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The prognostic value of biomarkers in the evaluation of glioblastoma multiforme

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THE PROGNOSTIC VALUE OF BIOMARKERS IN THE EVALUATION OF GLIOBLASTOMA MULTIFORME

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MARC-ANDRÉ GASCON

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Approved by

First Reader

James W. Holsapple, M.D.
Associate Professor of Neurosurgery

Second Reader

Oren Berkowitz, Ph.D., PA-C
Assistant Professor of Medicine

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MARC-ANDRE GASCON

ABSTRACT

Background

Glioblastoma multiforme (GBM) is a highly heterogeneous tumor of the central nervous system (CNS) that exhibits considerable variation in its clinical course. Recently, the World Health Organization (WHO) published a classification system for tumors of the CNS that combines histological features with molecular parameters to determine tumor grade. The incorporation of molecular biomarkers that carry both prognostic and predictive value adds another level of objectivity to the glioma grading system and will help guide clinical decision. As such, the assessment of biomarkers has become an integral part of tumor evaluation in neuro-oncology. This curriculum will discuss the clinical relevance of the most recently studied biomarkers with prognostic and predictive value in the evaluation of GBM. Biomarkers regularly used for the assessment of GBM include the IDH mutations, MGMT methylation status, and EGFRvIII. Furthermore, this review will offer a perspective on experimental approaches currently under investigation for treatment of GBM.

Literature Review Findings

MGMT methylation of the promoter region is associated with better treatment response from temozolomide (TMZ), an alkylating therapeutic. Treatment benefit was most prominent in the elderly population and therapy should be individualized for that age

group. Patients with GBM characterized by IDH1/IDH2 mutations carry a better overall prognosis primarily due to their higher sensitivity to chemo- and radiotherapy. The prognostic value of EGFRvIII remains controversial, although it may be associated with a worse prognosis. Nonetheless, EGFRvIII provides an ideal target for targeted molecular therapies as it is only found on tumor cells.

Proposed Methods

A curriculum aimed at educating primary care providers (PCPs) about the most clinically significant biomarkers in GBM will be developed. The curriculum will be in a PowerPoint format, and the hour-long lecture will be presented at continuing medical education national conferences. A pre- and post-test consisting of the same 10 multiple-choice questions will be administered on a voluntary basis to help evaluate knowledge acquisition from the curriculum. Results will be evaluated with a paired t-test analysis. The tests will be will be administered through Poll Everywhere, a smartphone survey application.

Conclusion

There is increasing evidence to suggest that therapies should be individualized according to specific biomarkers with predictive value. PCPs are in a position where they are often the first providers to suspect the diagnosis of a brain tumor. Therefore, it is imperative for PCPs to be aware of the latest development in the field of neuro-oncology so that they may appropriately counsel patients.

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LIST OF ABBREVIATIONS

APCs……..Antigen Presenting Cells

BTSCs……..…Brain Tumor Stem Cells

CME…….....Continuing Medical Education

CNS…….……Central Nervous System

DCs…….…..……Dendritic Cells

EGFR……..…….Endothelial Growth Factor Receptor

GBM……..…Glioblastoma Multiforme

IDH……Isocitrate Dehydrogenase

MGMT……...……O-6-Methylguanine-DNA Methyltransferase

NK…….Natural Killer

PCPs …….…….. Primary Care Providers

PDGF……...Platelet Derived Growth Factor

PDGFR….……Platelet Derived Growth Factor Receptor

TKRs……...Tyrosine Kinase Receptors

TAAs…….Tumor Associated Antigens

TMZ……...……Temozolomide

VEGF……...Vascular Endothelial Growth Factor

WHO…..…….……World Health Organization

INTRODUCTION

Background

Gliomas are malignancy of the CNS that primarily develop within the brain parenchyma. They are tumors that share histological features with normal glial cells. GBM, the most aggressive and commonly occurring glioma in adults, is generally associated with a poor prognosis and rapidly progressing disease[1]. In the United States, the reported annual incidence is 3.2 cases per 100,000 people, and despite its relatively rare incidence, the disease is responsible for a disproportionally high level of comorbidities and deaths[2]. GBM has a tendency to affect males and those above 64 years of age[2].

The diagnosis is based on histological features and molecular biomarkers that determine the tumor grade and potential for malignancy according to criteria established by the WHO[3]. The WHO classification system for CNS tumors was recently updated after recognition that tumors with morphologically similar features may harbor significantly different genetic alterations that carry different prognosis. The recent addition of biomarkers to the classification has helped redefine tumor subtypes within each WHO category and groups together entities with similar biological properties regardless of the putative cell of origin[3].

Prognostic biomarkers are used in oncology to predict the likely course of a disease without any given intervention. Predictive biomarkers offer information about a patient's likely response to a given therapy. There is increasing evidence that specific biomarkers associated with GBM may help guide clinical decisions.

Despite recent advancement in genomics, GBM remains an incurable disease and invariably results in deterioration of neurological function, ultimately leading to death. Following genome-wide analysis studies, several molecular alterations and aberrant growth signaling pathways involved in the pathogenesis of GBM have been identified. These findings have led to the development of molecular targeted therapies that aim to block signal transduction pathways involved in tumor growth, survival, and invasion[4]. However, following the results of multiple clinical trials, it is now becoming evident that molecular targeted therapies directed toward a single genetic alteration or signaling pathway will unlikely lead to disease remission[5].

Recent successes in proof-of-concept trials have opened the door to new therapeutic agents that aim to modulate and enhance the immune response against the tumor with the hope that a more durable and long-lasting response can be achieved in GBM patients. There is growing interest in the field of immunotherapy, and several studies are currently underway to evaluate the use of cell-based vaccines and oncolytic viruses that have the ability to target specific biomarkers expressed only on tumor cells. In studies thus far, immunotherapies have demonstrated satisfying safety profiles and limited adverse effects. However, to date, few large randomized controlled clinical trial have been conducted to determine the clinical efficacy of these therapies[5].

Additional research is required before remission can be achieved in GBM. The identification and characterization of key biomarkers is an essential step in the development of novel therapeutic strategies.

Statement of the Problem

The standard of care for newly diagnosed GBM includes maximal surgical resection to the extent possible and radiotherapy plus concomitant and adjuvant TMZ. Despite this multimodality treatment, most patients will recur within 6.9 months, and prognosis remains poor with a median survival time of only 14.6 months[6]. Given the poor prognosis associated with GBM, it is imperative that basic research continues to identify important biomarkers that can lead to clinical trial design and translate into improved clinical outcomes for patients.

The diagnosis of GBM has a drastic impact in a person's life. Whether a patient feels most comfortable discussing his/her diagnosis with a specialist or its PCP with whom he or she may have established long-term rapport, it is essential that all health care providers are knowledgeable of the most effective therapeutic options that make use of the latest developments in the field of neuro-oncology. As such, it is imperative for PCPs to understand the value of biomarkers in guiding their clinical decisions and treating their patients.

Hypothesis

Providing a CME curriculum will improve PCPs' knowledge and help them become acquainted with the prognostic and predictive value of specific biomarkers associated with GBM.

Objectives and specific aims

Providing a CME curriculum to educate PCPs about the value of well-characterized biomarkers associated with better prognosis or treatment responses to GBM will better equip clinicians in advising their patients through their disease, providing counsel on treatment options, and making appropriate recommendations.

1. To provide PCPs with a general understanding of the different treatment options available and current challenges related to the eradication of GBM.
2. To improve PCPs' understanding of biomarkers with prognostic and predictive value and ability to educate and counsel patients.
3. To compare pre and post-test results with a paired t-test analysis in order to evaluate knowledge acquisition following the presentation.

REVIEW OF THE LITERATURE

Overview

The brain is composed of both stromal and parenchymal tissue. The stroma plays a supportive or structural role. It is composed of both blood vessels that penetrate deeply into the brain parenchyma to provide oxygen and nutrients necessary for brain function, and connective tissue that provides additional structural support. The brain parenchyma is the functional tissue of the brain. It is used for cognition and motor function and is composed of neurons and glial cells[7].

Three types of glial cells maintain homeostasis by providing physical and metabolic support to the CNS. Oligodendrocytes surround and insulate neurons by creating the myelin sheath, while astrocytes provide structural support. Astrocytes are also involved in the removal of cellular debris and dead neuron cells, as do microglia, the last type of glial cell. Furthermore, astrocytes help maintain optimal neuronal function by regulating extracellular fluid composition and supporting the blood-brain barrier, which helps protect neural tissue from variations in blood composition and neurotoxic substances[7].

Primary brain tumors are neoplasms that originate from the brain tissue itself; whereas, secondary brain tumors are neoplasms that originate from somewhere else in the body, but involve the brain as a metastatic site. The most common primary brain tumors are gliomas, and although certain subtypes are histologically benign, the complexity of the brain often blurs the distinction between benign and malignant neoplasm[8]. A benign

tumor with little potential for malignancy may prove to be lethal if located in a region of the brain that interferes with vital bodily functions.

Gliomas are tumors that share histological features with normal glial cells and commonly involve the brain parenchyma. Although their origin remains unclear, they were traditionally believed to arise from normal glial cells. Recent evidence suggests that such tumors may originate from neural stem cells[9].

Epidemiology

Various international organizations track the incidence of gliomas through government cancer registries or personal health records. However, the complexity of the current classification of gliomas with regards to histological features, as well as differences in data collection techniques, make comparison among studies a challenge. In the United States, the Central Brain Tumor Registry of the United States estimates the annual incidence for malignant glioma is approximately 5 cases per 100,000 people. GBM, the most common and deadly glioma subtype occurring in adults, accounts for 60 to 70% of malignant gliomas with an annual age-adjusted incidence of 3.2 cases per 100,000 people[2,10]. Many studies have attempted to determine whether the incidence rate of malignant gliomas is increasing. Although the incidence rate appears to be generally rising over the last two decades, especially in the elderly, this is likely the result of improved diagnostic imaging[8,11]. At time of diagnosis, the median age of patients with GBM is 64 years old. The disease is about 60% more common in males than in females

and nearly twice as common in whites than in blacks[2]. Survival after diagnosis is highly variable and dependent on the tumor grade. GBM (WHO grade IV) is associated with the poorest prognosis amongst all gliomas. The overall survival at 5 years following diagnosis is merely 5.5%[2].

Approximately 5% of patients with malignant gliomas will report a family history of gliomas. Recent studies suggest a two-fold increased risk of developing brain tumors when a first-degree relatives is diagnosed with a glial tumor[12,13]. Genome-wide analysis has failed to identify a single genetic driver for familial cases of malignant gliomas that are not associated with rare genetic syndromes. In the absence of a single molecular driver contributing to the incidence rate of malignant gliomas, GBM is best explained with a polygenetic model where multiple mutations are involved and drive tumorigenesis[14,15].

Several observational studies have identified the relationship between radiation exposure and risk of malignancy. Ionizing radiation can induce DNA damage through molecular bond breakage and therefore, alter the molecular structure of DNA. In sufficient amounts, alteration in nucleic acid structure can overcome cellular DNA-repair mechanisms and lead to malignancy. Exposure to therapeutic doses or high-doses of radiation is the only clearly established risk factor for gliomas. Genetic factors can influence susceptibility from such risk factor[8]. Results of epidemiological studies assessing the risk of primary CNS brain tumor from diagnostic radiation imaging studies remain inconclusive to date. However, in the last two decades there has been a dramatic increase in the use of such diagnostic studies, which account for nearly half of per capita

radiation exposure. There is conclusive evidence that x- or gamma-irradiation exposure of at least 10-50 mSv is associated with a significant increased risk of malignancy[16]. This potential risk from radiologic diagnostic studies may be most relevant in the pediatric population because their brains are still growing and developing, however, data related to their risk following exposure is currently inconclusive.

Epidemiological studies of other proposed risk factors such as head injury, foods containing N-nitroso compounds, occupational risk factors and electromagnetic field exposure is also inconclusive to date[17]. Although there have been growing concerns that increased radiofrequency exposure to the brain from cellular phones may lead to brain malignancy, the largest studies tracking incidence rates of glioma in Nordic countries, the United States, and Israel have found no significant changes in trend of glioma incidence[11,18,19]. Interestingly, many epidemiological studies have consistently reported an inverse relationship between glioma risk and atopic disease[8,20,21]. Although the mechanism by which this protective effect is achieved remains unclear, it has been hypothesized that increased surveillance by the innate immune system may account for the reduced glioma risk observed in this patient population. Further studies are required to clarify an important but unknown link between immune mediators and malignant gliomas.

Classification

Current WHO classification of primary CNS tumor relies heavily on morphological features to classify gliomas into categories based on the predominant cell type present, and are later assigned a grade according to the presence or absence of standard pathological features. Although this system provides valuable information regarding tumor prognosis, considerable heterogeneity exists within the same tumor grade. Recent advancement in molecular analysis have revealed that tumors with similar morphological features may have vastly different molecular signatures, bearing significant implications for disease prognosis and treatment responses[22,23,24].

In response to mounting evidence supporting the prognostic value of certain molecular alterations, the WHO published an updated version of its CNS tumors classification system that considers both histological features and molecular parameters to define specific tumor entities[3]. The incorporation of phenotypic-genotypic diagnostic criteria for CNS tumor classification adds another level of objectivity. It is hoped that this new classification system will help characterize tumors into more biologically homogenous entities to improve patient management and allow for improved prognostic determination and treatment response. Additionally, defining CNS tumors more narrowly and with greater diagnostic accuracy may facilitate clinical, experimental and epidemiological studies, which can lead to improvements in the quality of life of patients living with brain tumors.

The updated 2016 WHO classification of tumors of the CNS primarily relies on light microscopy to establish tumor grade. However, for diagnostic categories that have always been difficult to define, the addition of genotypic parameters helps establish the diagnosis and trumps the histological phenotype[3]. Although all astrocytic and oligodendroglial tumors were previously grouped into separate categories, this novel approach now clumps all diffusely infiltrating gliomas together regardless of their putative cell of origin[3]. This provides a dynamic classification system of tumors that share common growth patterns, behaviors, and genetic drivers in the isocitrate dehydrogenase 1/2 (IDH1 and IDH2) genes. Moreover, it groups tumors with similar prognostic parameters and may guide both conventional and targeted therapies for entities that share biological and genetic properties. As a result of this new classification, adult diffuse gliomas include WHO grade II and grade III astrocytic tumors, grade IV glioblastoma, as well as grade II and III oligodendrogliomas[3].

Glioblastoma may arise *de novo* or from lower grade astrocytic gliomas and is generally associated with dense cellular infiltrate into the brain parenchyma, pleomorphism, high mitotic activity and either microvascular proliferation, necrosis or both[25]. Additionally, the 2016 CNS WHO further divides glioblastoma into three distinct sub-types according to their molecular parameters. Glioblastoma, IDH-wildtype accounts for nearly 90% of cases and correlates clinically with primary glioblastoma[3]. These tumors arise *de novo* without clinical or histopathological evidence of a pre-existing lower grade glioma and typically present with a short clinical history (<3 months) in patients over 55 years of age[3]. The second sub-type, glioblastoma, IDH-mutant accounts

for approximately 10% of cases[3]. It corresponds most closely with secondary glioblastoma and preferentially arises in younger patients through malignant progression from a lower grade diffuse astrocytic glioma. The time required for this progression varies considerably but ranges from less than one year to more than ten years with a mean interval of 4-5 years[3]. The final sub-type, glioblastoma, NOS, is a diagnosis that is reserved for tumors for which IDH evaluation cannot be completed[3].

Diagnosis

Patients affected by malignant glioma may present with a wide variety of signs and symptoms. The most common clinical presentation includes seizures, headaches, fatigue, focal neurological deficits, confusion, memory loss, and personality changes. Definitive diagnosis requires a biopsy with histologic confirmation to establish tumor type and grade[26]. Given the invasive nature of this procedure, imaging techniques such as magnetic resonance imaging or computed tomography are initially performed and can typically reveal findings that are strongly suggestive of an intracranial tumor. Malignant gliomas are defined by a heterogeneously enhancing mass with surrounding edema[27]. Furthermore, GBM is characterized by an area of central necrosis with peritumoral edema that is more prominent than that otherwise associated with lower grade gliomas[27]. Development of new imaging techniques such as diffusion-weighted imaging, diffusion tensor imaging, dynamic contrast-enhanced MRI, and perfusion imaging are increasingly used as diagnostic aids and provide additional information regarding vessel permeability

and cerebral blood flow volume. These new techniques may also be used as a mean to evaluate response to therapy[28].

When the diagnosis is uncertain, proton magnetic resonance spectroscopy can help discriminate between a tumor, an area of necrosis, or a benign lesion. This technique detects level of various metabolites in the brain that correlate with areas of increased membrane turn-over and decreased neuronal cellularity, findings consistent with malignant gliomas[27,28]. Positive-emission tomography uses isotopes and is currently being investigated for its usefulness in diagnosing and monitoring responses to therapy in malignant gliomas[29,30].

Standard of care

The standard of care for newly diagnosed GBM includes maximal surgical resection to the extent possible, followed by radiotherapy of the resection cavity in combination with temozolomide[31,32]. Surgical debulking of the tumor alone results in a median survival of 6 months. When combined with radiotherapy, the median survival rate improves to 12.1 months. The addition of TMZ, an oral alkylating agent that leads to DNA damage and induces apoptosis, has further extended the survival rate to 14.6 months with minimal added toxicity[6].

The standard of care involves 6 weeks of focal, fractioned external beam radiotherapy to the surgical cavity including an additional 2 cm margin of surrounding brain tissue and concomitant TMZ[6,33]. Furthermore, primary brain tumors are commonly

associated with complex medical problems that require symptomatic management concurrent to standard of care. Seizures, cerebral edema, infections, depression, cognitive dysfunction, fatigue, and venous thromboembolisms are common complications that can significantly impact quality-of-life in brain cancer patients.

The objective of surgery is to allow for maximal safe tumor resection without inadvertently damaging neurological functions[34]. Although this may not always be possible based on the surrounding anatomical structures involved, recent advancements in surgical imaging techniques have helped neurosurgeons distinguish neoplastic tissue from healthy surrounding neural tissue[34–36]. Fluorescence guided resection uses a dye that specifically binds to tumor cells and helps identify tumor margins when exposed to the appropriate wavelength. This technique results in increased gross total resection and improved progression-free survival [35–37].

Biodegradable wafers impregnated with carmustine, another alkylating agent, are an alternative therapy for treatment of GBM[38]. The implant is placed within the tumor resection cavity and allows for the direct delivery of carmustine within the tumor bed for several weeks while limiting systemic toxicities. Unfortunately, the implant is associated with increased risk of infections, cerebral edema, and impaired wound healing, and therefore it is not commonly used by most centers[38,39].

Despite multimodality treatment, most patients will recur within 6.9 months, and prognosis is poor with a mean survival rate of only 3.3% at 2 years[4,40]. Surgery and radiotherapy are often contraindicated when the disease recurs. Enrollment into clinical

trials is often the best option for these patients, and novel agents and delivery methods are typically tested in the recurrent setting first.

Gliomagenesis

Recent work in genomics has made significant progress to further our understanding of gliomagenesis. It is now recognized that malignant progression results from sequential accumulation of genetic mutations and dysregulation of important growth signaling pathways and corresponding intracellular cascades. Abnormal proliferation is sustained through unregulated expression of growth factors such as vascular endothelial growth factors (VEGF), epidermal growth factors, and loss of tumor suppressor genes such as phosphotensin analogue (PTEN)[4]. Furthermore, a mutation in the epidermal-growth factor receptor (EGFR) known as EGFRvIII in primary GBM or increased expression of PDGF-A receptor (PDGFR) commonly associated with secondary GBM results in permanent activation of two key tyrosine kinase receptors (TKRs) and increased signaling through the RAS and PI3K transduction cascade[4]. These intracellular signaling cascades regulate gene expression involved in cellular growth and proliferation, cytoskeletal arrangement, apoptosis, mobility, and angiogenesis[41]. Activation of the above pathways results in increased expression of VEGF and angiogenesis. VEGF expression has been shown to correlate clinically with time to recurrence and overall survival[34]. Together, EGFR, PDGFR, and VEGF-receptor play a critical role in the

normal development of the nervous system by promoting proliferation of multipotent stem cells that may also contribute to gliomagenesis as explained below[26,42].

IDH1/IDH2 mutation is predominantly found in low-grade gliomas (>80%) and secondary high-grade gliomas[24]. Mutation in the IDH1/IDH2 genes is a process that occurs early in gliomagenesis and results in enzymatic alterations that leads to accumulation of 2-hydroxyglutarate, a possible oncometabolite[24]. These mutations are currently the subject of intensive research for the development of novel treatment strategies.

Despite genetic differences, primary and secondary GBM are histologically indistinguishable and respond similarly to conventional therapy. By virtue of the specific biomarkers that define each subtype, they may respond differently to targeted molecular therapies or immunotherapies that are directed at specific molecular alteration. As such, identification and characterization of key genetic alterations in both primary and secondary GBM is an important step for the development of novel therapies that may improve clinical outcomes for patients.

The nervous system contains neural stem cells that are located deep within the tumor bed. These BTSCs exhibit self-renewal capabilities, and are able to proliferate and differentiate into distinct cell lineages[4,26,42]. BTSCs or related progenitor cells may give rise to malignant gliomas by escaping from regulatory mechanisms that control proliferation and differentiation. There is mounting evidence that BTSCs are an important driver of disease recurrence and progression in GBM[4,26,42]. They secrete soluble factors that suppress immunity both within the tumor bed and systemically. Immunosuppression

within the tumor microenvironment is another hallmark of GBM that contributes to cancer survival. Moreover, they promote angiogenesis and tumor growth through unregulated expression of VEGF[43]. By virtue of their relative senescent state, BTSCs are resistant to conventional therapies. Radio-resistance is mediated through preferential activation of DNA-damage-expression pathways, whereas chemo-resistance occurs mostly through increased expression of O-6-methylguanine-DNA methyltransferase (MGMT), up-regulation of multiple-drug resistance genes and inhibition of apoptosis[4,26]. Interestingly, BTSCs are characterized by CD133, a biomarker also known as prominin 1 that appears to be essential for the maintenance of BTSCs in GBM[44,45]. Together, these findings suggest that BTSCs may seed neoplastic cells that contribute to the primary malignancy. Eradication of GBM will likely require therapeutic strategies that effectively target this cell population. CD133 may provide a valuable target for future investigation[46].

Paul Ehrlich first introduced the notion that neoplastic cells originate spontaneously and that the immune system may recognize and eradicate them. Evidence of the immune system's role in cancer surveillance can be witnessed by the increased incidence of virally induced cancers in immunosuppressed patients and by the direct correlation between tumor-infiltrating lymphocytes and patient survival[47]. The ability of the immune system to control and "shape" cancer is defined as immunoediting and can be characterized by three processes: elimination, equilibrium, and evasion. There is increasing evidence that the interplay between the immune system and neoplastic cells shapes and edits tumors by driving their expression profile so that they may escape

immune surveillance and proliferate unhampered. The evidence suggests that the interaction between GBM tumor cells, the stroma, and the immune factors are important factors in disease progression and clonal evolution. Giomagenesis is promoted by an immunosuppressive microenvironment that suppresses the function of T cells, antigen presenting cells (APCs), and NK cells, while also inhibiting T helper cells and cytotoxic T lymphocytes development[47].

Significant efforts to identify key biomarkers and understand gliomagenesis and tumor resistance mechanisms have lead to the development of treatment strategies designed to block essential signaling pathways, surpass acquired resistance, and improve the penetrance of the blood brain barrier to new therapeutics.

Biomarkers

In recent years, the development of sequencing technologies and large scale gene-expression studies have helped recognize and confirm certain diagnostic, prognostic and predictive biomarkers which have advanced clinical trial designs and characterized GBM subgroups with more favorable prognosis and better response to therapy[23]. Furthermore, a greater understanding of the pathogenesis of GBM has opened the door to novel therapeutic avenues with expectation for better disease control and improved survival.

Alone, tumor grading provides some insight into the clinical behaviors of CNS tumors, however, the combination of tumor grade with diagnostic molecular markers provides clinicians with further information regarding the predicted course of the disease

and treatment options available. Prognostic and predictive markers are additional tools used in oncology that can aid providers with clinical decisions by further redefining CNS tumors within each WHO subtype. Prognostic markers provide insight into the likely course of the disease without any intervention, whereas, predictive markers help predict the likely outcome of an intervention and can help clinicians decide between multiple therapies when available. Together, they are particularly helpful for targeted therapies which only function in tumors characterized by specific molecular markers[23]. However, the heterogeneity of gliomas in terms of histological features, grade, clinical outcomes, and genomic greatly complicates the risk stratification associated with certain biomarkers[3]. Despites these challenges, there is mounting evidence that MGMT methylation status, the IDH 1/2 mutation, and EGFRvIII are important biomarkers that can help guide clinical decisions along with other traditional prognostic indicators such as age, performance status, tumor location, and the extent of surgical resection [23,24,48].

MGMT is an enzyme that repairs DNA damage in response to alkylating environmental pollutants, tobacco-specific carcinogens, and alkylating agents such as anti-cancer drugs. Since the amount of MGMT enzyme directly correlates with the ability of a cell to repair damaged DNA, MGMT expression levels provide key information regarding the susceptibility of a cell to alkylating agents. Therefore, MGMT expression is a marker of resistance in normal and neoplastic cells exposed to alkylating therapeutics[49]. Inversely, methylation of the MGMT promoter region results in epigenetic changes that lead to transcriptional inactivation of the MGMT gene and consequently lead to an increased susceptibility to alkylating agents. Methylation of the MGMT promoter region

is found in 35-45% of high-grade gliomas and approximately 80% of lower-grade gliomas[50]. MGMT methylation is a strong and independent prognostic marker associated with overall survival benefits and increased progression-free survival in high-grade gliomas[51,52]. In 2005, MGMT methylation status assessed by polymerase chain reaction in newly diagnosed glioblastoma was able to predict benefits from treatment with temozolomide, a potent alkylating therapeutic[53].

First identified in 2008, point mutations in the IDH1/IDH2 genes are now recognized as important diagnostic biomarkers. Although primary and secondary GBM are histologically indistinguishable, evidence strongly suggests that these tumors evolve through vastly different molecular pathways[23]. The mutation is easily detected through immunochemistry and is currently used to distinguish between primary glioblastoma, which does not harbor the mutated IDH genes, and secondary glioblastoma. Furthermore, non-glial cells never express the mutation, a feature that can be exploited to differentiate reactive gliosis from glioma[54]. Gliomas with the IDH1 and, less frequently, the IDH2 mutation bear better prognosis than their wild-type counter part[55]. Thus, IDH1/IDH2 status provides a better diagnostic and prognostic outlook for the clinician.

EGFRvIII is a mutant of the EGFR that results in constitutive activation of the TKR and downstream signal transduction pathways. The mutation is associated with increased cellular proliferation and tumor invasiveness, radio and chemo-therapeutic resistance, and apoptosis inhibition[56]. It is expressed in approximately 30% of primary GBM and is rarely seen in secondary GBM. EGFRvIII expression is typically associated with overexpression of the wild-type EGFR which can be detected in about 50% of

GBM[23,24,57]. Long-term survival may be worse in patients whose tumors harbor this mutation, however, the prognostic value of EGFRvIII remains uncertain. Nonetheless, EGFRvIII provides an ideal target for targeted therapies as the mutant receptor is localized only on tumor tissue. EGFRvIII positive-cells are able to secrete micro-vesicles containing the EGFRvIII mRNA[58]. The detection of such vesicles in the serum of patients with EGFRvIII-positive GBM provides a potential biomarker to monitor treatment response and relapses[58].

CD133 and CD155 are biomarkers that are associated with GBM and may play an important role in future therapies. CD155 is an oncofetal cell adhesion molecule that is upregulated in specific cancers, including GBM[59]. It is expressed in BTSCs and has recently been the target of an oncolytic virus in a phase I trial[59]. CD133 is another marker of BTSCs that appears to be essential to GBM maintenance[45]. Further research is required to determine the value of these biomarkers.

Despite these recent advancements in genomics, the extent of tumor resection, age at diagnosis and Karnofsky performance status remain the strongest prognostic factors associated with GBM[60]. Early age at diagnosis is significantly associated with survival benefit for all gliomas, but this relationship is most pronounced for GBM.

Novel Therapies

The discovery of distinct molecular expression profiles and specific genetic alterations has fostered interest in various molecular targeted agents. Preliminary data from phase

I/II clinical trials have supported the development EGFR antagonist, antiangiogenesis agents, and intracellular signaling pathway inhibitors[61]. Other approaches are currently investigating stem cell treatment and immunotherapies.

Many of these aforementioned mutations affect TKRs and downstream signaling pathways. Molecular targeted therapies aim to block these receptors and inhibit downstream signaling cascades. Molecular targeted therapies can be divided into small molecule inhibitors and monoclonal antibodies. Small molecule inhibitors, many of which are tyrosine kinase inhibitors (TKIs), are lipophilic organic compounds able to cross cell membranes and function by selectively inhibiting intracellular tyrosine kinase domains or adaptor proteins involved in downstream signaling cascade[5]. Monoclonal antibodies are too large to cross the cell phospholipid bilayer and function by blocking extracellular proteins or disrupting ligand-receptor interaction at the cell surface[5]. In clinical trials, experimental treatments are typically tested once the disease recurs. However, they may also be conducted in combination with standard of care. Overall, these agents have demonstrated only modest therapeutic benefit with treatment responses between 0 to 15% and limited improvement in progression free survival[62].

Erlotinib and Gefitinib are both oral small molecule EGFR TKIs that selectively inhibit the receptor. Preclinical data showed that these agents exhibit prominent anti-proliferative and anti-invasiveness effects along with potent EGFR inhibition and prolonged survival. Clinical trials have failed to demonstrate therapeutic benefits and have not improved clinical outcomes[63].

Cetuximab is a monoclonal antibody that prevents EGFR activation by hindering ligand-receptor interaction. Cetuximab binding also results in internalization and downregulation of the EGFR[4]. Although preclinical models demonstrated decreased tumor growth and improved survival, clinical trials have shown only limited therapeutic benefit without significant improvement in patients' outcomes[5].

Imatinib is another small molecule inhibitor that targets multiple kinases. It functions by inhibiting activation of the PDGFR and other RTKs. Clinical trials have failed to demonstrate therapeutic efficacy[4,5].

Angiogenesis is an important process that characterizes GBM and is mediated by VEGF secretion in the tumor microenvironment. Recent evidence suggests that VEGF expression correlates clinically with a shorter recurrence rate and is associated with a poorer prognosis. As a result, several molecular targeted therapies were developed to inhibit this growth pathway[4,5].

Bevacizumab is a monoclonal antibody that blocks ligand-receptor interaction by binding VEGF in the extracellular matrix. Clinical trials performed in the recurrent setting demonstrated improved clinical outcomes when bevacizumab was used in conjunction with radiotherapy and TMZ. In 2009, Bevacizumab was approved for used in GBM patients after disease recurrence[64,65]. However, bevacizumab failed to demonstrate overall survival benefits in new diagnosed GBM. It was associated with higher rates of toxicities that significantly decreased quality of life[66].

Several multikinase TKIs have also been investigated for their anti-angiogenic property in GBM. Cediranib and sunitinib exhibit predominant VEGF inhibition with

additional PDGFR and c-kit inhibition[5]. In spite of promising preclinical data, clinical trials have failed to demonstrate therapeutic benefit[5]. Vatalanib, another multikinase TKIs, with anti-VEGF and anti-PDGFR properties was also found to produce limited clinical benefits[4,5].

So far, small molecule TKIs have failed to demonstrate therapeutic benefit in patients with GBM. These findings are likely the result of multiple genetic alterations and signaling pathways that drive tumor growth in GBM. Intra-tumor heterogeneity and the lack of single dominant oncogenic mutation make small molecule TKIs that target a single growth pathway unlikely to be successful therapeutic options for GBM[5]. The ability to target multiple growth pathways at once through combination of multiple molecular targeted agents or designing small molecule inhibitors with specificity for multiple TKRs may offer greater efficacy. However, these approaches may also be associated with increased risk of toxicities from systemic inhibition[31]. To date, no combination therapy has proven to be superior to single agents[4,5].

Therapeutic strategies that combine both conventional therapies while harnessing the immune system to fight cancer are now available. Multiple options that aim to control and enhance the immune system are currently being investigated including checkpoint inhibitors, gene therapies, vaccine therapies, and oncolytic viruses[46].

Peptide vaccines are among the major immunotherapeutic strategies being investigated. They involve the direct administration of tumor associated antigens (TAAs) and trigger a specific immune response against antigens present solely on tumor cells to minimize the risk of autoimmunity. Rindopepimut, a well-known peptide vaccine, is

directed against EGFRvIII harboring tumor cells. Recently, a phase II clinical trial demonstrated that concurrent administration of Rindopepimut with temozolomide in the adjuvant phase of newly diagnosed GBM patients was safe and efficacious. Overall, survival ranged from 21.8 to 26 months, and no symptomatic immune adverse effects were observed[67]. A phase III, international, randomized, double-blind, controlled study of rindopepimut/GM-CSF with adjuvant TMZ in newly diagnosed, surgically resected, EGFRvIII-positive GBM is currently underway. Results are expected to be available in early 2017[59].

Dendritic cell (DCs) vaccines have been extensively studied for the treatment of GBM. DCs are professional APCs that play an important role in activating CD4+ and CD8+ lymphocytes. DCs vaccines require autologous activated DCs that are extracted through leukapheresis. Following extraction, the DCs are loaded with TAAs by pulsing apoptotic tumor cells, tumor lysate, or peptides. After reintroduction of activated DCs cells into the patient, they activate and stimulate a cytotoxic CD8+ and T helper CD4+ response to induce tumor cell death[68, 69]. Preliminary data from 20 patients treated with DCVax in combination with radiation therapy and TMZ showed a median overall survival rate of 36 months. Interestingly, two patients survived more than ten years after diagnosis[70].

A number of viruses exhibit selective tumor cell killing properties with the ability to trigger a range of inflammatory and immune stimulatory effects on the tumor itself, the brain tumor stromal components, and the peripheral immune system. The aim is to recruit an effector adaptive immune response against TAAs that can produce robust and long-

lasting immunity. The PVS-RIPO virus is a nonpathogenic, recombinant Poliovirus: Rhinovirus chimera with tropism for tumor cell expressing CD155, an oncofetal cell adhesion molecule upregulated in ectodermal/neuroectodermal neoplasms including GBM[59]. The virus genome consists of the live attenuated poliovirus serotype 1 (Sabin) vaccine with its internal ribosomal entry site replaced with that of the human rhinovirus type 2[59]. This IRES exchange completely ablates the inherent polio-virus pathogenicity in healthy neuronal tissue. No incidence of poliomyelitis were reported in safety studies[71]. The first in-human phase I clinical trial was started at Duke University in 2012 with histologically confirmed GBM patients whom were infused intratumorally with the PVS-RIPO virus. So far, three patients have been disease-free for 23, 35 and 36 months after treatment[72].

Existing research

Alkylating chemotherapeutic

Prior to 2005, several clinical trials investigated the addition of various chemotherapeutic regimens to radiotherapy with limited clinical success. Following the results of a promising pilot phase II trial assessing the efficacy of concomitant and adjuvant administration of temozolomide with fractioned radiotherapy, a large randomized, multicenter, phase III trial was sponsored to compare radiotherapy plus TMZ versus

standard of care. All patients in the study had histologically confirmed newly diagnosed GBM[6].

The study included 573 patients distributed over a total of 85 institutions. Candidates were randomly assigned to receive radiotherapy alone (n= 286) or radiotherapy plus temozolomide (n=287). Baseline characteristics were well-balanced, and cofounders such as age, prior debulking surgery, corticosteroid use, mini-mental status exam, and performance status were distributed similarly amongst both arms of the trial. Eighty-five percent of patients had their diagnosis reviewed and confirmed through the submission of histologic slides to an independent pathologist[6].

The primary goal of the study included overall survival, and the secondary goal was comprised of progression-free survival, safety, and quality of life. Statistics were analyzed with a Kaplan-Meier method, and the study was designed to have an 80% power at a statistical significance level of 0.05 to detect a 33% increase in median survival. Median follow up occurred at 28 months. At that time, 480 patients (84%) had died from their disease.

Addition of TMZ to radiotherapy when compared to radiotherapy alone was statistically significant with an unadjusted hazard ratio of 0.63 (95% CI, 0.52-0.75; P< 0.001). The overall median survival benefit from TMZ was 2.5 months. The median survival was 14.6 months (95% CI, 13.2-16.8) for radiotherapy plus TMZ and 12.1 months (95% CI, 11.2-13.0) for radiotherapy alone. The two year survival rate was 26.5% (95% CI, 21.2-31.7%) for radiotherapy plus TMZ and 10.4% (95% CI, 6.8-14.1%) for radiotherapy alone. Median progression-free survival was 6.9 months (95% CI, 5.8-

8.2) for radiotherapy plus TMZ and 5.0 months (95% CI, 4.2-5.5) for radiotherapy alone. When adjusted for age, steroid use at the time of enrollment, gender, score on mini-mental status exam, and tumor location, the hazard ratio for death in the radiotherapy-plus-temozolomide group was 0.62 (95% CI, 0.51-0.75). Overall, the radiotherapy group did not show any high-grade hematologic toxic effects. On the other hand, 19 patients (7%) had grade 3 or 4 toxic effects in the radiotherapy-plus-temozolomide arm[6].

In this study, Stupp et al. (2005) demonstrated that the addition of concomitant and adjuvant TMZ to radiotherapy early in the course of GBM provides statistically and clinically significant survival benefit. The addition of TMZ was associated with a median increase in survival of 2.5 months or a relative reduction in the risk of death of 37% with minimal added toxicities[6].

Prognostic and predictive value of MGMT promoter methylation

Hegi et al. (2005) demonstrated the prognostic value of MGMT. Methylation of the promoter region was determined to be an independent prognostic factor regardless of treatment. MGMT promoter methylation (vs. unmethylated) was associated with a hazard ratio of 0.45 (95% CI, 0.32-0.61; $P<0.001$). Additionally, MGMT promoter methylation showed survival benefits from the addition of TMZ to radiotherapy with a median survival of 21.7 months (95% CI, 17.4-30.4) compared to 15.3 months (95% CI, 13-20.9). Analysis by Kaplan-Meier estimate showed significant survival differences between the two groups ($P= 0.007$). Furthermore, absence of MGMT promoter

methylation showed statistically insignificant survival difference between the TMZ-radiotherapy group and the radiotherapy-alone group. Thus, the results of this study suggest that therapy should be individualized to patients according to their MGMT promoter methylation status as they are most likely to benefit from the addition of TMZ[53].

In 2013, Gilbert et al. studied whether dose-dense (DD) TMZ offers survival benefits over conventional TMZ. DD TMZ depletes MGMT enzyme levels within mononuclear cells and possibly tumor cells, which may result in increased sensitivity to TMZ. Therefore, all 833 patients received radiotherapy and concomitant TMZ. At the conclusion of radiotherapy, they were randomly assigned to receive either adjuvant standard TMZ or DD-TMZ. Patients were stratified according to age, karnofsky performance status, extent of tumor resection, neurological function, and MGMT promoter methylation status[51].

The study revealed no statistically significant overall survival benefits from the addition of DD-TMZ compared to standard TMZ with a hazard ratio of 1.03 (95% CI, 0.88-1.20; P=0.63). Median progression free survival was also not statistically significant with a hazard ratio of 0.87 (95% CI, 0.75-1.00; P=0.06). The study failed to demonstrate increased sensitivity to alkylating agents based on MGMT methylation status. However, the value of MGMT promoter methylation as a prognostic factor was further demonstrated from the result of this study. MGMT promoter methylation was associated with an improved overall survival rate compared to unmethylated promoter with a hazard ratio of 0.58 (95% CI, 0.58-0.69; P<0.001)[51].

Although most studies failed to reveal increased sensitivity to temozolomide in MGMT methylated tumors, a sub-group analysis has revealed the potential predictive value of MGMT in two sub-populations of high-grade gliomas. In 2012, two randomized clinical trials in elderly patients with glioblastoma demonstrated that methylated MGMT promoter is a strong predictive biomarker that is associated with benefit from treatment with temozolomide. The results of these randomized clinical trials strongly suggests that MGMT methylation status should be standardized and treatment options individualized for that age group[51]. The second sub-group investigated whether the IDH1 status influenced the prognostic versus predictive value of MGMT promoter methylation in anaplastic gliomas. The study concluded that MGMT promoter methylation is a predictive biomarker that confers benefits from alkylating agents in IDH1-wild-type but not IDH1-mutant malignant gliomas of WHO grade III-IV[52].

Prognostic value of EGFR

A recent meta-analysis conducted by Thuy et al. (2015) attempted to determine the prognostic value of EGFR-positive GBM tumors. The median survival difference for patients characterized by EGFR-positive tumor was 0.15 month (95% CI, -0.20-0.49; P=0.394) when compared to EGFR-negative tumors. Additionally, the median survival difference for GBM patients characterized by the EGFRvIII variant was -0.6 month (95% CI, -2.18-0.98; P=0.457). Together, the results of this study suggest that EGFR bears no significance with respect to disease prognosis.

The study had several limitations. First, the study included observational studies (prospective and retrospective), case series, and data from clinical trials and each study design was treated equally during the analysis. Case series do not provide the same power as clinical trials and should not be considered to provide the same level of evidence. Furthermore, methods of data collection differed significantly between studies and ranged from immunohistochemistry, in situ hybridization, and polymerase chain reaction. These methodologies do not have the same sensitivity and this variability introduces a degree of uncertainty that could be accounted for. Finally, the authors designed the study to categorize biomarkers into categories based on what they deemed clinically significant. Thus, a survival benefit >3 months was arbitrarily defined as a meaningful change. Nonetheless, the methodology offered by Thuy et al. (2015) was able to replicate results from several studies, confirming the prognostic value of MGMT and IDH1. More studies are required to determine the prognostic value of EGFR in GBM[73].

METHODS

Study design

A curriculum for continuing medical education (CME) will be developed in a lecture-based format to inform PCPs of the different prognostic value and treatment options associated with specific biomarkers.

Study population and sampling

The hour-long lecture will be offered at medical conferences in partnership with national primary care organizations. This curriculum will allow PCPs to gain CME credits in return for their participation. The lecture will also be available to health care providers and researchers with a special interest in the field. The objective of the lecture is to increase health care providers' knowledge of newly discovered biomarkers in GBM patients that bear prognostic value.

The purpose of this curriculum is to provide a meaningful increase in knowledge acquisition. The parameters of the study entail an 80% passing grade and an expected improvement score of 15% in the post-test following the lecture with a statistical significance level of 0.05 and 80 percent power. In order to meet the sample size criteria, the curriculum should be offered to a minimum of 28 persons[74]. Offering the curriculum at a minimum of three conferences, one for each association, will meet the minimum requirement for statistical significance.

Recruitment

Participants of the study will be solicited amongst providers attending CME national conventions where the curriculum will be presented. Enrollment into the study will be voluntary and will occur after informed consent is provided. Advertisement of the lecture will include website publications by each participating organization, email and letter delivery to subscribed members of each association, and mass distribution to universities, hospitals, and research institutions. Offering the curriculum at a wide range of conferences and locations will maximize our ability to reach providers throughout the country and spread awareness of novel biomarkers with prognostic value as well as novel therapeutic approaches currently being investigated.

In return for their participation, attendees will receive one hour of CME credit. The disproportionally high level of comorbidities and deaths associated with GBM will also provide an incentive for PCPs to attend the lecture. Being educated on the state of the art development in the field of neuro-oncology will allow PCPs to share this knowledge with their patients and better guide clinical decision.

Curriculum

The presentation will be developed using Microsoft PowerPoint. It will begin with a concise outline of the learning objectives, which are listed below in Table 1. The learning objectives will provide guidelines to the attendees and help focus their attention to core aspects we hope they retain from the presentation. Additional key issues for discussion will include:

- Brief review of the epidemiology and pathogenesis of GBM
- Current therapeutic modalities
- Prognostic and predictive value of biomarkers
- Involvement of BTSCs in disease recurrence and treatment failure
- Outlook on future therapies

Table 1. Learning Objectives of the Curriculum

By completing the curriculum, the learner will be qualified to:
1. Recognize the key molecular alterations and growth pathways involved in the malignant progression of GBM
2. List the different treatment modalities available for patients with GBM
3. Evaluate the risks versus benefits of diagnostic imaging, especially in the pediatric population
4. Recognize the therapeutic value of important biomarkers associated with GBM
5. Understand the role of BTSCs in disease recurrence and resistance to conventional therapy
6. Understand the emerging role of immunotherapies and latest research development in the field

Curriculum Assessment

A pre- and post-test will be the main method of assessment. All attendees of the lecture will be asked to complete these tests. The tests will be administered using the attendees' mobile phones and results will be collected through Poll Everywhere, a mobile device survey application. Data from the pre-test will help assess the baseline understanding of current therapeutic strategies used to eradicate GBM. The post-test will consist of the same questions used before and will be administered at the end of the lecture. Data from

the post-test will allow for a comparison of their acquired knowledge from the presentation and its effectiveness.

Study variables and measures

The study variables will consist of a pre- and post-test, each consisting a minimum of ten multiple-choice questions. The questions will cover topics included in the learning objectives as well as the following points:

1. Key demographic affected by GBM
2. Important growth factors involved in malignant gliomas
3. Surgical techniques
4. Logistics of fractioned external beam radiotherapy and TMZ combination therapy
5. Antiangiogenic therapy in the recurrent setting
6. Symptomatic management of commonly associated medical issues
7. Risk associated with ionizing radiation
8. MGMT methylation status and IDH1/IDH2 biomarker
9. Importance of the tumor microenvironment and BTSCs in disease recurrence and treatment failure
10. Outlooks on immunotherapies currently under investigation

Furthermore, the survey will collect basic demographic information to gain a greater understanding of the participant involved in the study. The information collected will include: age, gender, medical specialty, work setting, and geographic location.

Data collection

The pre-test will be performed prior to the start of the lecture. Data will be collected with the use of a mobile device survey application that allows answers to be pooled and analyzed. Immediately following the lecture, the post-test will be administered and results will be pooled with the pre-test data. Finally, the post-test will be graded with a passing score of 80%, and correct answers will be displayed on the screen. This method will allow attendees to determine whether knowledge gained from the lecture helped them performed better during the post-test. Additionally, all pre- and post-test data collected during the year will be pooled together for further analysis. Other survey methods such as pen and paper, or emailed surveys are unlikely to yield the same participation rate.

Data analysis

Following data collection, a paired t-test will be conducted comparing pre-test and post-test results. The mean of both tests will be analyzed and compared to determine if the presentation represents an effective curriculum for delivering the latest information regarding the prognostic value and treatment options associated with specific biomarkers. Furthermore, an item analysis will be conducted to determine which aspects of the presentation work well and which are in need of improvement. The results of these analyses will assist us in determining whether the learning objectives have been achieved.

Timeline and resources

The presentation will be submitted to CME accredited conferences to gain approval and ensure that high educational standards are maintained throughout. Accreditation from national organizations such as the AAPA will be required to perform the lecture at national medical conventions. Accreditation from these organizations will ensure that a standard level of criteria is met.

Several medical conferences are held throughout the year at various locations in the United States. After completion of the accreditation process, specific conferences will be selected with the aim of offering CME to a wide variety of PCPs including family physicians, physician assistants, and nurse practitioners. Targeting a large number of providers involved in primary care, whom are often first to suspect a diagnosis of brain tumor, will increase the extent of our reach among vast patient populations and ensure that proper treatment is provided.

Institutional Review Board

The proposed methodology will be presented for IRB approval to the Boston Medical Center IRB under 45 CFR 46.101 (b) criteria for educational purposes.

CONCLUSION

Discussion

This work provides a review of the most recent literature pertaining to clinically significant prognostic biomarkers and therapeutic options available to patients with GBM. Despite considerable research, this review highlights the need for the development of new therapeutic strategies in order to tackle the insidious nature of GBM. This curriculum will allow health care providers to gain a greater understanding of the molecular drivers of GBM. Furthermore, it will provide an important perspective into the most recent development and challenges associated with treatment of GBM.

This curriculum will be offered at select national conferences to educate all PCPs such as physicians, physician assistants, and nurse practitioners whom are interested in learning about conventional and experimental therapeutic strategies.

Limitations to the curriculum include a low attendance rate at CME conferences. This study may not reach statistical significance if less than 28 participants attend the lecture. Given that many of the approaches presented above are still experimental, limited information from large randomized double-blind placebo-controlled trials is available. As such, additional research will be required before these approaches can be translated into clinical practice.

CME accreditation will compensate PCPs for their attendance while serving as an additional incentive for their participation. Targeting multiple providers at various conferences hosted by nationally recognized organizations allows us to extend our reach

to more patients and ultimately help them make better informed decisions regarding their course of treatment.

Summary

GBM is the most invasive and commonly occurring glioma subtype in adults. The disease is associated with a poor prognosis, and at the time of its diagnosis, it is often rapidly progressing. To date, first line therapy includes maximal safe surgical resection, radiotherapy, and concomitant and adjuvant chemotherapy in newly diagnosed GBM patients. Unfortunately, disease recurrence is common. A variety of therapies have been tested in the recurrent setting with the hope of improved clinical outcomes.

Recently, several biomarkers that correlate with better prognosis have been identified. These markers have also been the focus of intensive research efforts for the development of novel therapies. The following summary provides a brief introduction into the most clinically relevant biomarkers, a rationale for treatment failure observed with current therapies, and a perspective on future therapies.

Genome-wide analysis studies have identified IDH1/IDH2, MGMT methylation status, and EGFRvIII as important biomarkers that bear prognostic and predictive value. Mutation in the IDH1 and IDH2 genes provides both diagnostic and prognostic information to health care providers. The mutation is diagnostic for secondary GBM, which is associated with a slightly better prognosis, primarily because it reflects greater sensitivity to radio- and chemotherapy. MGMT expression is associated with resistance to alkylating agents. There is growing evidence to support that chemotherapy with TMZ

should be individualized according to a patient's MGMT methylation status. EGFRvIII is a mutation almost exclusively located on primary GBM. While the mutation may be associated with a poor prognosis, the value of this biomarker remains controversial. The receptor is the target of multiple small molecule inhibitors and monoclonal antibodies. Thus far, no therapies targeting the EGFR and downstream intracellular signaling cascade have resulted in meaningful clinical improvements in the standard of care.

Multiple lines of evidences have highlighted the role of BTSCs in disease recurrence and resistance to conventional therapies. It has been hypothesized that BTSCs exist within the tumor bed and contribute to primary tumors as well as disease recurrence. These cells exhibit self-renewal capabilities and inherent resistance to radio- and chemotherapy by virtue of their relative senescent state. Additionally, BTSCs contribute to the immunosuppressive microenvironment that is characteristic of GBM by expressing cell surface molecules and secreting soluble factors that suppress the immune system. Together, these important processes drive clonal evolution, a mechanism by which tumor cells become resistant to therapy and evade immune detection.

In recent years, it has become apparent that molecular targeted therapies with specificity for a single receptor or growth pathway will not be effective in eradicating a highly heterogeneous tumor characterized by multiple genetic alterations. Therapeutic strategies that target BTSCs will likely be required to completely eradicate GBM. Approaches that combine the use of standard therapies with immunotherapies are currently being investigated. Theoretically, cell-based vaccines or oncolytic viruses have

the ability to stimulate a robust and long-lasting immune response that can specifically target BTSCs and tumor cells that express specific immunogenic markers.

No widespread platform currently exists for providers to learn about the latest development in neuro-oncology because many of these therapies are still being investigated. This curriculum serves to educate primary health care providers on state of the art development in research and latest literature in the field. In the near future, immunotherapies alone or in combination with conventional therapies may become available to patients. It is crucial that providers learn about these therapies so they may appropriately advise their patients.

Clinical and/or public health significance

Despite its relatively rare incidence, GBM is associated with a disproportionally high level of comorbidities and deaths. In recent years, several therapeutic approaches have failed to demonstrate significant clinical benefit. Recognition of important prognostic biomarkers and potential new therapies is an important step to appropriately counsel patients on potential treatment options.

LIST OF JOURNAL ABBREVIATIONS

AAFP	American Academy of Family Physicians
Acta Neuropathol (Berl)	Acta Neuropathologica (Berl)
Am J Neuroradiol (AJNR)	American Journal of Neuroradiology
Annu Rev Pathol	Annual Review of Pathology: Mechanisms of Disease
Arch Neurol	Archives of Neurology
Asian Pac J Cancer Prev (APJCP)	Asian Pacific Journal of Cancer Prevention
Biochim Biophys Acta (BBA)	Biochimica et Biophysisca Acta
The BMJ	BMJ: British Medical Journal
Brain Pathol Zurich Switz	Brain Pathology (Zurich, Switzerland)
CA Cancer J Clin	CA: A Cancer Journal for Clinicians
Cancer Chemother Pharmacol	Cancer Chemotherapy and Pharmacology
Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol	Cancer Epidemiology, Biomarkers & Prevention Published by the American Association for Cancer Research Cosponsored by the American Society of Preventive Oncology
Cancer Genet	Cancer Genetics
Cancer Res	Cancer Research

Chin J Cancer	Chinese Journal of Cancer
Clin Med Insights Oncol	Clinical Medicine Insights: Oncology
Cochrane Database Syst Rev	Cochrane Database System of Reviews
Curr Pharm Des	Current Pharmaceutical Design
Epidemiol Camb Mass	Epidemiology (Cambridge, Mass.)
Eur J Neurol	European Journal of Neurology
Expert Rev Anticancer Ther	Expert Review of Anticancer Therapy
Front Immunol	Frontiers in Immunology
Genet Epidemiol	Genetic Epidemiology
Handb Clin Neurol	Handbook of Clinical Neurology
Hematol Oncol Clin North Am	Hematology/Oncology Clinics of North America
Int J Biochem Cell Biol	The International Journal of Biochemistry & Cell Biology
J Clin Oncol Off J Am Soc Clin Oncol	Journal Clinical Oncology Official Journal of American Society of Clinical Oncology
J Clin Neurosci	Journal of Clinical Neuroscience
J Mol Diagn (JMD)	Journal of Molecular Diagnostics
J Mol Med Berl Ger	Journal of Molecular Medicine (Berlin, Germany)
J Natl Cancer Inst	Journal of the National Cancer Institute
J Nucl Med Off Publ Soc Nucl Med	The Journal of Nuclear Medicine Official Publication of the Society Nuclear Medicine

JAMA	The Journal of the American Medical Association
Lancet Oncol	The Lancet Oncology
N Engl J Med	New England Journal of Medicine
Nat Med	Nature Medicine
Neuro-Oncol	Neuro-Oncology
Neuroimaging Clin N Am	Neuroimaging Clinics of North America
Neurol Clin	Neurologic Clinics
Neurosurg Clin N Am	Neurosurgery Clinics of North America
Neurol Res Int	Neurology Research International
Neurosurg Rev	Neurosurgical Review
Oncol Williston Park N	Oncology (Williston Park, NY)
Pharmacol Ther	Pharmacology & Therapeutics
Stem Cells Dayt Ohio	Stem Cells (Dayton, Ohio)
Surg Neurol Int	Surgical Neurology International
Trends Mol Med	Trends in Molecular Medicine

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