in GBM. Moreover, while several studies have established surgical resection as a predictor of overall survival in rGBM (Audureau et al., 2018; Chaichana et al., 2013; D'Amico et al., 2015), other studies have suggested that surgery at progression may not be a prognostic marker for survival outcomes in rGBM patients. For example, Clarke et al. (2011) studied two

independent data sets of 511 and 247 rGBM patients and found no statistically significant difference in 6-month PFS or OS between patients with and without surgery at progression (Clarke et al., 2011). Despite these discrepancies, age, KPS, surgical resection, and chemotherapy at progression should be considered as important prognostic factors in rGBM and factored in as covariates when investigating any other specific prognostic factors.

Molecular markers like IDH-1 gene mutation have been extensively studied as prognostic factors in GBM, but not in the recurrent setting. Even following initial diagnosis of GBM, the prognostic value of IDH-1 mutation is being debated in view of studies that have demonstrated both weak and strong association between IDH-1 mutation and overall survival (Amelot et al., 2015; Mukasa et al., 2012). A few studies have examined the role of IDH-1 mutation in rGBM, but only in the clinical trial setting and the results were not conclusive (Mandel et al., 2016; Taal et al., 2014). These studies suggested that patients with an IDH-1 mutated tumor show an improved trend in overall survival at first recurrence. However, IDH-1 mutation did not result in prolonged PFS or OS compared to IDH-1 wild-type tumors in recurrent GBM trials (Mandel et al., 2016). Moreover, studies conducted by Mandel (2016) and Taal (2014) included a total of only 10 patients (five patients in each study) with IDH-1 mutation. The authors indicated that the results of these studies must be interpreted with caution, considering the very small sample size, and suggested additional studies to better understand the role of IDH-1 mutation in rGBM.

Current Therapies and Unmet Medical Need

Newly diagnosed GBM subjects have a median overall survival (mOS) of 12 to 15 months and a 2-year OS rate of up to 27% (Omuro & DeAngelis, 2013). Subjects who have experienced multiple recurrences have a poor prognosis, with an mOS of 6 to 7 months; OS in subjects who have failed TMZ and bevacizumab, or equivalent salvage chemotherapy, is reported to be as short as 3 to 5 months (Iwamoto et al., 2009; Omuro & DeAngelis, 2013). The poor median OS rates in rGBM, resulting from the available treatment options not extending the subjects' OS beyond 6 or 7 months, highlights the seriousness of recurrent or progressive GBM as well as the unmet medical need in treating this disease.

The current FDA-approved therapies – bevacizumab, carmustine wafer, NovoTTF-100A, and lomustine – are marginally effective in extending OS in subjects with recurrent or progressive GBM (Davis, 2016). Despite the scientific advances in immunotherapies and monoclonal antibodies, a new standard of care for GBM has not been established in over 10 years. TMZ following RT is still the standard of care and it was established in 2005 following a Phase 3 trial that was led by Roger Stupp and sponsored by the European Organization for Research and Treatment of Cancer and the NCI - Clinical Trials Group (Davis, 2016; Stupp et al., 2009). The lack of standard salvage therapies has prompted the use of unsatisfactory treatment options, such as nitrosoureas, temozolomide re-challenge, and other targeted agents (Davis, 2016). In addition to surgical resection and approved therapies, a better understanding of tumor microenvironment and the potential role of immune regulation and epigenetic pathways

in disease pathophysiology has led to the initiation of clinical trials with various immune modulators, including monoclonal antibodies, vaccines, PD-1/PDL-1 checkpoint inhibitors, and DNA methyltransferase inhibitors (Artene et al., 2018; Chin et al., 2018; Jain, 2018; Paolillo et al., 2018; Zhang et al., 2019). These therapies, if proven effective and safe either as monotherapies or in combination, can provide additional treatment options for GBM patients.

Summary of Research Approach in Literature

GBM is considered to be of the most aggressive and untreatable forms of cancer (Paolillo et al., 2018). The topics of research in GBM aimed at understanding the pathophysiology of the disease and meeting the needs of patients through more effective treatments are expansive and rapidly evolving. On the one hand, researchers are focusing on molecular, genetic, epigenetic, and immunological markers in GBM, not only to better classify the disease but also to evaluate the prognostic value of these markers in terms of disease outcomes. On the other hand, researchers are also evaluating the role of clinical prognostic factors, such as age at diagnosis, tumor site, surgical resection, and its timing, KPS, and chemotherapeutic regimens, in the disease outcome. However, there are complex interactions between these molecular and clinical prognostic factors and researchers are aware that they need to adjust for other potential prognostic factors, both molecular and clinical, when evaluating the role of any specific factor for its prognostic value. This study took similar considerations into account in its design.

Apart from some prospective clinical trials conducted for novel therapies targeting underlying genetic, epigenetic, and immunological markers in disease

pathophysiology, most of the research conducted in this space has been retrospective. Because these retrospective studies are typically single- or limited-center studies, similar patient molecular and clinical information is available to make reasonable comparisons for the variables of interest. Furthermore, the number of such retrospective studies conducted over the years has created a database that is adequate for more in-depth analysis, as indicated by meta-analyses that have evaluated the prognostic value of MGMT promoter methylation status, IDH mutation status, and surgical resection for the long-term survival of GBM patients. However, there are some general limitations to conducting GBM research, which are further accentuated in retrospective studies conducted at limited centers. First, GBM is a rare disease with just over 100,000 patients in the United States; this affects the sample size and design of the studies conducted. Second, the GBM patient population is very heterogenous in terms of the genetic, epigenetic, and immunological markers that they express and the treatment modalities that they receive in the course of their disease (e.g., surgical resections and the timings of these resections, chemotherapies, and immunotherapies). This overall heterogeneity, along with the rareness of the disease and the small sample size, makes it difficult to completely match the groups in a comparative study on the variable of interest.

Summary and Conclusions

The body of knowledge on rGBM, including studies evaluating its prognostic factors, is less extensive than on GBM overall. While it is reasonable to draw inferences from the results of studies that evaluated prognostic factors for GBM, the prognostic value of these molecular and clinical factors needs to be evaluated in the recurrent setting

as well to inform the most suitable treatment course for GBM patients following recurrence. This literature review has indicated that for rGBM researchers have focused more on clinical prognostic factors like tumor location and timing of surgical resection than on molecular prognostic factors. A few studies have explored the prognostic value of molecular markers like IDH mutation in rGBM, but the sample size was too small to draw any reasonable conclusions. Considering that there is still some debate about the prognostic value of factors like MGMT promoter methylation and IDH mutation even in GBM, further studies are warranted that specifically evaluate the prognostic value of such factors in rGBM.

The focus of this research study was to evaluate the prognostic value of IDH-1 mutation status in rGBM. Because GBM is not curable, all patients relapse: disease recurs at some point following remission after initial treatment. While the overall knowledge of clinical and molecular prognostic factors in GBM also informs treatment-modalities selected for patients at recurrence, a better understanding of the relevance of these prognostic factors in the recurrent setting may provide further insight into treatment decisions for rGBM patients. For example, understanding the role of IDH-1 in rGBM is relevant because over the years clinical data has shown a benefit from the aggressive treatment of glioma patients with IDH-1 mutation, thereby making upfront and initial treatment with RT and chemotherapy a standard of care for patients with IDH-1 mutant gliomas (Miller et al., 2017). Because the role of IDH-1 mutation in rGBM is not well understood, it is difficult to determine whether similar aggressive treatment at

recurrence will confer any significant benefit to the patients, given the potential risks associated with RT and chemotherapy.

Other relevant molecular and clinical prognostic factors need to be considered as covariates in investigating the prognostic value of IDH-1 in rGBM. This literature review suggests that MGMT promoter methylation (molecular marker), age, KPS, surgery for resection, and chemotherapy at progression (clinical factors) would be important covariates when comparing the PFS and OS of rGBM patients with and without IDH-1 mutation.

Consistent with other studies conducted to determine the association between prognostic factors and clinical outcomes like PFS and OS, this study was a retrospective cohort study based on quantitative data collected through retrospective chart reviews of adult patients diagnosed with GBM at select hospitals in Massachusetts. Individual chart reviews for patients provided data on initial diagnosis and IDH-1 mutation status as well time to first recurrence, time to disease progression or subsequent recurrence, and death. Disease outcomes – time to recurrence from initial diagnosis, time to disease progression following first recurrence, and overall survival – was evaluated to determine whether IDH-1 mutation is a prognostic factor for adult rGBM patients. The Cox proportional-hazard model was used to assess the effect (hazard ratio) of IDH-1 mutation status on time to disease progression and survival. In addition, a Kaplan-Meir curves were generated to obtain survival rate for patients with and without IDH-1 mutation. Cox regression analysis was also conducted to test the effects of other key covariates –

MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression–on progression and survival.

Chapter 3: Research Method

Introduction

The purpose of this study was to determine whether isocitrate dehydrogenase-1 (IDH-1) mutation is a prognostic factor in recurrent glioblastoma (rGBM) considering other molecular and clinical prognostic factors as covariates. In a retrospective cohort study, time to first recurrence from initial diagnosis and time to disease progression or death from first recurrence was evaluated in GBM patients with IDH-1 mutated and wild-type tumors. The effect of key variables (i.e., O^6-methylguanine-DNA methyl-transferase (MGMT) promoter methylation, age, Karnofsky Performance Score (KPS), surgery for resection, and chemotherapy at progression) on correlation between IDH-1 mutation status and disease progression and survival was evaluated. The study also determined the overall prevalence of GBM as a type of nervous system cancers reported at select centers in Massachusetts.

This chapter provides an overview of the research design and rationale to address specific research questions pertaining to potential prognostic role of IDH-1 mutation in disease progression and overall survival of rGBM patients as well the effects of other covariates on prognostic effect of IDH-1 mutation. The population and sampling sections describe the population of GBM patients that was included in the study and the inclusion criteria that was used to include subject specific data in the overall evaluable sample from the retrospective cohort. The data analysis plan section describes how each research question was addressed based on the key study variables utilizing appropriate statistical methods and quantitative analysis with IBM SPSS Statistics (version 25) software

obtained through Walden University. Chapter also covers aspects of external and internal validity based on the overall scope of the study and its design and limitations. A brief overview of ethical considerations is included before the overall summary of this chapter.

Research Design and Rationale

Study Variables

In a retrospective cohort study design, relevant data on GBM patients was collected at two selected Massachusetts hospitals (i.e., Brigham and Women's and St. Vincent Hospitals). Table 1 outlines the patient-level independent and dependent variables that were selected to address specific research questions. The status of IDH-1 mutation was the key independent variable and was categorized as a nominal variable. Key dependent variables were time-to-disease progression and survival. These dependent variables were categorized as interval variables (i.e., number of days) and were measured from initial diagnosis of GBM in each subject as well from first and/or subsequent recurrences in the same subject. In addition to demographic information, independent variables included key covariates selected for the study including MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy a progression.

Table 1

Dataset Variables

Variable	Coding Description	Level of Measurement
Independent Variables (Exposure Variable)		
IDH-1 mutation status	Yes = 1; No = 2	Nominal
Gender	Female = 1; Male = 2	Nominal
Age	≥ 18 and <55 = 1; 56-65 = 2; 66-75 = 3; ≥76 = 4	Nominal
Ethnicity	Black = 1; White = 2; Hispanic = 3; Asian = 3 Other/Unknown = 4	Nominal
Hospital	BWH* = 1; SVH** = 2	Nominal
MGMT promoter methylation	Yes = 1; No = 2 Missing data = 3	Nominal
Karnofsky Performance Score (KPS)	>70 = 1; ≤70 =2 Missing data = 3	Nominal
Surgery for resection	Yes = 1; No = 2	Nominal
Chemotherapy at recurrence	Yes = 1; No = 2	Nominal
Dependent Variables (Outcome Variable)		
Time to disease progression or first recurrence from initial diagnosis	Number of days	Interval

Variable	Coding Description	Level of Measurement
Time to subsequent recurrence from first recurrence	Number of days	Interval
Time to death from initial diagnosis	Number of days	Interval
Time to death from first recurrence	Number of days	Interval

*BWH = Brigham and Women's Hospital.

**SVH = St. Vincent Hospital.

The two hospitals included in this study represented two major centers, one each in the Eastern (BWH) and Western (SVH) part of Massachusetts. There are other hospitals in the region that refer GBM patients to BWH. The retrospective analysis timeframe for this study included a period of 12 years from January 2008 to 2020.

Study Design

This study was a retrospective cohort study based on quantitative data collected through retrospective chart reviews of adult patients diagnosed with GBM at select hospitals in Massachusetts. An observational study design, rather than an experimental design, was selected considering the scope of the study that aimed to evaluate the association between exposure (i.e., IDH-1 mutation) and disease outcome (i.e., time to disease progression or progression free survival and survival) (Euser et al., 2009; Song & Chung, 2010). Study cohorts, in terms of exposure, and outcome measures are summarized below.

Exposure and Cohorts

IDH-1 gene mutation in tumors of GBM patients was considered as the exposure of interest for the purposes of this study; therefore, the two cohorts were defined based on this exposure status that is, GBM patients that had IDH-1 mutation and GBM patients without IDH-1 mutation or wild-type IDH-1 gene. In GBM, testing for IDH-1 mutation status is done utilizing sensitive diagnostic tests at the time of the initial diagnosis of the disease and the result of this testing is included in the medical records along with other clinical and diagnostic assessments conducted as part of the initial diagnosis. In this study, the date of exposure (IDH-1 mutation status) for each patient in each cohort was the same as the date of their initial diagnosis of GBM as this testing was conducted per the standard practices as part of the initial diagnosis and the information was available during the initial chart review; therefore, for the purposes of this study the exposure in each patient in each cohort occurred before the outcomes described below.

Study Outcomes

Time to disease progression or progression free survival (PFS) and survival were the two main outcomes selected for this study. PFS was considered as the duration of time from initial diagnosis and treatment of disease to first recurrence and duration of time from first recurrence to subsequent recurrence or disease progression. Survival was considered as either the duration of time from initial diagnosis of the disease to death or duration of time from first recurrence of disease to death. In this study, time in days was calculated to determine PFS and survival in patients with and without mutated IDH-1 gene. In addition to the date of initial diagnosis, the medical record of each patient

included a date, along with a documented imaging evidence, of disease recurrence that allowed to determine the duration of time from initial diagnosis to first and subsequent recurrences. Similarly, the date of death was also recorded in the medical records that allowed to determine the survival duration from initial diagnosis and from first disease recurrence. As noted above, the date of the IDH-1 mutation status (exposure) was considered the same as the date of initial diagnosis as this information was available in the records for each patient at initial diagnosis. The events of disease progression and death, that respectively determined the outcomes of PFS and survival, happened after the initial diagnosis of disease and determination of IDH-1 mutation status; therefore, the exposure in each patient in each cohort occurred before the selected study outcomes.

Individual chart reviews for patients provided data on initial diagnosis and IDH-1 mutation status as well time to first recurrence, time to disease progression or subsequent recurrence, and death. Disease outcomes – time to recurrence from initial diagnosis, time to disease progression following first recurrence, and overall survival – were evaluated to determine whether IDH-1 mutation is a prognostic factor for adult GBM and rGBM patients. Retrospective cohort design was selected mainly for efficiency because a prospective cohort study would have been costly and time-consuming making it impractical for this dissertation project. Moreover, the proposed study was aligned with the conceptual framework of outcomes research, particularly outcomes research in oncology that has been generally adopted by researchers (Apolone, 2003; Fay et al., 2015; Kovvali, 2014; Lee et al., 2000; Melamed et al., 2017; Roberts et al., 2019). Outcomes research addresses a broad range of questions and oncology-related endpoints,

like overall survival (OS) and progression-free survival (PFS), which are now being studied utilizing administrative databases and cohort or case-control study designs (Apolone, 2003; Fay et al., 2015; Kovvali, 2014; Lee et al., 2000). The study utilized clinical outcomes, OS and PFS, that are suggested in the outcomes research framework and were based on data obtained from electronic medical records (EMR) at select hospitals. EMR provides detailed clinical data that are relevant for outcomes research and is now increasingly used in clinical oncology and epidemiology studies (Lau et al., 2011).

Methodology

Population

The target population consisted of subjects with confirmed diagnosis of GBM in the EMR database of the two hospitals in Massachusetts. Some subjects obtained their initial diagnosis of GBM at other hospitals in the region and were referred to these hospitals for treatment. The databases were searched through a Research Patient Data Repository (RPDR) query using the International Classification of Disease-10 (ICD-10) code. Since GBM or rGBM does not have a specific ICD-10 code, initial search was conducted using the ICD-10 code C71 for “malignant neoplasm of the brain.” As anticipated the search based on ICD-10 code C71 resulted in approximately 1200-1500 cases that included all neoplasms of the brain. It was not feasible to review individual patient records of all these cases to identify patients that would qualify for evaluable population (i.e., subjects included in the analysis plan) to determine the prognostic value of IDH-1 mutation; therefore, to make the database search manageable, the strategy

outlined below in sampling and sampling procedure was used instead to identify evaluable study population based on the key variables of the study that is, IDH-1 mutation status and GBM patients with documented recurrence of their disease.

Sampling and Sampling Procedures

This study was based on secondary analysis through retrospective chart review of quantitative data collected on adult subjects with GBM at select hospitals in Massachusetts. Since it was not possible to review the 1200 to 1500 cases that resulted from suing the ICD-10 code C71 for "malignant neoplasm of the brain", the database search strategy was revised to identify relevant cases based on the key variables of the study that is, IDH-1 mutation status and GBM patients with documented recurrence of their disease. The database was first queried for all patients who had been tested for IDH-1 mutation and this search yielded a total of 588 cases. Since IDH-1 mutation can be of interest in a variety of oncologic conditions (e.g., myeloid leukemia, breast cancer, and lung cancer) (Bledea et al., 2019; Hodges et al., 2013), the ICD-10 code C71 for neoplasm of the brain was then applied to these 588 cases to further narrow the cases to relevant study population and this step reduced the sample size to 405 cases. In the final step, individual patient records for all 405 cases were reviewed and per the inclusion/exclusion criteria those patients that had non-glioblastoma tumors, or did not have confirmed diagnosis of GBM through radiographic imaging, or GBM patients with no documented evidence of recurrence were excluded from the final dataset. This strategy resulted in a final sample size of 177 cases, whose charts were then reviewed in detail to collect all relevant information for detailed analyses to address the research

questions. Each subject's protected health information, except for demographic information, was de-identified and all relevant data was entered on an Excel spreadsheet and cross-checked against the EMR generated output for accuracy. These data were then uploaded from the Excel spreadsheet into SPSS v25 for analysis.

An a priori analysis conducted using G*Power3 indicated that a sample size of 108 subjects was needed to detect a small effect size (d = .15) at an expected power of .90 and an alpha of .05 (Faul et al., 2007). The sample size was also estimated using the method proposed by Hsieh and Lavori (2000) that provides a conservative sample size estimation specifically for Cox proportional hazard regression model (Hsieh & Lavori, 2000). Although the inclusion criteria restricting the study sample to only rGBM patients with known IDH-1 mutation status narrowed the overall study population, the final sample size (177 cases) still exceeded the sample size of 108 that was estimated a priori using G*Power3 (Faul et al., 2007).

Data Collection

The study was designed as a retrospective cohort study. A major step in the data collection process was the selection of participating hospitals to ensure that enough relevant and reliable data was available for GBM patients to address the research questions and the relative ease with which this data could be accessed for the study. Initial assessment was done by interviewing neuro oncologists within my professional network to determine the number of GBM cases seen or referred to select hospitals in the areas, the availability of medical records for these patients, interest in collaboration on the study, and the flexibility in the institutional process to allow access to these data for

collaborating researchers. Brigham and Women's Hospital (BWH) and St. Vincent Hospital (SVH) were selected for this study because it was anticipated that there were approximately 1500 hundred subjects with neoplasms of the brain in the combined databases that could provide required sample size per the inclusion/exclusion criteria of the study particularly key variables of the study that is, patients with known IDH-1 mutation status and document evidence of recurrent GBM. Furthermore, both hospitals have a robust EMR system in place for patient medical records that could be searched and retrieved for required information. BWH and SVH are key hospitals, respectively, in the Eastern and Western part of Massachusetts and GBM patients from other hospitals in the region are also referred to BWH. Dr. Timothy Smith, Director, Computational Neuroscience Outcomes Center and Assistant Professor of Neurosurgery, Brigham and Women's Hospital and Harvard Medical School and Dr. Daniyal Siddiqui, Chief, Division of Hematology and Medical Oncology, St. Vincent Hospital agreed to collaborate on the study. A brief outline of the research study was submitted to both collaborating oncologists and these clinicians facilitated institutional/IRB approval.

Ability to access patient level data, as a researcher not employed at the hospital, was also an important factor in selecting BWH and SVH as participating hospitals in this study. The EMR data at BWH was accessed as part of an already approved broader GBM research protocol under the supervision of collaborating and principal investigator, Dr. Timothy Smith. Dr. Smith included me in his research team, and I completed all BWH required training prior to the start of data collection. Similarly, Dr. Daniyal Siddiqui obtained necessary approval from SVH hospital and included me in his team as a

collaborating researcher. Once appropriate approvals from Walden University were obtained, including IRB approval, data collection step was initiated for the study. The BWH and SVH's institution policy allowed me to view the EMR, but I was not able to query the data since only employed medical personnel are given EMR log-in information. I created the data query based on the inclusion criteria and study variables of interest and queried the EMR for the required datasets in collaboration with and under the supervision of Dr. Smith and Dr. Siddiqui's designated residents on the team. Confidentiality of the retrieved EMR data was maintained by ensuring that the data were not disclosed to any unauthorized user at any time and any data that were not properly de-identified was held with Drs. Smith and Siddiqui at the hospital with limited access by only authorized individuals on their team. Once the data were retrieved, each subject's protected private health information, except for demographic information, was de-identified. The de-identified data were then entered on an Excel spreadsheet and cross-checked against the EMR generated output for accuracy. The de-identified data was downloaded from the Excel spreadsheet into SPSS v25 for analysis.

Electronic medical records provide detailed clinical data that is relevant for outcomes research and is now increasingly used in clinical oncology and epidemiology studies (Lau et al., 2011). It was assumed for purposes of this study that patient data in the EMR was collected and entered in a reliable and accurate manner since both participating hospitals follow robust processes and standard operating procedures to ensure data integrity. It was not feasible to conduct an independent audit of the data due to hospital policies, magnitude of the database, and patient privacy concerns under the

Health Insurance Portability and Accountability Act (HIPAA) of 1996 (Rights (OCR), 2009) . Similarly, GBM patients are under the care of trained neuro oncologists; therefore, it was assumed that the medical information they entered about their patients in the EMR was accurate because of their extensive training in the medical practice of neuro oncology.

Data Analysis Plan

Data analysis was conducted using the SPSS v25 obtained through Walden University. Data in SPSS was directly uploaded from an Excel spreadsheet and manual check was performed to ensure accuracy of data transfer. The data analysis addressed the following study specific research questions and hypothesis. These research questions along with key variables, their level of measurement, and statistical methods that were used to address each question are outlined in Table 2.

RQ1: Is there a significant difference in time to disease progression and overall survival after first recurrence between rGBM patients with IDH-1 mutation and those without IDH-1 mutation?

$H_0$1: Based on IDH-1 mutation status, there is no statistically significant difference in the time to disease progression and/or overall survival of rGBM patients.

$H_1$1: Based on IDH-1 mutation status, there is a statistically significant difference in the time to disease progression and/or overall survival of rGBM patients.

RQ2: Is the correlation between IDH-1 mutation status and disease progression and survival after first recurrence affected by the covariates of MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression?

$H_0$2 – The correlation between IDH-1 mutation status and disease progression and survival is not affected by covariates MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression.

$H_1$2 - The correlation between IDH-1 mutation status and disease progression and survival is affected by covariates MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression.

Table 2

Research Questions, Variables, and Statistical Methods

Research Questions	Dependent Variable		Independent Variables		Statistical Method
	Variable	Measurement	Variable	Measurement	
Is there a significant difference in time to disease progression and overall survival after first recurrence between rGBM patients with IDH-1 mutation and those without IDH-1 mutation?	Time to disease progression or first recurrence from initial diagnosis.	Total number of days (for each variable listed)	IDH-1 mutation	Present (mutated IDH-1) or absent (wild-type IDH-1)	Cox proportional hazard analysis (hazard ratio)
	Time to death from initial diagnosis.				Kaplan-Meir Curve
	Time to death from first recurrence.				

Research Questions	Dependent Variable		Independent Variables		Statistical Method
	Variable	Measurement	Variable	Measurement	
Is the correlation between IDH-1 mutation status and disease progression and survival after first recurrence affected by the covariates of MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression?	Time to disease progression or first recurrence from initial diagnosis. Time to death from initial diagnosis and first recurrence	Total number of days (for both variables).	IDH-1 mutation	Present (mutated IDH-1) or absent (wild-type IDH-1)	Cox regression analyses
			MGMT promoter methylation	Methylated or unmethylated	
			Age	Years	
			KPS	>70 or $\underline{< 70}$	
			Surgery for resection	Yes or No	
			Chemotherapy at progression	Yes or No	

The Cox proportional-hazards model was used to assess the effect (hazard ratio) of IDH-1 mutation status on time to disease progression and survival. In addition, Kaplan-Meir curves were generated to obtain survival rate for patients with and without the IDH-1 mutation. A Cox regression analysis was conducted to test the effects of key covariates (i.e., MGMT promoter methylation, age, KPS, surgery for resection and chemotherapy at progression) on disease progression and survival. Cox proportional hazard model is the most commonly used statistical method in epidemiological and

clinical studies that investigate time-to-event outcomes like death and disease progression, which were also used in this study (Delgado et al., 2014; George et al., 2014; Koletsi & Pandis, 2017). Similarly, the Kaplan-Meir estimation is commonly used for survival analysis and to compare the survival distribution of two groups (George et al., 2014). Cox proportional hazard model is also considered appropriate for time-to-event based studies because its regression analysis allows to evaluate the independent predictive value of selected covariates on the outcome measures like survival and time to disease progression (Delgado et al., 2014; George et al., 2014; Koletsi & Pandis, 2017). Since the study aimed to look at the effect of key covariates on disease progression and overall survival in GBM patients, Cox regression analysis was considered the most appropriate statistical method to assess the effect of selected covariates.

Research in GBM is focused on molecular, genetic, epigenetic, and immunological markers in GBM, not only to better classify the disease but also to evaluate the prognostic value of these markers in terms of disease outcomes (Omuro & DeAngelis, 2013). Researchers are also evaluating the role of clinical prognostic factors, such as age at diagnosis, tumor site, surgical resection and its timing, KPS, and chemotherapeutic regimens, in the disease outcome. There are complex interactions between these molecular and clinical prognostic factors and appropriate adjustments must be made when evaluating the role of any specific factor for its prognostic value. This study took similar considerations into account in its design. The literature review conducted for this project suggested that MGMT promoter methylation (molecular marker), age, KPS, surgery for resection, and chemotherapy at progression (clinical

factors) would be important covariates when comparing the PFS and OS of rGBM patients with and without IDH-1 mutation.

Cox proportional hazard analysis was conducted to determine the effect of IDH-1 mutation on OS and PFS utilizing the hazard ratio. Hazard ratio (HR) is used to interpret the Cox model and it is defined as the predicted hazard function in relation to two different conditions of a predictor variable (Delgado et al., 2014; George et al., 2014; Koletsi & Pandis, 2017). A hazard ratio of greater than one and less than one respectively indicates that the event is more likely or less likely to occur, whereas a HR of one indicates that the predictor has no effect on the hazard of the event (George et al., 2014). In this study the hazard ratio of IDH-1 mutation status on OS or PFS was determined by this model. Cox regression analysis was also conducted to test the effects of other key covariates on OS and PFS. Kaplan-Meir method was also used to determine the survival rate between the IDH-1 mutated and wildtype groups.

Cox regression assumes proportional hazard and this assumption must be satisfied to ensure proper interpretation of the data using this model (Delgado et al., 2014; George et al., 2014; Koletsi & Pandis, 2017). While there may be a change over time in the underlying hazard, the model assumes proportional hazards for the values of predictors, which may be affected by time-varying covariates (George et al., 2014). Most of the covariates selected for this study (e.g., MGMT promoter methylation status, surgery for resection, and chemotherapy at recurrence) were categorical and did not affect the proportional hazard assumption of the regression model. One of the covariates selected for the study was Karnofsky Performance Score (KPS) that may change over time and

affect the proportional hazard assumption of the regression model. Since KPS as a covariate did not have any effect on the OS, no additional methods were used to test the proportional hazard assumption for example, defining this covariate as a time-dependent covariate in the SPSS and then run the Cox regression with both time-fixed and time-dependent covariates (Delgado et al., 2014).

Threats to Validity

The study was designed as a retrospective cohort study based on quantitative data collected on adult GBM patients at select hospitals in Massachusetts that is, BWH and SVH. These hospitals were selected to make the research feasible through ease of collaboration with oncologists at these centers and better access to EMR, which required the researcher to go through training at these hospitals. This approach would not have been possible if too many cancer centers had been selected, particularly in other states. The two centers were selected to ensure appropriate representation of the population within the state. Although no GBM patients at SVH met the inclusion criteria of having documented recurrence and IDH-1 mutation status, the BWH database included patients that were treated at or referred from other major hospitals in Massachusetts that is, Massachusetts General Hospital (MGH), Beth Israel Deaconess Center (BIDC), Dana Farber Cancer Institute (DFCI), Vermont Health Network (VHN), and Wentworth Douglass Hospital (WDH); therefore, study sample comprised of patients from multiple centers across the state. In addition, there is no evidence to suggest that the pathophysiology and clinical course of GBM would be different across the United States and the demographic information of patients in this study was consistent with the

previously reported demographic information for GBM patients suggesting that appropriate and representative sample of GBM was included in this study (Ostrom et al., 2018). Furthermore, EMR provides detailed clinical data that is relevant for outcomes research and is now increasingly used in clinical oncology and epidemiology studies (Lau et al., 2011); therefore, the risk to external validity was considered minimal for this study.

In terms of internal validity, the study assumed that the patient data in the EMR was collected and entered in a reliable and accurate manner, particularly for the key variables. This assumption was appropriate considering the robust processes and standard operating procedures that each participating hospital have in place to ensure data integrity. It was not feasible to conduct an independent audit of the data due to hospital policies, magnitude of the database, and patient privacy concerns under the HIPAA (Rights (OCR), 2009). The other key assumption in context of internal validity was regarding the clinical assessment of recurrence and disease progression in GBM patients. While radiographic imaging was used as an objective measure to determine recurrence and progression of disease, the assessment was still made by a clinician. GBM patients are under the care of trained neurooncologists and such radiographical assessments are part of regular clinical practice, including oncology.

Cohort studies may also be susceptible to selection, information, and comparison bias and as such can affect internal validity (Euser et al., 2009). Studies that include objective assessment of exposure and outcome adequately addresses these potential biases. In terms of selection bias, the inclusion of the GBM subjects in the study with the exposure of interest (IDH-1) mutation was not dependent on the likelihood of them

having the outcome of interest (disease progression and survival). Moreover, the diagnosis of GBM, both initial and recurring, were based on radiographic assessment and therefore it eliminated the selection bias due to differential referral or diagnosis (Euser et al., 2009). Similarly, information bias due to misclassification was unlikely since presence or absence of IDH-1 mutations is made by established laboratory diagnostic test. The use of objective measures for key independent and dependent variables also addressed any potential concern of construct validity that requires use of correct instruments and accurate measures of key variables (Strauss & Smith, 2009).

Considering that the prognostic value of the IDH-1 mutation for the rGBM patients in this study was determined by comparing outcomes like time to disease progression and mortality, the confounding variables were factored in as covariates in the final data analysis. Cox regression analysis were conducted to address this limitation and to evaluate the effect of these confounding variables on the outcome of interest. The small sample size did not allow regression analysis after matching the groups for these covariates. Inability to match groups for confounding variables is a general limitation and challenge that researchers face when conducting research in a rare disease like GBM. In rare diseases, the sample size is relatively small to begin with and patient heterogeneity makes it difficult to adjust for all confounding variables while still maintaining a reasonable sample size for statistical analysis.

Ethical Procedures

The study was conducted under the appropriate oversight of Institutional Review Board (IRB) for human subject protection. The EMR data at BWH was accessed as part

of an approved GBM protocol (protocol number: 2015P002352) under the supervision of collaborating and principal investigator, Dr. Timothy Smith, Director, Computational Neuroscience Outcomes Center and Assistant Professor of Neurosurgery, Harvard Medical School. Similarly, approval was obtained from SVH IRB (email communication dated July 09, 2019 – submitted to Walden University for IRB approval) to access EMR data under the supervision of collaborating investigator Dr. Daniyal Siddiqui, Chief, Division of Hematology and Medical Oncology. A brief outline of the proposed research study was submitted to both collaborating oncologists and these clinicians facilitated institutional/IRB approval. All required training at BWH was completed and this training was also accepted by SVH prior to accessing the EMR data. Training courses for BWH included HIPAA, Protecting Patient Privacy, and Ethical Standards along with other general courses like Hazard Communication, Patient Care Assessment and Patient Safety. These courses were administered by HealthStream® and a certificate was issued upon successful completion of each course. Institutional Review Board approval was also obtained from Walden University (approval number: 08-19-20-0036388). Confidentiality of retrieved EMR data was maintained by ensuring that the data were not disclosed to any unauthorized user at any time and any data that were not properly de-identified was held with Drs. Smith and Siddiqui at the hospital with limited access by only authorized individuals on their team. Raw data with patient identifiable information were stored with Drs. Smith and Siddiqui at their respective hospitals and will be appropriately destroyed after the completion of this dissertation. Further precautions were taken to safeguard subjects' protected health information (PHI) by de-identifying the data for confidentiality

for example, medical record number was used for each subject included in the study rather than the use of name or initials. De-identified data were entered in the Excel sheet and subsequently uploaded in the SPSS software for the purposes of data analysis.

Summary

This chapter provided an overview of the methodological approach that was used for the study. It defined the key independent variable (IDH-1) and dependent variables (PFS and OS) that were selected for the study along with important covariates that were considered in the data analysis. The overall study design, including study population, sampling method, and inclusion criteria was defined in context of the overall scope of the study. Potential threats to external and internal validity were also addressed. While the study was conducted only at two major hospitals in Massachusetts, threat to external validity was considered minimal because: a) the BWH database was a combined database of BWH and MGH and included patients referred to these hospitals from other centers in the region as well; and b) there is no evidence to suggest that the pathophysiology and clinical course of GBM is different across the United States. Similarly, the objective measures and assessment used for both dependent and independent variables minimized the threat to internal and construct validity. A brief description of the statistical method was also provided; Cox proportional hazard model and regression analysis were used to interpret the study results and address specific research questions and hypothesis. The results of the study are presented in Chapter 4.

Chapter 4: Results

Introduction

The purpose of this study was to determine whether isocitrate dehydrogenase-1 (IDH-1) mutation is a prognostic factor in recurrent glioblastoma (rGBM) considering other molecular and clinical prognostic factors as covariates. In a retrospective cohort study, time to disease progression or death from first recurrence was evaluated in rGBM patients with IDH-1 mutated and wild-type tumors. The effect of key variables (i.e., O^6-methylguanine-DNA methyl-transferase (MGMT) promoter methylation, age, Karnofsky Performance Score (KPS), surgery and/or chemotherapy at progression) was evaluated on the correlation between IDH-1 mutation status and disease progression and survival. The research questions and hypothesis that this study intended to answer were:

RQ1: Is there a significant difference in time to disease progression and overall survival after first recurrence between rGBM patients with IDH-1 mutation and those without IDH-1 mutation?

$H_0$1: Based on IDH-1 mutation status, there is no statistically significant difference in the time to disease progression and/or overall survival of rGBM patients.

$H_1$1: Based on IDH-1 mutation status, there is a statistically significant difference in the time to disease progression and/or overall survival of rGBM patients.

RQ2: Is the correlation between IDH-1 mutation status and disease progression and survival after first recurrence affected by the covariates of MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression?

$H_0$2 – The correlation between IDH-1 mutation status and disease progression and survival is not affected by covariates MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression.

$H_1$2 - The correlation between IDH-1 mutation status and disease progression and survival is affected by covariates MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression.

This chapter provides an overview of how the actual data collection process went during the research study, particularly focusing on some of the challenges and limitations that were not anticipated prior to the start of data collection. The descriptive demographic characteristics of the study population is also discussed along with the external validity of the data. Data collection section is followed by detailed study results including statistical analysis organized by research questions and hypothesis. Additional post-hoc analyses, that were conducted based on the findings from primary analysis, are also presented in the results section. Answers to the key research questions are then summarized at the end of the chapter.

Data Collection

The target population consisted of adult subjects with confirmed diagnosis of GBM and the study was based on secondary analysis through retrospective chart review of quantitative data collected on these subjects at select hospitals in Massachusetts. Data was collected from Electronic Medical Records (EMR) of the Brigham and Women's Hospital (BWH), which turned out to be a combined database of BWH and Massachusetts General Hospital (MGH), and St. Vincent Hospital (SVH). The BWH and

MGH database also included patients that were referred to these centers from Beth Israel Deaconess Center (BIDC), Dana Farber Cancer Institute (DFCI), Vermont Health Network (VHN), and Wentworth Douglass Hospital (WDH). That database was searched for the study population and corresponding study-variables of interest during the period of 2008 and 2020.

The major unanticipated challenge faced was that the databases were not searchable for disease specific "key-terms" like GBM or rGBM as initially planned. Rather, the databases could be searched only through a Research Patient Data Repository (RPDR) query using the International Classification of Disease-10 (ICD-10) code and GBM or rGBM does not have a specific ICD-10 code that could have been used to search the database. Instead, ICD-10 code C71 for "malignant neoplasm of the brain" had to be used but it includes other cancers of the brain besides GBM. As anticipated the search based on ICD-10 code C71 resulted in approximately 1200-1500 cases that included all neoplasms of the brain. It would not have been feasible to review individual patient records of all these cases to identify patients that would qualify for inclusion in the study. To make the database search manageable, the following strategy was used instead to identify patients based on the key variables of the study that is, IDH-1 mutation status and GBM patients with documented recurrence of their disease; therefore, the database was first queried for all patients who had been tested for IDH-1 mutation and this search yielded a total of 588 cases. Since IDH-1 mutation can be of interest in a variety of oncologic conditions (e.g., myeloid leukemia, breast cancer, lung cancer, etc.) (Bledea et al., 2019; Hodges et al., 2013), the ICD-10 code for neoplasm of the brain was then

applied to these 588 cases to further narrow the cases to relevant study population and this step reduced the sample size to 405 cases. In the final step, individual patient records for all 405 cases were reviewed and per the inclusion/exclusion criteria those patients that had non-glioblastoma tumors or GBM patients with no documented evidence of recurrence were excluded from the dataset. This strategy resulted in a final sample size of 177 cases, whose charts were then reviewed in detail to collect all relevant information for detailed analyses to address the research questions. GBM is a rare disease and it was anticipated that the overall sample size for the study is going to be small. The inclusion/exclusion criteria of having only rGBM patients that have IDH-1 testing result, narrowed the overall study population; however, the sample size still exceeded the sample size of 108 that was estimated a priori using G*Power3 to detect a small effect size at an expected power of .90 and alpha of .05 (Faul et al., 2007).

The baseline demographic characteristics of the population was not different from the final dataset since this was a retrospective cohort study. Data on age, gender, ethnic background was collected as part of the study and is described in the results section. The final dataset also did not change the minimal risk to external validity that was assumed prior to data collection. The study was initially planned based on two centers (i.e., BWH and SVH) and while SVH did not contribute any relevant cases to the study, the database of BWH was a combined database of BWH and MGH and this combined database also included patients that were referred to these hospitals from other major medical institutions and networks (i.e., BIDC, DFCI, VHN, and WDH) in Massachusetts; therefore, the dataset provided good representation of rGBM data in Massachusetts.

While caution needs to be taken in the generalizability of the data and results to the broader U.S. population, there is no evidence to suggest that the pathophysiology and clinical course of GBM would be different across the U.S.; therefore, it is anticipated that the results of this study provide fair representation of the overall rGBM patient population in the U.S.

The univariate analyses indicated that IDH-1 is not a prognostic factor in recurrent GBM albeit with some data limitations. Multivariate analyses with selected molecular and clinical covariates were conducted to complete the planned analyses and to determine if the results were independent of other variables selected in the study. These multivariate analyses also indicated that IDH-1 is not a prognostic factor in rGBM within the boundaries of this study. Post-hoc analyses were conducted to evaluate the role of IDH-1 mutation over the entire GBM disease span and to compare the results of this study in context of the existing body of knowledge on the role of IDH-1 mutation status in GBM and rGBM.

Results

Descriptive Statistics

This section provides: a) general information on the demographics and key variables of the study population; and b) primary data collected on progression-free survival (PFS) and survival to support the statistical analyses for study research questions.

Demographic and Key Study Variables

The general demographic information and distribution frequency of key study variables are summarized in Table 3. The study population was predominantly White males of 65 years or younger; the median age of the study population was 60 (25, 87) years. Majority of patients' tumors had wildtype IDH-1 (92%) whereas MGMT promoter methylation status was somewhat evenly distributed between methylated (41%) and unmethylated (51%). Clinical-based variables indicate that 60% of study population had a KPS of >70 and patients that had surgery or received chemotherapy at recurrence were 31% and 84%, respectively. Although the patients in the database were from six different institutions, most of the patients (88%) were treated at MGH.

Table 3

General Demographics and Key Variables

Attribute	N	Percentage
Age		
≤65 years	120	67.8%
>65 years	57	32.2%
Gender		
males	117	66.1%
females	60	33.9%
Race		
whites	150	84.7%
blacks	3	1.7%
Hispanics	2	1.1%
Asians	3	1.7%
American Indians	2	1.1%
others	8	4.5%
not provided	9	5.1%
IDH-1		
wildtype	163	92.1%
mutated	13	7.3%
missing	1	0.6%
MGMT		
methylated	72	40.7%

Attribute	N	Percentage
unmethylated	90	50.8%
missing	15	8.5%
KPS		
≤70	57	32.2%
>70	107	60.5%
missing	13	7.3%
Surgery at Recurrence		
yes	37	31%
no	81	68%
Chemotherapy at Recurrence		
yes	102	84.3%
no	19	15.7%
Hospitals		
BWH	2	1.1%
MGH	156	88.1%
DFCI	15	8.5%
BIDMC	2	1.1%
VHN	1	0.6%
WDH	1	0.6%
SVH	0	0%

Since IDH-1 mutation status is the key variable of the study, the distribution of other key variables selected for the study was also assessed in context of IDH-1 mutation status. Data tabulated for each variable in Table 4 excludes those subjects with missing values for the stated variable. As anticipated the overall number of patients with tumors carrying IDH-1 mutation was small (13%) with equal number of males and females and all of these patients were ≤65 years. MGMT promoter methylation status was equally distributed in the IDH-1 positive patients and most of them had either chemotherapy or surgery at recurrence.

Table 4

Demographic Information and Key Covariates in Context of IDH-1 Mutation Status

Variables	IDH-1 Wildtype	IDH-1 Mutated	Total
Age			
≤65	107 (60%)	13 (7%)	120 (68%)
>65	57 (32%)	0	57 (32%)
Gender			
male	110 (62%)	7 (4%)	117 (66%)
female	54 (31%)	6 (3%)	60 (34%)
MGMT			
methylated	64 (39%)	7 (54%)	71 (44%)
unmethylated	85 (52%)	5 (38.5%)	90 (56%)
KPS			
≤70	54 (33%)	3 (2%)	57 (35%)
>70	96 (59%)	10 (6%)	106 (65%)
Surgery at recurrence			
yes	33 (28%)	4 (3%)	37 (31%)
no	78 (66%)	3 (2%)	81 (69%)
Chemotherapy at recurrence			
yes	94 (78%)	7 (6%)	101(84%)
no	19 (16%)	0	19 (16%)

Progression-Free Survival and Overall Survival

Time in months was calculated to determine the median progression-free survival (PFS) and median overall survival (mOS) in patients with wildtype and mutated IDH-1 gene (Table 5). PFS was defined as time from initial diagnosis to first recurrence and time from first recurrence to subsequent recurrence. Survival was defined as time from initial diagnosis to death and time from first recurrence to death. Data suggests that compared to time from initial diagnosis, median PFS and OS is shorter in the recurrent setting irrespective of IDH-1 status. Furthermore, compared to their wildtype

counterparts, patients with IDH-1 mutation seems to have better outcomes in median PFS and OS except for time-to-death from first recurrence.

Table 5

Median Progression Free Survival (PFS) and Overall Survival (OS)

IDH-1 Mutation Status	PFS		OS	
	Initial Diagnosis to First Recurrence	First Recurrence to Second Recurrence	Initial Diagnosis to Death	First Recurrence to Death
Wildtype				
Mdn (months)	8	4	13	4.5
SD	9	5	11	4
N	116	42	50	42
Mutated				
Mdn (months)	12	6.5	25	3
SD	15	2	23	3.5
N	7	2	3	3

Statistical Analyses

Statistical analyses first focused on the specific research questions and then additional analyses were conducted to gain an understanding of the role of IDH-1 mutation in the overall disease prognosis in GBM. Cox regression assumes proportional hazard and this assumption must be satisfied to ensure proper interpretation of the data using this model (Delgado et al., 2014; George et al., 2014; Koletsi & Pandis, 2017). Almost all the variables selected for this study (i.e., IDH-1 mutation status, MGMT promoter methylation status, surgery for resection, and chemotherapy at recurrence) are categorical and did not affect the proportional hazard assumption of the regression model.

Research Question 1

The first research question (RQ1) was: Is there a significant difference in time to disease progression and overall survival after first recurrence between rGBM patients with IDH-1 mutation and those without IDH-1 mutation? Univariate analysis of variance (ANOVA) was conducted to evaluate the difference between the IDH-1 mutated and wildtype groups for PFS (i.e., time from first recurrence to subsequent recurrence) and survival (i.e., time from first recurrence to death) (Table 6). Homogeneity of variance assumption (Levene's test) was met for all covariates in the ANOVA. The analyses indicated that there was no statistically significant difference in either PFS [$F(1, 789.3) = .03$, $p = .86$] or survival [$F(1, 668.6) = .04$, $p = .83$].

Table 6

IDH-1 Mutation as Prognostic Factor in Recurrent GBM

Measure	Sum of Squares	*df*	Mean Square	F	Significance	Partial Eta Squared
PFS (first recurrence to second recurrence)	789.30	1	789.30	.03	.86	.001
OS (first recurrence to death)	668.57	1	668.57	.04	.83	.001

Survival analysis were also conducted to test if there was a difference between the two IDH-1 groups in terms of days from first recurrence to death. There was no

significant difference noted in median survival times utilizing Kaplan-Meir cumulative survival analysis (Table 7 and Figure 2) and Cox regression analysis (Table 8).

Table 7

Median Survival Time and IDH-1 Mutation Status in Recurrent GBM

Variable	DF	Parameter Estimate	Standard Error	95% CI	Chi-Square	Significance
IDH-1 mutation	1	181.42	18.20	64.70, 209.30	.19	.66

Figure 2

Survival Function and IDH-1 Mutation Status in Recurrent GBM

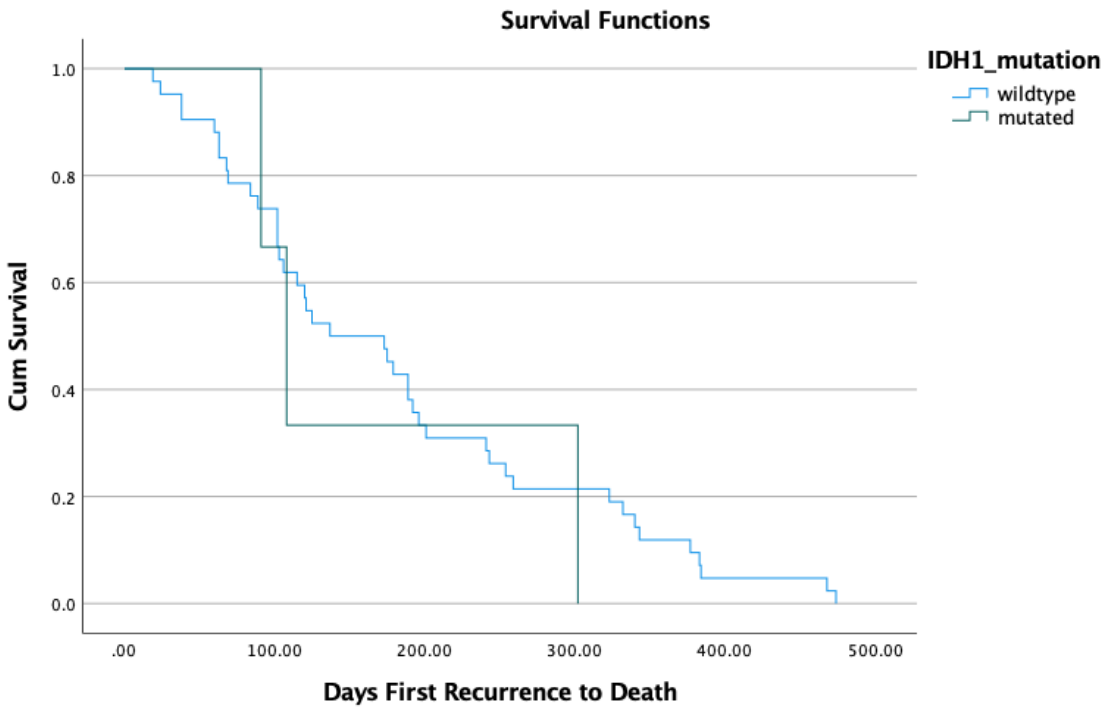

Table 8

Cox Regression with IDH-1 Mutation Status and Survival in Recurrent GBM

Variable	B	SE	Wald	*df*	Exp(B) (95% CI)	Significance
IDH-1 mutation	.26	.60	.18	1	1.3 (.39, 4.24)	.67

Since there was no statistically significant difference between IDH-1 wildtype and mutated groups in PFS [$F(1, 789.3) = .03$, $p = .86$] and survival (HR 1.3; 95% CI, .39, 4.24; $p = .67$), the null hypothesis for RQ 1 was not rejected (i.e., $H_0$1: Based on IDH-1 mutation status, there is no statistically significant difference in the time to disease progression and/or overall survival of rGBM patients).

Research Question 2

The second research question (RQ2) was: Is the correlation between IDH-1 mutation status and disease progression and survival after first recurrence affected by the covariates of MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression. While the overall comparison of rGBM patients with IDH-1wildtype and mutated genes did not indicate any difference in PFS and survival, it was important to determine if there is an effect, or lack thereof, of selected covariates on these outcomes. Both univariate and multivariate regression analyses was conducted evaluating days from first recurrence to second recurrence (PFS) and first recurrence to death

(survival) with predictors of age, gender, MGMT methylation status, KPS, and surgery or chemotherapy at recurrence.

In terms of PFS, the univariate analysis indicated that none of the covariates had any significant contribution to the outcome (Table 9). Similarly, there was no significant difference in PFS when factoring in all the predictors, $F(6, 198713) = 1.23$, $p = .31$ and none of the covariates were found to be significant contributors in PFS (Table 10).

Table 9

IDH-1 Mutation and PFS in Recurrent GBM – Effect of Covariates (Univariate Analyses)

Measure	Sum of Squares	*df*	Mean Square	F	Significance	Partial Eta Squared
IDH-1	789.30	1	789.30	.30	.86	.00
Age	40785.33	1	40785.33	1.44	.24	.03
Gender	68991.50	1	68991.50	2.50	.12	.05
MGMT methylation	69180.32	1	69180.32	2.32	.14	.06
KPS	49151.17		49151.17	1.71	.20	.04
Surgery and/or chemotherapy	20463.10	1	20463.10	.71	.40	.02

Table 10

IDH-1 Mutation and Progression Free Survival in Recurrent GBM – Effect of Covariates

Variable	B	SE	*t*	Significance	95% CI
Age	52.18	61.76	.84	.40	-73.57, 177.73

Variable	B	SE	*t*	Significance	95% CI
Gender	-61.52	56.94	-1.08	.29	-177.37, 54.319
MGMT Methylation	79.77	55.02	1.45	.16	-32.15, 191.71
KPS	-66.41	61.19	-1.08	.28	-190.91, 58.08
Surgery and/or Chemotherapy	12.06	125.29	.09	.92	-242.86, 266.98

In terms of survival, the univariate analysis indicated that none of the covariates had any significant contribution to the outcome (Table 11). Similar to PFS, there was no statistically significant difference noted for survival when factoring in all the predictors, $F(6, 99178.32) = 1.17$, $p = .34$ and none of the covariates were found to be significant contributors in survival (Table 12).

Table 11

IDH-1 Mutation and Survival in Recurrent GBM – Effect of Covariates (Univariate Analyses)

Measure	Sum of Squares	*df*	Mean Square	F	Significance	Partial Eta Squared
Age	16086.40	1	16086.40	1.08	.30	.02
Gender	317.96	1	317.96	.02	.89	.00
MGMT methylation	25525.80	1	25525.80	1.74	.19	.04
KPS	21341.35	1	21341.35	1.50	.23	.04

Measure	Sum of Squares	*df*	Mean Square	F	Significance	Partial Eta Squared
Surgery and/or chemotherapy	27120.25	1	27120.25	1.86	.18	.04

Table 12

IDH-1 Mutation and Survival in Recurrent GBM - Effect of Covariates

Variable	B	SE	*t*	Significance	95% CI
Age	-45.69	45.50	-1.00	.32	-138.27, 46.88
Gender	3.05	43.35	.07	.94	-85.14, 91.24
MGMT Methylation	76.36	45.59	1.67	.10	-16.39, 169.11
KPS	70.48	41.43	1.70	.10	-13.80, 154.77
Surgery and/or Chemotherapy	37.38	57.99	.64	.52	-80.60, 155.36

The null hypothesis for RQ2 (i.e., $H_0$2 – The correlation between IDH-1 mutation status and disease progression and survival is not affected by covariates of age, gender, MGMT promoter methylation, KPS, surgery and/or chemotherapy at progression) was not rejected because: a) there was no significant difference between IDH-1 wildtype and mutated groups after factoring in covariates of age, gender, MGMT methylation status, KPS, and surgery and/or chemotherapy at recurrence for both PFS [$F(6, 198713) = 1.23$, $p = .31$] and survival [$F(6, 99178.32) = 1.17$, $p = .34$]; and b) none of the covariates

showed any significant contribution to the PFS and survival after recurrence (Tables 10 and 12, respectively).

Post-hoc Analyses

Post-hoc analyses focused on evaluating the role of IDH-1 mutation in PFS and overall survival (OS) from initial diagnosis that is, over the entire GBM disease span rather than after recurrence, which was evaluated as part of specific research questions. The intention was to: a) compare the data from this study with other limited studies conducted to evaluate the role of IDH-1 in GBM; and b) assess the underlying premise of this study that while IDH-1 mutation may be a prognostic factor in GBM if evaluated from initial disease diagnosis, it may not be of prognostic significance once the disease recurs or rGBM. The PFS and OS for the purposes of this post-hoc analyses was defined as days from initial diagnosis to first recurrence and days from initial diagnosis to death, respectively.

Univariate analysis of variance (ANOVA) was conducted to evaluate the difference between the IDH-1 mutated and wildtype groups for PFS and OS (Table 13). The analyses indicated that while there was no statistically significant difference in PFS [$F(1, 189074.27) = 2.26$, $p = .14$], the difference in OS was statistically significant [$F(1, 724286.53) = 5.50$, $p = .02$].

Table 13

IDH-1 Mutation as Prognostic Factor in GBM

Measure	Sum of Squares	*df*	Mean Square	F	Significance	Partial Eta Squared
PFS (initial diagnosis to first recurrence)	189074.27	1	189074.27	2.26	.14	.02
OS (initial diagnosis to death)	724286.53	1	724286.53	5.50	.02	.01

Survival analysis were also conducted to test if there was a difference between the two IDH-1 groups in terms of days from initial diagnosis to death. There was no significant difference noted in median survival times utilizing Kaplan-Meir cumulative survival analysis (Table 14 and Figure 3) and Cox regression analysis (Table 15).

Table 14

Median Survival Time and IDH-1 Mutation Status in GBM

Variable	DF	Parameter Estimate	Standard Error	95% CI	Chi-Square	Significance
IDH-1 mutation	1	475.75	51.95	329.67, 472.33	2.12	.15

Figure 3

Survival Function and IDH-1 Mutation Status in GBM

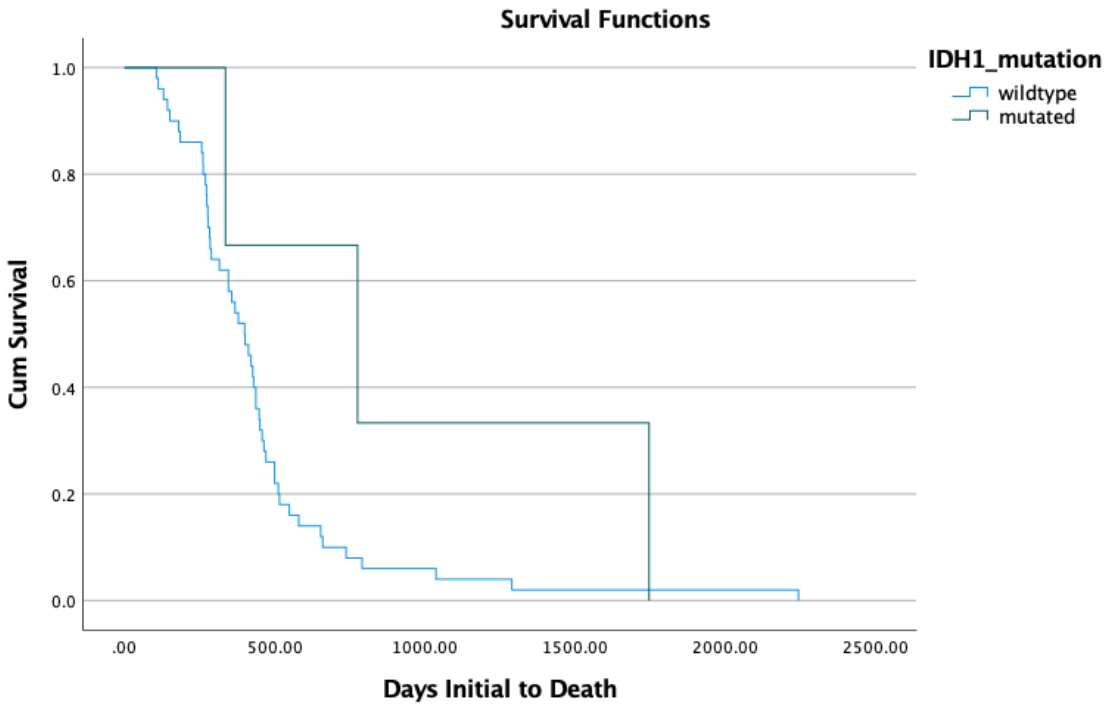

Table 15

Cox Regression with IDH-1 Mutation Status and Overall Survival in GBM

Variable	B	SE	Wald	df	Exp(B) (95% CI)	Significance
IDH-1 mutation	-.86	.61	2.00	1	.423 (.13, 1.40)	.16

Since a statistically significant difference was noted for OS (Table 13), both univariate and multivariate regression analysis were conducted evaluating days from initial diagnosis to death (OS) with predictors of age, gender, MGMT methylation status, KPS, and surgery and/or chemotherapy. In the univariate analysis, gender and age were found to be significant contributing covariates in OS and the covariate of age showed a strong trend (Table 16). In the multivariate analysis, there was a significant difference in OS when factoring in all the predictors, $F(6, 2049021.89) = 2.48$, $p = .04$; however, only covariates MGMT methylation status was found to be significant contributors in OS (Table 17).

Table 16

IDH-1 Mutation and Overall Survival in GBM – Effect of Covariates (Univariate Analyses)

Measure	Sum of Squares	*df*	Mean Square	F	Significance	Partial Eta Squared
Age	393946.28	1	393946.28	2.85	.09	.05
Gender	606677.90	1	606677.90	4.53	.04	.08
MGMT methylation	739158.35	1	739158.35	5.34	.02	.10
KPS	361769.20	1	49151.17	2.47	.12	.05
Surgery and/or chemotherapy	206987.90	1	206987.90	1.37	.25	.03

Table 17

IDH-1 Mutation and Overall Survival in GBM - Effect of Covariates

Variable	B	SE	*t*	Significance	95% CI
Age	-141.56	142.20	-1.00	.33	-430.87, 147.74
Gender	-42.40	135.46	-.31	.76	-318.00, 233.19
MGMT Methylation	413.90	142.47	2.90	.007	124.04, 703.77
KPS	35.27	129.47	.27	.78	-228.13, 298.67
Surgery and/or Chemotherapy	132.31	181.23	.73	.47	-236.40, 501.02

Based on these results, Cox regression analysis was conducted with both IDH-1 mutation and MGMT methylation status in the model considering the significant contribution of the latter as a covariate in overall survival (Table 18). The analysis indicated that both IDH-1 mutation and MGMT methylation were negatively correlated with mortality and while IDH-1 mutation showed a strong statistical trend for its contribution in OS (HR .31; 95% CI, .08, 1.12; p = .07), contribution of MGMT methylation in OS was statistically significant (HR .38; 95% CI, .18, .80; p = .01); however, this prognostic effect of methylated MGMT promoter was not seen following disease recurrence in this study (HR .51; 95% CI, .23, 1.13; p = .10). The sample size of IDH-1 mutated group was not sufficient to do further subgroup survival analysis of MGMT methylated and unmethylated groups.

Table 18

Cox Regression: IDH-1 Mutation and MGMT Methylation Status and Overall Survival in GBM

Variable	B	SE	Wald	df	Exp(B) (95% CI)	Significance
IDH-1 mutation	-1.16	.65	3.18	1	.31 (.08, 1.12)	.07
MGMT methylation	-.97	.38	6.39	1	.38 (.18, .80)	.01

Cox regression analyses were also conducted with IDH-1 mutation and gender and age in the model. The analysis indicated that contribution of gender was not statistically significant in OS (HR 1.54; 95% CI, .82, 2.90; p = .18) but age was negatively correlated with mortality and showed a strong statistical trend (HR .57; 95% CI, .31, 1.04; p = .07) for its contribution in OS (Tables 19 and 20).

Table 19

Cox Regression: IDH-1 Mutation and Gender and Overall Survival in GBM

Variable	B	SE	Wald	df	Exp(B) (95% CI)	Significance
IDH-1 mutation	.59	.65	.84	1	1.81 (.51, 6.44)	.36
Gender	.43	.32	1.82	1	1.54 (.82, 2.90)	.18

Table 20

Cox Regression: IDH-1 Mutation and Age and Overall Survival in GBM

Variable	B	SE	Wald	df	Exp(B) (95% CI)	Significance
IDH-1 mutation	.701	.619	1.28	1	2.01 (.60, 6.77)	.26
Age	-.56	.30	3.35	1	.57 (.31, 1.04)	.07

Summary

Research questions for this study aimed to assess the prognostic value of IDH-1 mutation in recurrent GBM. The following specific research questions were postulated for the study purpose and the results related to these questions are hereby summarized:

- The first research question was: Is there a signification difference in time to disease progression and overall survival after first recurrence between rGBM patients with IDH-1 mutation and those without IDH-1 mutation? Time to disease progression or progression free survival (PFS) was defined as time from first recurrence to second recurrence and survival was defined as time from first recurrence to death. The results of the study indicated that there was no statistically significant difference in either PFS [$F(1, 789.3) = .03$, $p = .86$] or survival [$F(1, 668.6) = .04$, $p = .83$] of rGBM patients with IDH-1 wildtype and mutated tumors. Furthermore, survival analysis also indicated statistically insignificant difference (HR 1.3; 95% CI, .39, 4.24; $p = .67$) between the two groups; therefore, the null hypothesis of this research question was not rejected (i.e., $H_0$1: Based

on IDH-1 mutation status, there is no statistically significant difference in the time to disease progression and/or overall survival of rGBM patients).

- The second research question was: Is the correlation between IDH-1 mutation status and disease progression and survival after first recurrence affected by the covariates of MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression. While the overall comparison of rGBM patients with IDH-1wildtype and mutated tumors did not indicate any difference in PFS and survival, it was important to determine if there is an effect, or lack thereof, of selected covariates on these outcomes. The results of the study indicated that there was no significant difference in PFS [$F(6, 198713) = 1.23$, $p = .31$] and OS [$F(6, 99178.32) = 1.17$, $p = .34$] after factoring in all the predictors and none of the covariates showed any significant contribution to either PFS or survival after recurrence; therefore, the null hypothesis of this research question was not rejected (i.e., $H_0$2: The correlation between IDH-1 mutation status and disease progression and survival is not affected by covariates MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression).

The role of IDH-1 mutation as a prognostic factor in GBM remains unclear, particularly in the recurrent disease. The results of this study, albeit with its limitations, suggests that IDH-1 mutation is not a prognostic factor in recurrent GBM. Post-hoc analyses conducted in this study evaluated the prognostic value of IDH-1 mutation over the entire GBM disease span with the intention to: a) compare the data from this study with other limited studies conducted to evaluate the role of IDH-1 in GBM; and b) assess the underlying premise of this study that while IDH-1 mutation may be a prognostic

factor in GBM if evaluated from initial disease diagnosis, it may not be of prognostic significance once the disease recurs defined as rGBM. The next chapter presents the findings of this study in context of the existing body of knowledge and interprets the results considering the limitations of the study. The following chapter also includes implications of this study, contribution to a positive social change in relation to clinical practice in rGBM, and recommendations for future research.

Chapter 5: Discussion, Conclusion, and Recommendations

Introduction

The purpose of this study was to determine whether IDH-1 mutation is a prognostic factor in rGBM considering other molecular and clinical prognostic factors as covariates. The prognostic value of IDH-1 mutation is debated even following initial diagnosis of GBM since studies have demonstrated both weak and strong association between IDH-1 mutation and overall survival (Amelot et al., 2015; Mukasa et al., 2012; Zou et al., 2013). A few studies have examined the role of IDH-1 mutation in rGBM, but only in the clinical trial setting and with inconclusive results (Mandel et al., 2016; Taal et al., 2014). This study was a retrospective cohort study based on quantitative data collected through retrospective chart reviews of adult patients diagnosed with GBM at select hospitals in Massachusetts. Time to disease progression or death from first recurrence was evaluated in rGBM patients with IDH-1 mutated and wild-type tumors. The effect of key variables (i.e., MGMT promoter methylation, age, KPS, surgery for resection, and chemotherapy at progression) was also evaluated in context of the association between IDH-1 mutation status and disease progression and survival. The results of this study indicated that there was no statistically significant difference in either time to disease progression or survival of rGBM patients with IDH-1 wildtype and mutated tumors. Similarly, the results also indicated that there was no significant difference in time to disease progression or survival after factoring in all the predictor variables and none of these variables showed any significant contribution to either time to disease progression or survival after disease recurrence.

Interpretation of the Findings

The characteristics of the study population were found to be generally consistent with the existing knowledge on GBM. In the study database after adjusting for IDH-1 mutation, there were approximately 44% GBM patients among patients diagnosed with neoplasm of the brain which is consistent with earlier reports that indicate that GBM accounts for approximately 48% of all primary malignant brain tumors (*Quick Brain Tumor Facts*, n.d.). The study population was predominantly White (85%) males (66%) of 65 years or younger; the median age of the study population was 60 (25, 87) years. These findings were consistent with previous reports that indicate a median age of 65 year at diagnosis of GBM with higher incidence in adults aged 75-85 years (Ostrom 2018). Similarly, incidence rate of GBM is considered 1.6 times higher in males and approximately twice as greater in Whites than Blacks (Ostrom et al., 2018). Majority of patients' tumors in this study had wildtype IDH-1 (92%) whereas MGMT promoter methylation status was somewhat evenly distributed between methylated (41%) and unmethylated (51%). These results were consistent with previous reports indicating that approximately 95% of primary GBM tumors have wildtype IDH-1 and about 50% have methylated MGMT promoters (Lieberman, 2017; Olar & Aldape, 2014; Yang et al., 2015).

The primary objective of this study was to determine the prognostic value of IDH-1 mutation in recurrent GBM that is, progression free survival and survival following first recurrence. The study results indicated that there was no statistically significant difference in either PFS [$F(1, 789.3) = .03$, $p = .86$] or survival (HR 1.3; 95% CI, .39,

4.24; p = .67) of rGBM patients with IDH-1 wildtype and mutated tumors. The results of this study appears to be consistent with couple of studies that have examined the role of IDH-1 mutation in rGBM, albeit in a clinical trial setting, that showed that IDH-1 mutation did not result in prolonged PFS or survival compared to IDH-1 wild-type tumors in recurrent GBM (Mandel et al., 2016; Taal et al., 2014). While the number of IDH-1 mutated patients in this study was approximately three times more (13 patients) compared to the five rGBM patients with mutated IDH-1 included in the study by Mandel (2016) and Taal (2014), analyses is still overall limited by the small number of IDH-1 mutated patients and results must be considered with caution. The results of the study also indicated that selected predictors (i.e., MGMT promoter methylation status, age, KPS, and surgery and/or chemotherapy at recurrence) do not affect PFS and survival following disease recurrence. The effects of these predictors on PFS and survival have not been studied in rGBM and multiple factors must be considered to evaluate the role of these predictors in disease prognosis. For example, recent studies have highlighted the timing of resection and its association with PFS and survival in GBM with a lower risk of death noted with repeat resection without taking the timing of resection into account but a higher risk of death was noted after adjustment for the timing of resection (Goldman et al., 2018; Y.-H. Zhao et al., 2019). Considering the small sample size of IDH-1 mutated group, it was not feasible to conduct further subset analyses by matching the two groups with specific parameters for each selected covariate. Although the results of the study indicated that the selected covariates (i.e., age, MGMT promoter methylation status, KPS, and surgery or chemotherapy at recurrence) did not affect the PFS and survival in

recurrent GBM, the complexity of disease pathophysiology and interactions between molecular and clinical markers of prognosis make it challenging to conclusively determine effect of these factors in GBM, including rGBM (Audureau et al., 2018; Chaichana et al., 2013; Clarke et al., 2011).

The post-hoc analyses also compared the effect of IDH-1 mutation on PFS and overall survival (OS) from initial diagnosis to first recurrence and death. The analyses indicated that while there was no statistically significant difference in PFS [$F(1, 189074.27) = 2.26$, $p = .14$], the difference in OS was statistically significant [$F(1, 724286.53) = 5.50$, $p = .02$]. Furthermore, Cox regression analysis was conducted with both IDH-1 mutation and MGMT methylation status in the model considering the significant contribution of the latter as a covariate in overall survival. The analysis indicated that both IDH-1 mutation and MGMT methylation were negatively correlated with mortality and while IDH-1 mutation showed a strong statistical trend for its contribution in OS (HR .31; 95% CI, .08, 1.12; p = .07), contribution of MGMT methylation in OS was statistically significant (HR .38; 95% CI, .18, .80; $p = .01$). The strong trend, but inconclusive evidence of association, noted in this study for the prognostic value of IDH-1mutation from initial diagnosis to death seems to be reflective of previously reported data that suggests both weak and strong association between IDH-1 mutation and overall survival (Amelot et al., 2015; Chen et al., 2016; Mandel et al., 2016; Mukasa et al., 2012; Xia et al., 2015; Zou et al., 2013). A larger sample size of patients with IDH-1 mutation in this study may have provided a clearer perspective on the association between IDH-1 mutation and survival following initial diagnosis. It is

worth noting that some of the studies showing strong association between IDH-1 mutation and overall survival did not adjust for some of key contributing variables, like MGMT promoter methylation status (Mandel et al., 2016). This study showed a statistically significant effect of MGMT promoter methylation status on OS from initial diagnosis and this finding is consistent with previous reports, including three meta-analysis, that have shown that OS was significantly better in patients with methylated MGMT promoter (Olson et al., 2011; H. Zhao et al., 2016; Y.-H. Zhao et al., 2018). The prognostic effect of methylated MGMT promoter, however, was not seen following disease recurrence in this study. The prognostic value of methylated MGMT promoter in rGBM has been studied in combination with other factors like radiosurgery and researchers have suggested additional studies to specifically evaluate the role of MGMT promoter methylation status in rGBM (Kim et al., 2017). Cox regression analysis was also conducted with both IDH-1 mutation and age in the model considering the significant contribution of the latter as a covariate in the univariate analysis. The median age in the IDH-1 mutated group was 45 (35, 65) years. The analysis indicated that age was negatively correlated with mortality and showed a strong statistical trend for its contribution in OS (HR .57; 95% CI, .31, 1.04; p = .07). These results were consistent with previous findings that have reported age 50 years as the typical cut-off from the perspective of a prognostic value, with a higher risk of death seen in patients over 70 years; however, the shorter survival rate for older GBM patients is most likely due to comorbidities and inability to tolerate the effects of the cancer itself and treatments like surgery and chemotherapy (Ostrom et al., 2018; Thakkar et al., 2014).

The study was aligned with the conceptual framework of outcomes research, particularly outcomes research in oncology as proposed by Lee (2000) and generally adopted by other researchers (Apolone, 2003; Fay et al., 2015; Kovvali, 2014; Lee et al., 2000; Melamed et al., 2017; Roberts et al., 2019). Utilizing the outcomes measures of PFS and survival included in the outcomes research framework, the results of this study indicated that IDH-1 mutation was not a prognostic factor in rGBM. The results of this study contributed to the body of knowledge on the molecular and clinical prognostic factors that should be considered in the treatment and management of rGBM patients; therefore, it broadly addressed both the prediction rules and the treatment options as well as application aspect of the outcomes research framework (Lee et al., 2000).

Limitations of the Study

Even though the overall sample size of the study (177) exceeded the sample size of 108 that was estimated a priori for statistical analyses, these analyses were limited by the relatively small number of patients with mutated IDH-1 status (7.3%) and the results must be interpreted with caution. An overall small sample size, including number of patients with mutated IDH-1 status, was anticipated considering that GBM is a rare disease, testing for IDH-1 mutation status only recently became a standard practice after its inclusion in the classification of gliomas, and only 5% of primary GBM tumors have mutated IDH-1 (Lieberman, 2017; Miller et al., 2017; Olar & Aldape, 2014; Ostrom et al., 2018). These facts were evident in the data collected for this study where the original database included about 1500 patients with neoplasm of the brain, but the final study sample size was reduced to 177 once the key inclusion criteria were applied such as

documented evidence of IDH-1 mutation status and disease recurrence. The results of this study would need to be interpreted in context of its limitations, but they could be considered reliable since these results were overall congruent with earlier reports, as discussed above, on the prognostic value of IDH-1 in GBM both from initial diagnosis and following recurrence.

This study also met the parameters for external validity that were assumed prior to data collection and analyses. The study was planned at two clinical centers in Massachusetts, Brigham and Women's Hospital (BWH) and St. Vincent Hospital (SVH), to ensure appropriate representation of the population within the state. Although no GBM patients at SVH met the inclusion criteria of having documented recurrence and IDH-1 mutation status, the BWH database included patients that were treated at or referred from other major hospitals in Massachusetts that is, Massachusetts General Hospital (MGH), Beth Israel Deaconess Center (BIDC), Dana Farber Cancer Institute (DFCI), Vermont Health Network (VHN), and Wentworth Douglass Hospital (WDH). The age, gender, and ethnic characteristics of this study population was consistent with the previously reported demographic information for GBM suggesting that appropriate and representative sample of GBM was included in this study. Overall, this study is considered to have good external validity because there is no evidence to suggest that the pathophysiology and clinical course of GBM would be different across the United States, it included patients from multiple clinical centers across Massachusetts, and the demographic characteristics of these patients were consistent with previously reported demographic data for GBM (Ostrom, 2018).

Recommendations

The primary focus of this study was to evaluate the prognostic value of IDH-1 mutation status in recurrent GBM taking into consideration other key predictors. The limited sample size particularly for IDH-1 mutated tumors in this study underscored the overall challenges of conducting studies in rare diseases like GBM and these challenges are further compounded with stricter inclusion exclusion criteria typically selected to limit the scope of the study. Following recommendations are proposed for future studies to further confirm the findings of this study:

- A larger study sample size to ensure that there is a higher number of patients with mutated IDH-1 tumors in the overall study population of rGBM. Although this retrospective study relied on a database that had patient records from major hospitals in Massachusetts, the total number of patients with mutated IDH-1 tumors was still relatively small (7.3%). Considering that testing for IDH-1 mutation status only recently became a standard practice in GBM and only 5% of GBM tumors carry IDH-1 mutation, future studies would most likely have to be conducted as multicenter studies across the United States to increase the overall sample size thereby ensuring enough patients with mutated IDH-1 tumors.
- The effect of predictors like age, MGMT promoter methylations status, KPS, and surgery or chemotherapy at recurrence on PFS and survival based on IDH-1 mutation status should be further evaluated in rGBM. Although the results of this study indicated that these predictors are not associated with PFS and survival based on IDH-1 mutation status, these results cannot be considered conclusive based on the small number of IDH-1

mutated patients; the two IDH-1 groups would have to be appropriately matched for these predictors to provide more conclusive evidence.

- The results of this study indicated that methylated MGMT promoter was a key predictor of survival from initial diagnosis but not after disease recurrence. Previous studies have mainly focused on the prognostic value of MGMT promoter methylation status in GBM after initial diagnosis but not in recurrent GBM; therefore, future studies can further explore the prognostic value of MGMT promoter methylation in recurrent setting to confirm the results of this study. Recent studies have suggested that MGMT promoter methylation status may change over time and following relapse (Feldheim et al., 2019; Storey et al., 2019); therefore, future studies should also consider retesting of the MGMT promoter methylation status at recurrence.
- In this study IDH-1 mutation was negatively correlated with mortality and showed a strong statistical trend for its contribution in overall survival from initial diagnosis (HR .31; 95% CI, .08, 1.12; p = .07). This finding is consistent with the previous reports that have shown both strong and weak association between IDH-1 mutation and overall survival (Amelot et al., 2015; Chen et al., 2016; Mandel et al., 2016; Mukasa et al., 2012; Xia et al., 2015; Zou et al., 2013). Additional studies with larger sample size are recommended to further investigate the correlation of IDH-1 mutation and overall survival in GBM. Consistent with previous studies, this study showed that methylated MGMT promoter is a key prognostic factor in overall survival of GBM patients from initial diagnosis; therefore, future studies should at least factor in MGMT promoter

methylation status when evaluating the prognostic value of IDH-1 mutation status in overall survival.

Implications

It is anticipated that the results of this study will bring about a positive social change by affecting both patient treatment and health care practice in recurrent GBM. The presumed role of IDH-1 mutation as an overall prognostic factor upon initial diagnosis of GBM typically results in the selection of treatment modalities that are relatively aggressive, including a combination of resection, chemotherapy, and adjuvant therapy, with an intent to improve progression free survival and overall survival; however, this prognostic value of IDH-1 in recurrent GBM has not been extensively studied. The results of this study, albeit with its limitations, showed that IDH-1 mutation is not a prognostic factor in recurrent GBM; therefore, continuation of an aggressive treatment approach that is based on IDH-1 mutation status at initial diagnosis will most likely not confer any clinical or survival advantage following disease recurrence. Considering that there are significant risks associated with aggressive treatments like chemotherapy, the results of this study may mitigate unnecessary exposure of rGBM patients to the safety risks that are associated with treatments selected at initial diagnosis. It is anticipated that the results of this study will also contribute to a positive social change by informing the clinical practice guidelines to treat and manage GBM patients following disease recurrence. It should continue to advance the conversation on how prognostic factors like IDH-1 mutation may need to be considered differently in recurrent setting versus initial diagnosis and patients' quality of life and overall risks/benefits of

treatments should be considered when selecting an optimal treatment course for patients with recurrent GBM. Moreover, if these costly treatments that are a financial burden for both patients and health care system are not found necessary in the recurrent disease, then positive social change may also be affected over time through substantial savings in the overall health care costs associated with the management of GBM and rGBM.

The results of this study underscored the relevance and utility of outcomes research in oncology (Lee et al., 2000) and it added to the existing evidence that prediction rules, treatment options, and application aspect of outcomes research framework can be appropriately utilized in future studies of similar purpose and scope. This study was designed as a retrospective cohort study based on data collected from electronic medical records and this study design is considered relevant for outcomes research (Lau et al., 2011). Retrospective cohort design was also selected for efficiency because a prospective study would have been costly and time-consuming making it impractical for this dissertation project; however, the results of this study do make a case for prospective studies in future to further evaluate the role of prognostic factors, including IDH-1 mutation, in recurrent GBM. The rare nature of this disease and complex interactions between molecular and clinical prognostic factors mainly limits the retrospective studies in terms of overall sample size, matching of the groups for contributing variables, and occurrence of events like disease progression and death needed for outcome analyses. Although a prospective study would take longer to complete, it may be better suited to address research questions by mitigating some of the limitations of retrospective study, particularly in rare diseases like GBM.

Conclusion

Glioblastoma is an aggressive form of brain cancer that has a high recurrence rate and very poor prognosis (Ostrom et al., 2018). The prognostic value of various molecular markers (e.g., IDH-1 mutation, MGMT promoter methylation, etc.) and clinical factors (e.g., age, KPS, surgery and chemotherapy, etc.) has been studied in GBM after initial diagnosis but not as extensively in the recurrent GBM. Utilizing a retrospective cohort design and framework of outcomes research in oncology, this study evaluated the prognostic value of IDH-1 mutation in recurrent GBM in the context of key predictor variables of age, MGMT promoter methylation, KPS, and surgery and chemotherapy at recurrence. The results of this study indicated, albeit with its limitations, that IDH-1 mutation was not a prognostic factor in recurrent GBM. The prognostic value of IDH-1 mutation from initial diagnosis in this study was inconclusive consistent with previous reports. The results of this study also indicated that although methylated MGMT promoter was a strong prognostic factor from initial diagnosis as previously reported, it was not a prognostic factor in recurrent GBM. Overall, the results of this study suggest that the pathophysiology and prognosis of GBM may need to be considered differently at initial diagnosis and following disease recurrence. Molecular markers like IDH-1 mutation and MGMT promoter methylation status are used as prognostic factors to make treatment decisions for GBM patients at initial diagnosis. The results of this study indicate that these molecular markers may not carry the same prognostic value after disease recurrence and treatment decision that are made based on these markers at initial diagnosis may not be relevant or accurate at disease recurrence. Considering that there

are significant risks associated with aggressive treatments like combination of chemotherapies that are selected based on prognostic factors like IDH-1 mutation and MGMT promoter methylation at initial diagnosis, the results of this study may mitigate unnecessary exposure of rGBM patients to the safety risks that are associated with such treatments; therefore, it is anticipated that the results of this study will bring about a positive social change by affecting both patient treatment and health care practice in recurrent GBM.

References

Amelot, A., De Cremoux, P., Quillien, V., Polivka, M., Adle-Biassette, H., Lehmann-Che, J., Françoise, L., Carpentier, A. F., George, B., Mandonnet, E., & Froelich, S. (2015). IDH-Mutation Is a Weak Predictor of Long-Term Survival in Glioblastoma Patients. *PloS One*, *10*(7), e0130596. https://doi.org/10.1371/journal.pone.0130596

Apolone, G. (2003). Clinical and outcome research in oncology. The need for integration. *Health and Quality of Life Outcomes*, *1*, 3. https://doi.org/10.1186/1477-7525-1-3

Archavlis, E., Tselis, N., Birn, G., Ulrich, P., & Zamboglou, N. (2014). Combined salvage therapies for recurrent glioblastoma multiforme: Evaluation of an interdisciplinary treatment algorithm. *Journal of Neuro-Oncology*, *119*(2), 387–395. https://doi.org/10.1007/s11060-014-1500-8

Artene, S. A., Tuţă, C., Dragoi, A., Alexandru, O., Stefana Oana, P., Tache, D. E., Dănciulescu, M. M., Boldeanu, M. V., Siloşi, C. A., & Dricu, A. (2018). Current and emerging EGFR therapies for glioblastoma. *Journal of Immunoassay & Immunochemistry*, *39*(1), 1–11. https://doi.org/10.1080/15321819.2017.1411816

Audureau, E., Chivet, A., Ursu, R., Corns, R., Metellus, P., Noel, G., Zouaoui, S., Guyotat, J., Le Reste, P.-J., Faillot, T., Litre, F., Desse, N., Petit, A., Emery, E., Lechapt-Zalcman, E., Peltier, J., Duntze, J., Dezamis, E., Voirin, J., … Club de Neuro-Oncologie of the Société Française de Neurochirurgie. (2018). Prognostic factors for survival in adult patients with recurrent glioblastoma: A decision-tree-

based model. *Journal of Neuro-Oncology, 136*(3), 565–576. https://doi.org/10.1007/s11060-017-2685-4

Bledea, R., Vasudevaraja, V., Patel, S., Stafford, J., Serrano, J., Esposito, G., Tredwin, L. M., Goodman, N., Kloetgen, A., Golfinos, J. G., Zagzag, D., Weigelt, B., Iafrate, A. J., Sulman, E. P., Chi, A. S., Dogan, S., Reis-Filho, J. S., Chiang, S., Placantonakis, D., … Snuderl, M. (2019). Functional and topographic effects on DNA methylation in IDH1/2 mutant cancers. *Scientific Reports, 9*. https://doi.org/10.1038/s41598-019-53262-7

Brown, T. J., Brennan, M. C., Li, M., Church, E. W., Brandmeir, N. J., Rakszawski, K. L., Patel, A. S., Rizk, E. B., Suki, D., Sawaya, R., & Glantz, M. (2016). Association of the Extent of Resection With Survival in Glioblastoma. *JAMA Oncology*, *2*(11), 1460–1469. https://doi.org/10.1001/jamaoncol.2016.1373

Calvert, A. E., Chalastanis, A., Wu, Y., Hurley, L. A., Kouri, F. M., Bi, Y., Kachman, M., May, J. L., Bartom, E., Hua, Y., Mishra, R. K., Schiltz, G. E., Dubrovskyi, O., Mazar, A. P., Peter, M. E., Zheng, H., James, C. D., Burant, C. F., Chandel, N. S., … Stegh, A. H. (2017). Cancer-Associated IDH1 Promotes Growth and Resistance to Targeted Therapies in the Absence of Mutation. *Cell Reports*, *19*(9), 1858–1873. https://doi.org/10.1016/j.celrep.2017.05.014

Cancer of the Brain and Other Nervous System—Cancer Stat Facts. (n.d.). SEER. Retrieved April 27, 2019, from https://seer.cancer.gov/statfacts/html/brain.html

Chaichana, K. L., Zadnik, P., Weingart, J. D., Olivi, A., Gallia, G. L., Blakeley, J., Lim, M., Brem, H., & Quiñones-Hinojosa, A. (2013). Multiple resections for patients

with glioblastoma: Prolonging survival. *Journal of Neurosurgery*, *118*(4), 812–820. https://doi.org/10.3171/2012.9.JNS1277

Chen, J.-R., Yao, Y., Xu, H.-Z., & Qin, Z.-Y. (2016). Isocitrate Dehydrogenase (IDH)1/2 Mutations as Prognostic Markers in Patients With Glioblastomas. *Medicine*, *95*(9), e2583. https://doi.org/10.1097/MD.0000000000002583

Cheng, H.-B., Yue, W., Xie, C., Zhang, R.-Y., Hu, S.-S., & Wang, Z. (2013). IDH1 mutation is associated with improved overall survival in patients with glioblastoma: A meta-analysis. *Tumour Biology: The Journal of the International Society for Oncodevelopmental Biology and Medicine*, *34*(6), 3555–3559. https://doi.org/10.1007/s13277-013-0934-5

Chin, C., Lunking, E. S., de la Fuente, M., & Ayad, N. G. (2018). Immunotherapy and Epigenetic Pathway Modulation in Glioblastoma Multiforme. *Frontiers in Oncology*, *8*, 521. https://doi.org/10.3389/fonc.2018.00521

Clarke, J. L., Ennis, M. M., Yung, W. K. A., Chang, S. M., Wen, P. Y., Cloughesy, T. F., Deangelis, L. M., Robins, H. I., Lieberman, F. S., Fine, H. A., Abrey, L., Gilbert, M. R., Mehta, M., Kuhn, J. G., Aldape, K. D., Lamborn, K. R., Prados, M. D., & North American Brain Tumor Consortium. (2011). Is surgery at progression a prognostic marker for improved 6-month progression-free survival or overall survival for patients with recurrent glioblastoma? *Neuro-Oncology*, *13*(10), 1118–1124. https://doi.org/10.1093/neuonc/nor110

Cloughesy, T. F., Cavenee, W. K., & Mischel, P. S. (2014). Glioblastoma: From molecular pathology to targeted treatment. *Annual Review of Pathology*, *9*, 1–25. https://doi.org/10.1146/annurev-pathol-011110-130324

Costa, B. M., Caeiro, C., Guimarães, I., Martinho, O., Jaraquemada, T., Augusto, I., Castro, L., Osório, L., Linhares, P., Honavar, M., Resende, M., Braga, F., Silva, A., Pardal, F., Amorim, J., Nabiço, R., Almeida, R., Alegria, C., Pires, M., … Reis, R. M. (2010). Prognostic value of MGMT promoter methylation in glioblastoma patients treated with temozolomide-based chemoradiation: A Portuguese multicentre study. *Oncology Reports*, *23*(6), 1655–1662. https://doi.org/10.3892/or_00000808

Czapski, B., Baluszek, S., Herold-Mende, C., & Kaminska, B. (2018). Clinical and immunological correlates of long term survival in glioblastoma. *Contemporary Oncology (Poznan, Poland)*, *22*(1A), 81–85. https://doi.org/10.5114/wo.2018.73893

D'Amico, R. S., Cloney, M. B., Sonabend, A. M., Zacharia, B., Nazarian, M. N., Iwamoto, F. M., Sisti, M. B., Bruce, J. N., & McKhann, G. M. (2015). The Safety of Surgery in Elderly Patients with Primary and Recurrent Glioblastoma. *World Neurosurgery*, *84*(4), 913–919. https://doi.org/10.1016/j.wneu.2015.05.072

Davis, M. E. (2016). Glioblastoma: Overview of Disease and Treatment. *Clinical Journal of Oncology Nursing*, *20*(5 Suppl), S2-8. https://doi.org/10.1188/16.CJON.S1.2-8

Delgado, J., Pereira, A., Villamor, N., López-Guillermo, A., & Rozman, C. (2014). Survival analysis in hematologic malignancies: Recommendations for clinicians. *Haematologica*, *99*(9), 1410–1420. https://doi.org/10.3324/haematol.2013.100784

Deng, L., Xiong, P., Luo, Y., Bu, X., Qian, S., Zhong, W., & Lv, S. (2018). Association between IDH1/2 mutations and brain glioma grade. *Oncology Letters*, *16*(4), 5405–5409. https://doi.org/10.3892/ol.2018.9317

Euser, A. M., Zoccali, C., Jager, K. J., & Dekker, F. W. (2009). Cohort studies: Prospective versus retrospective. *Nephron. Clinical Practice*, *113*(3), c214-217. https://doi.org/10.1159/000235241

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–191. https://doi.org/10.3758/bf03193146

Fay, M., Head, R., & Martin, J. (2015). Where is the radiobiology and pharmacology research to improve outcomes in glioblastoma? *Journal of Neuro-Oncology*, *124*(1), 1–3. https://doi.org/10.1007/s11060-015-1816-z

Feldheim, J., Kessler, A. F., Monoranu, C. M., Ernestus, R.-I., Löhr, M., & Hagemann, C. (2019). Changes of O6-Methylguanine DNA Methyltransferase (MGMT) Promoter Methylation in Glioblastoma Relapse-A Meta-Analysis Type Literature Review. *Cancers*, *11*(12). https://doi.org/10.3390/cancers11121837

George, B., Seals, S., & Aban, I. (2014). Survival analysis and regression models. *Journal of Nuclear Cardiology: Official Publication of the American Society of Nuclear Cardiology*, *21*(4), 686–694. https://doi.org/10.1007/s12350-014-9908-2

Goldman, D. A., Hovinga, K., Reiner, A. S., Esquenazi, Y., Tabar, V., & Panageas, K. S. (2018). The relationship between repeat resection and overall survival in patients with glioblastoma: A time-dependent analysis. *Journal of Neurosurgery*, *129*(5), 1231–1239. https://doi.org/10.3171/2017.6.JNS17393

Golub, D., Iyengar, N., Dogra, S., Wong, T., Bready, D., Tang, K., Modrek, A. S., & Placantonakis, D. G. (2019). Mutant Isocitrate Dehydrogenase Inhibitors as Targeted Cancer Therapeutics. *Frontiers in Oncology*, *9*, 417. https://doi.org/10.3389/fonc.2019.00417

Hegi, M. E., Diserens, A.-C., Gorlia, T., Hamou, M.-F., de Tribolet, N., Weller, M., Kros, J. M., Hainfellner, J. A., Mason, W., Mariani, L., Bromberg, J. E. C., Hau, P., Mirimanoff, R. O., Cairncross, J. G., Janzer, R. C., & Stupp, R. (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. *The New England Journal of Medicine*, *352*(10), 997–1003. https://doi.org/10.1056/NEJMoa043331

Hodges, T. R., Choi, B. D., Bigner, D. D., Yan, H., & Sampson, J. H. (2013). Isocitrate dehydrogenase 1: What it means to the neurosurgeon: a review. *Journal of Neurosurgery*, *118*(6), 1176–1180. https://doi.org/10.3171/2013.3.JNS122282

Hsieh, F. Y., & Lavori, P. W. (2000). Sample-size calculations for the Cox proportional hazards regression model with nonbinary covariates. *Controlled Clinical Trials*, *21*(6), 552–560. https://doi.org/10.1016/s0197-2456(00)00104-5

Iwamoto, F. M., Abrey, L. E., Beal, K., Gutin, P. H., Rosenblum, M. K., Reuter, V. E., DeAngelis, L. M., & Lassman, A. B. (2009). Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. *Neurology*, *73*(15), 1200–1206. https://doi.org/10.1212/WNL.0b013e3181bc0184

Jain, K. K. (2018). A Critical Overview of Targeted Therapies for Glioblastoma. *Frontiers in Oncology*, *8*, 419. https://doi.org/10.3389/fonc.2018.00419

Juratli, T. A., Kirsch, M., Geiger, K., Klink, B., Leipnitz, E., Pinzer, T., Soucek, S., Schrock, E., Schrok, E., Schackert, G., & Krex, D. (2012). The prognostic value of IDH mutations and MGMT promoter status in secondary high-grade gliomas. *Journal of Neuro-Oncology*, *110*(3), 325–333. https://doi.org/10.1007/s11060-012-0977-2

Kaminska, B., Czapski, B., Guzik, R., Król, S. K., & Gielniewski, B. (2019). Consequences of IDH1/2 Mutations in Gliomas and an Assessment of Inhibitors Targeting Mutated IDH Proteins. *Molecules (Basel, Switzerland)*, *24*(5). https://doi.org/10.3390/molecules24050968

Kazda, T., Dziacky, A., Burkon, P., Pospisil, P., Slavik, M., Rehak, Z., Jancalek, R., Slampa, P., Slaby, O., & Lakomy, R. (2018). Radiotherapy of Glioblastoma 15 Years after the Landmark Stupp's Trial: More Controversies than Standards? *Radiology and Oncology*, *52*(2), 121–128. https://doi.org/10.2478/raon-2018-0023

Kim, B. S., Kong, D.-S., Seol, H. J., Nam, D.-H., & Lee, J.-I. (2017). MGMT promoter methylation status as a prognostic factor for the outcome of gamma knife

radiosurgery for recurrent glioblastoma. *Journal of Neuro-Oncology*, *133*(3), 615–622. https://doi.org/10.1007/s11060-017-2478-9

Koletsi, D., & Pandis, N. (2017). Survival analysis, part 3: Cox regression. *American Journal of Orthodontics and Dentofacial Orthopedics: Official Publication of the American Association of Orthodontists, Its Constituent Societies, and the American Board of Orthodontics*, *152*(5), 722–723. https://doi.org/10.1016/j.ajodo.2017.07.009

Kovvali, G. (2014). Systems oncology: A new paradigm in cancer research. *Journal of Carcinogenesis*, *13*, 6. https://doi.org/10.4103/1477-3163.128641

Labussiere, M., Sanson, M., Idbaih, A., & Delattre, J.-Y. (2010). IDH1 gene mutations: A new paradigm in glioma prognosis and therapy? *The Oncologist*, *15*(2), 196–199. https://doi.org/10.1634/theoncologist.2009-0218

Lamborn, K. R., Yung, W. K. A., Chang, S. M., Wen, P. Y., Cloughesy, T. F., DeAngelis, L. M., Robins, H. I., Lieberman, F. S., Fine, H. A., Fink, K. L., Junck, L., Abrey, L., Gilbert, M. R., Mehta, M., Kuhn, J. G., Aldape, K. D., Hibberts, J., Peterson, P. M., Prados, M. D., & North American Brain Tumor Consortium. (2008). Progression-free survival: An important end point in evaluating therapy for recurrent high-grade gliomas. *Neuro-Oncology*, *10*(2), 162–170. https://doi.org/10.1215/15228517-2007-062

Lau, E. C., Mowat, F. S., Kelsh, M. A., Legg, J. C., Engel-Nitz, N. M., Watson, H. N., Collins, H. L., Nordyke, R. J., & Whyte, J. L. (2011). Use of electronic medical records (EMR) for oncology outcomes research: Assessing the comparability of

EMR information to patient registry and health claims data. *Clinical Epidemiology*, *3*, 259–272. https://doi.org/10.2147/CLEP.S23690

Lee, S. J., Earle, C. C., & Weeks, J. C. (2000). Outcomes research in oncology: History, conceptual framework, and trends in the literature. *Journal of the National Cancer Institute*, *92*(3), 195–204. https://doi.org/10.1093/jnci/92.3.195

Li, R., Chen, X., You, Y., Wang, X., Liu, Y., Hu, Q., & Yan, W. (2015). Comprehensive portrait of recurrent glioblastoma multiforme in molecular and clinical characteristics. *Oncotarget*, *6*(31), 30968–30974. https://doi.org/10.18632/oncotarget.5038

Lieberman, F. (2017). Glioblastoma update: Molecular biology, diagnosis, treatment, response assessment, and translational clinical trials. *F1000Research*, *6*, 1892. https://doi.org/10.12688/f1000research.11493.1

Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., Ohgaki, H., Wiestler, O. D., Kleihues, P., & Ellison, D. W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: A summary. *Acta Neuropathologica*, *131*(6), 803–820. https://doi.org/10.1007/s00401-016-1545-1

Lu, V. M., Goyal, A., Graffeo, C. S., Perry, A., Burns, T. C., Parney, I. F., Quinones-Hinojosa, A., & Chaichana, K. L. (2019). Survival Benefit of Maximal Resection for Glioblastoma Reoperation in the Temozolomide Era: A Meta-Analysis. *World Neurosurgery*, *127*, 31–37. https://doi.org/10.1016/j.wneu.2019.03.250

Mandel, J. J., Cachia, D., Liu, D., Wilson, C., Aldape, K., Fuller, G., & de Groot, J. F. (2016). Impact of IDH1 mutation status on outcome in clinical trials for recurrent glioblastoma. *Journal of Neuro-Oncology*, *129*(1), 147–154. https://doi.org/10.1007/s11060-016-2157-2

Melamed, A., Rauh-Hain, J. A., & Schorge, J. O. (2017). Clinical outcomes research in gynecologic oncology. *Gynecologic Oncology*, *146*(3), 653–660. https://doi.org/10.1016/j.ygyno.2017.06.016

Miller, J. J., Shih, H. A., Andronesi, O. C., & Cahill, D. P. (2017). Isocitrate dehydrogenase-mutant glioma: Evolving clinical and therapeutic implications. *Cancer*, *123*(23), 4535–4546. https://doi.org/10.1002/cncr.31039

Mohammed, S. I., Springfield, S., & Das, R. (2012). Role of epigenetics in cancer health disparities. *Methods in Molecular Biology (Clifton, N.J.)*, *863*, 395–410. https://doi.org/10.1007/978-1-61779-612-8_25

Mukasa, A., Takayanagi, S., Saito, K., Shibahara, J., Tabei, Y., Furuya, K., Ide, T., Narita, Y., Nishikawa, R., Ueki, K., & Saito, N. (2012). Significance of IDH mutations varies with tumor histology, grade, and genetics in Japanese glioma patients. *Cancer Science*, *103*(3), 587–592. https://doi.org/10.1111/j.1349-7006.2011.02175.x

Nathan, D. G. (1998). Clinical research: Perceptions, reality, and proposed solutions. National Institutes of Health Director's Panel on Clinical Research. *JAMA*, *280*(16), 1427–1431. https://doi.org/10.1001/jama.280.16.1427

Nobusawa, S., Watanabe, T., Kleihues, P., & Ohgaki, H. (2009). IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *15*(19), 6002–6007. https://doi.org/10.1158/1078-0432.CCR-09-0715

Notterman, D. A., & Mitchell, C. (2015). Epigenetics and Understanding the Impact of Social Determinants of Health. *Pediatric Clinics of North America*, *62*(5), 1227–1240. https://doi.org/10.1016/j.pcl.2015.05.012

Olar, A., & Aldape, K. D. (2014). Using the molecular classification of glioblastoma to inform personalized treatment. *The Journal of Pathology*, *232*(2), 165–177. https://doi.org/10.1002/path.4282

Olson, R. A., Brastianos, P. K., & Palma, D. A. (2011). Prognostic and predictive value of epigenetic silencing of MGMT in patients with high grade gliomas: A systematic review and meta-analysis. *Journal of Neuro-Oncology*, *105*(2), 325–335. https://doi.org/10.1007/s11060-011-0594-5

Omuro, A., & DeAngelis, L. M. (2013). Glioblastoma and other malignant gliomas: A clinical review. *JAMA*, *310*(17), 1842–1850. https://doi.org/10.1001/jama.2013.280319

Ostrom, Q. T., Bauchet, L., Davis, F. G., Deltour, I., Fisher, J. L., Langer, C. E., Pekmezci, M., Schwartzbaum, J. A., Turner, M. C., Walsh, K. M., Wrensch, M. R., & Barnholtz-Sloan, J. S. (2014). The epidemiology of glioma in adults: A "state of the science" review. *Neuro-Oncology*, *16*(7), 896–913. https://doi.org/10.1093/neuonc/nou087

Ostrom, Q. T., Gittleman, H., Liao, P., Rouse, C., Chen, Y., Dowling, J., Wolinsky, Y., Kruchko, C., & Barnholtz-Sloan, J. (2014). CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. *Neuro-Oncology*, *16 Suppl 4*, iv1-63. https://doi.org/10.1093/neuonc/nou223

Ostrom, Q. T., Gittleman, H., Liao, P., Vecchione-Koval, T., Wolinsky, Y., Kruchko, C., & Barnholtz-Sloan, J. S. (2017). CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014. *Neuro-Oncology*, *19*(suppl_5), v1–v88. https://doi.org/10.1093/neuonc/nox158

Ostrom, Q. T., Gittleman, H., Truitt, G., Boscia, A., Kruchko, C., & Barnholtz-Sloan, J. S. (2018). CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2011-2015. *Neuro-Oncology*, *20*(suppl_4), iv1–iv86. https://doi.org/10.1093/neuonc/noy131

Paolillo, M., Boselli, C., & Schinelli, S. (2018). Glioblastoma under Siege: An Overview of Current Therapeutic Strategies. *Brain Sciences*, *8*(1). https://doi.org/10.3390/brainsci8010015

Quick Brain Tumor Facts. (n.d.). National Brain Tumor Society. Retrieved June 9, 2020, from https://braintumor.org/brain-tumor-information/brain-tumor-facts/

Rights (OCR), O. for C. (2009, November 20). *Summary of the HIPAA Security Rule* [Text]. HHS.Gov. https://www.hhs.gov/hipaa/for-professionals/security/laws-regulations/index.html

Ringel, F., Pape, H., Sabel, M., Krex, D., Bock, H. C., Misch, M., Weyerbrock, A., Westermaier, T., Senft, C., Schucht, P., Meyer, B., Simon, M., & SN1 study group. (2016). Clinical benefit from resection of recurrent glioblastomas: Results of a multicenter study including 503 patients with recurrent glioblastomas undergoing surgical resection. *Neuro-Oncology*, *18*(1), 96–104. https://doi.org/10.1093/neuonc/nov145

Roberts, R. D., Lizardo, M. M., Reed, D. R., Hingorani, P., Glover, J., Allen-Rhoades, W., Fan, T., Khanna, C., Sweet-Cordero, E. A., Cash, T., Bishop, M. W., Hegde, M., Sertil, A. R., Koelsche, C., Mirabello, L., Malkin, D., Sorensen, P. H., Meltzer, P. S., Janeway, K. A., … Crompton, B. D. (2019). Provocative questions in osteosarcoma basic and translational biology: A report from the Children's Oncology Group. *Cancer*, *125*(20), 3514–3525. https://doi.org/10.1002/cncr.32351

Song, J. W., & Chung, K. C. (2010). Observational studies: Cohort and case-control studies. *Plastic and Reconstructive Surgery*, *126*(6), 2234–2242. https://doi.org/10.1097/PRS.0b013e3181f44abc

Storey, K., Leder, K., Hawkins-Daarud, A., Swanson, K., Ahmed, A. U., Rockne, R. C., & Foo, J. (2019). Glioblastoma Recurrence and the Role of O6-Methylguanine-DNA Methyltransferase Promoter Methylation. *JCO Clinical Cancer Informatics*, *3*, 1–12. https://doi.org/10.1200/CCI.18.00062

Strauss, M. E., & Smith, G. T. (2009). Construct validity: Advances in theory and methodology. *Annual Review of Clinical Psychology*, *5*, 1–25. https://doi.org/10.1146/annurev.clinpsy.032408.153639

Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J. B., Janzer, R. C., Ludwin, S. K., Allgeier, A., Fisher, B., Belanger, K., Hau, P., Brandes, A. A., Gijtenbeek, J., Marosi, C., Vecht, C. J., Mokhtari, K., Wesseling, P., Villa, S., Eisenhauer, E., … National Cancer Institute of Canada Clinical Trials Group. (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *The Lancet. Oncology*, *10*(5), 459–466. https://doi.org/10.1016/S1470-2045(09)70025-7

Stupp, R., Wong, E. T., Kanner, A. A., Steinberg, D., Engelhard, H., Heidecke, V., Kirson, E. D., Taillibert, S., Liebermann, F., Dbalý, V., Ram, Z., Villano, J. L., Rainov, N., Weinberg, U., Schiff, D., Kunschner, L., Raizer, J., Honnorat, J., Sloan, A., … Gutin, P. H. (2012). NovoTTF-100A versus physician's choice chemotherapy in recurrent glioblastoma: A randomised phase III trial of a novel treatment modality. *European Journal of Cancer (Oxford, England: 1990)*, *48*(14), 2192–2202. https://doi.org/10.1016/j.ejca.2012.04.011

Sun, H., Yin, L., Li, S., Han, S., Song, G., Liu, N., & Yan, C. (2013). Prognostic significance of IDH mutation in adult low-grade gliomas: A meta-analysis. *Journal of Neuro-Oncology*, *113*(2), 277–284. https://doi.org/10.1007/s11060-013-1107-5

Taal, W., Oosterkamp, H. M., Walenkamp, A. M. E., Dubbink, H. J., Beerepoot, L. V., Hanse, M. C. J., Buter, J., Honkoop, A. H., Boerman, D., de Vos, F. Y. F., Dinjens, W. N. M., Enting, R. H., Taphoorn, M. J. B., van den Berkmortel, F. W. P. J., Jansen, R. L. H., Brandsma, D., Bromberg, J. E. C., van Heuvel, I., Vernhout, R. M., … van den Bent, M. J. (2014). Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): A randomised controlled phase 2 trial. *The Lancet. Oncology*, *15*(9), 943–953. https://doi.org/10.1016/S1470-2045(14)70314-6

Tateishi, K., Wakimoto, H., & Cahill, D. P. (2017). IDH1 Mutation and World Health Organization 2016 Diagnostic Criteria for Adult Diffuse Gliomas: Advances in Surgical Strategy. *Neurosurgery*, *64*(CN_suppl_1), 134–138. https://doi.org/10.1093/neuros/nyx247

Terasaki, M., Ogo, E., Fukushima, S., Sakata, K., Miyagi, N., Abe, T., & Shigemori, M. (2007). Impact of combination therapy with repeat surgery and temozolomide for recurrent or progressive glioblastoma multiforme: A prospective trial. *Surgical Neurology*, *68*(3), 250–254. https://doi.org/10.1016/j.surneu.2006.11.042

Thakkar, J. P., Dolecek, T. A., Horbinski, C., Ostrom, Q. T., Lightner, D. D., Barnholtz-Sloan, J. S., & Villano, J. L. (2014). Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by*

the American Society of Preventive Oncology, *23*(10), 1985–1996. https://doi.org/10.1158/1055-9965.EPI-14-0275

Tommasini-Ghelfi, S., Murnan, K., Kouri, F. M., Mahajan, A. S., May, J. L., & Stegh, A. H. (2019). Cancer-associated mutation and beyond: The emerging biology of isocitrate dehydrogenases in human disease. *Science Advances*, *5*(5), eaaw4543. https://doi.org/10.1126/sciadv.aaw4543

van den Bent, M. J., Dubbink, H. J., Sanson, M., van der Lee-Haarloo, C. R., Hegi, M., Jeuken, J. W. M., Ibdaih, A., Brandes, A. A., Taphoorn, M. J. B., Frenay, M., Lacombe, D., Gorlia, T., Dinjens, W. N. M., & Kros, J. M. (2009). MGMT promoter methylation is prognostic but not predictive for outcome to adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors: A report from EORTC Brain Tumor Group Study 26951. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *27*(35), 5881–5886. https://doi.org/10.1200/JCO.2009.24.1034

Verhaak, R. G. W., Hoadley, K. A., Purdom, E., Wang, V., Qi, Y., Wilkerson, M. D., Miller, C. R., Ding, L., Golub, T., Mesirov, J. P., Alexe, G., Lawrence, M., O'Kelly, M., Tamayo, P., Weir, B. A., Gabriel, S., Winckler, W., Gupta, S., Jakkula, L., … Cancer Genome Atlas Research Network. (2010). Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*, *17*(1), 98–110. https://doi.org/10.1016/j.ccr.2009.12.020

Waitkus, M. S., Diplas, B. H., & Yan, H. (2016). Isocitrate dehydrogenase mutations in gliomas. *Neuro-Oncology*, *18*(1), 16–26. https://doi.org/10.1093/neuonc/nov136

WHO | The determinants of health. (n.d.). WHO. Retrieved April 27, 2019, from https://www.who.int/hia/evidence/doh/en/

Wick, W., Osswald, M., Wick, A., & Winkler, F. (2018). Treatment of glioblastoma in adults. *Therapeutic Advances in Neurological Disorders*, *11*, 1756286418790452. https://doi.org/10.1177/1756286418790452

Wilson, T. A., Karajannis, M. A., & Harter, D. H. (2014). Glioblastoma multiforme: State of the art and future therapeutics. *Surgical Neurology International*, *5*. https://doi.org/10.4103/2152-7806.132138

Xavier-Magalhães, A., Nandhabalan, M., Jones, C., & Costa, B. M. (2013). Molecular prognostic factors in glioblastoma: State of the art and future challenges. *CNS Oncology*, *2*(6), 495–510. https://doi.org/10.2217/cns.13.48

Xia, L., Wu, B., Fu, Z., Feng, F., Qiao, E., Li, Q., Sun, C., & Ge, M. (2015). Prognostic role of IDH mutations in gliomas: A meta-analysis of 55 observational studies. *Oncotarget*, *6*(19), 17354–17365. https://doi.org/10.18632/oncotarget.4008

Yan, H., Parsons, D. W., Jin, G., McLendon, R., Rasheed, B. A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G. J., Friedman, H., Friedman, A., Reardon, D., Herndon, J., Kinzler, K. W., Velculescu, V. E., Vogelstein, B., & Bigner, D. D. (2009). IDH1 and IDH2 mutations in gliomas. *The New England Journal of Medicine*, *360*(8), 765–773. https://doi.org/10.1056/NEJMoa0808710

Yang, P., Zhang, W., Wang, Y., Peng, X., Chen, B., Qiu, X., Li, G., Li, S., Wu, C., Yao, K., Li, W., Yan, W., Li, J., You, Y., Chen, C. C., & Jiang, T. (2015). IDH mutation and MGMT promoter methylation in glioblastoma: Results of a prospective registry. *Oncotarget*, *6*(38), 40896–40906. https://doi.org/10.18632/oncotarget.5683

Zang, L., Kondengaden, S. M., Che, F., Wang, L., & Heng, X. (2018). Potential Epigenetic-Based Therapeutic Targets for Glioma. *Frontiers in Molecular Neuroscience*, *11*, 408. https://doi.org/10.3389/fnmol.2018.00408

Zhang, J.-L., Zhong, X.-S., Yang, S.-B., Kang, X., Li, Y., Chen, J.-X., & Li, W.-B. (2019). Features and therapeutic potential of T-cell receptors in high-grade glioma. *Chinese Medical Journal*, *132*(12), 1435–1440. https://doi.org/10.1097/CM9.0000000000000282

Zhao, H., Wang, S., Song, C., Zha, Y., & Li, L. (2016). The prognostic value of MGMT promoter status by pyrosequencing assay for glioblastoma patients' survival: A meta-analysis. *World Journal of Surgical Oncology*, *14*(1), 261. https://doi.org/10.1186/s12957-016-1012-4

Zhao, Y.-H., Wang, Z.-F., Cao, C.-J., Weng, H., Xu, C.-S., Li, K., Li, J.-L., Lan, J., Zeng, X.-T., & Li, Z.-Q. (2018). The Clinical Significance of O6-Methylguanine-DNA Methyltransferase Promoter Methylation Status in Adult Patients With Glioblastoma: A Meta-analysis. *Frontiers in Neurology*, *9*, 127. https://doi.org/10.3389/fneur.2018.00127

Zhao, Y.-H., Wang, Z.-F., Pan, Z.-Y., Péus, D., Delgado-Fernandez, J., Pallud, J., & Li, Z.-Q. (2019). A Meta-Analysis of Survival Outcomes Following Reoperation in Recurrent Glioblastoma: Time to Consider the Timing of Reoperation. *Frontiers in Neurology*, *10*, 286. https://doi.org/10.3389/fneur.2019.00286

Zou, P., Xu, H., Chen, P., Yan, Q., Zhao, L., Zhao, P., & Gu, A. (2013). IDH1/IDH2 mutations define the prognosis and molecular profiles of patients with gliomas: A meta-analysis. *PloS One*, *8*(7), e68782. https://doi.org/10.1371/journal.pone.0068782

Appendix

Copyright Permission for Lee et al (2000, p.200)

N

no-reply@copyright.com
Tue 1/21/2020 10:07 PM
To: Haroon Hashmi

Header

Thank you for your order!

Dear Mr. Haroon Hashmi,

Thank you for placing your order through Copyright Clearance Center's RightsLink® service.

Order Summary

Licensee:	Mr. Haroon Hashmi
Order Date:	Jan 21, 2020
Order Number:	4753950781859
Publication:	Journal of the National Cancer Institute
Title:	Outcomes Research in Oncology: History, Conceptual Framework, and Trends in the Literature
Type of Use:	Thesis/Dissertation
Order Total:	0.00 USD

View or print complete details of your order and the publisher's terms and conditions.

Sincerely,

Copyright Clearance Center

Tel: +1-855-239-3415 / +1-978-646-2777
customercare@copyright.com
https://myaccount.copyright.com