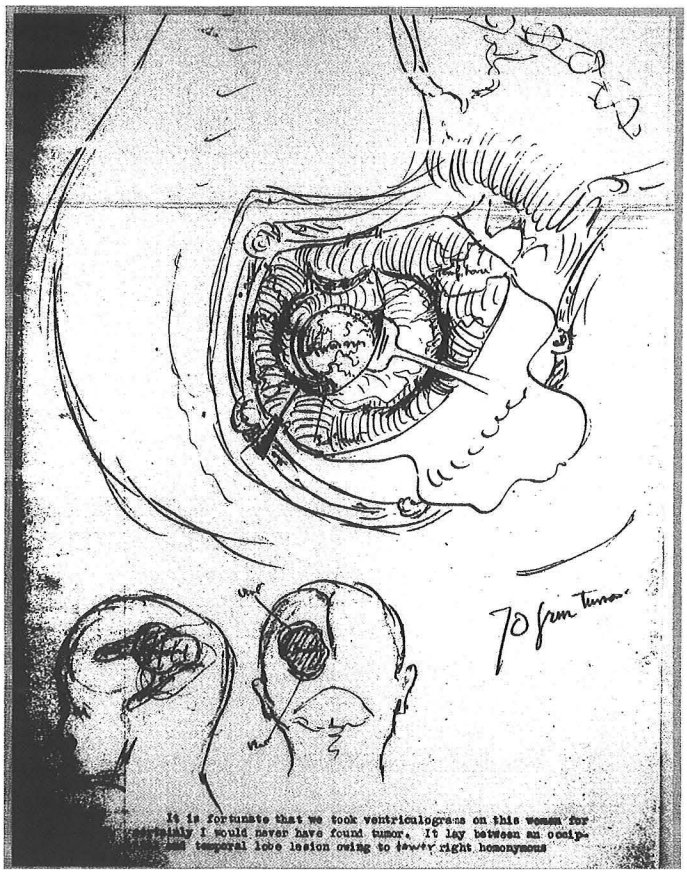
The Management of Glioblastoma Multiforme



Internal Medicine Grand Rounds

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This is to acknowledge that Barry Levinson, M.D. has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Levinson will be discussing "off-label" uses in his presentation.

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Interests include clinical research in a variety of solid tumors including melanoma, renal cell carcinoma, and brain tumors.

INTRODUCTION

It is estimated that in the United States in 2002, there will be 17,000 new cases of primary brain tumors (9600 men, 7400 women) and approximately 13,100 deaths (7200 men, 5900 women) from these disorders. Brain tumors comprise only 2% of all adult cancers but they are among the most debilitating malignant diseases. Brain tumors are responsible for 2% of cancer deaths among women, and less than 2% among men. The estimated annual incidence of new primary brain tumors is 7-17 per 100,000 per year.[1]

Most primary brain tumors arise from neuroectodermal cells, primarily from the glia that support the neurons. Glioma is a general term that refers to all tumors of glial origin. That includes astrocytomas, oligodendrogliomas, ependymomas, and choroid plexus tumors. Glioblastoma is the most malignant form of astrocytoma. In the adult, glioblastoma constitutes approximately 40% of diagnosed brain tumors, and 55% of all gliomas. Glioblastoma remains the most intractable primary brain tumor and constitutes the largest number of cases. It exhibits the poorest survival time and predominates in an older population. It has peak incidence in adults and represents only 6% of primary tumors in patients 20 years old and younger. The relative risk of males compared with females for glioblastomas is approximately 1.6. The five-year overall survival rate for glioblastoma is only 3.4%.[2]

Glioblastoma multiforme typically exists in two forms; as a primary, or de novo tumor, and as a secondary tumor that is derived from a pre-existing, less aggressive glioma, which then progresses to a more aggressive form. Primary glioblastomas tend to occur in older patients, whereas secondary glioblastomas tend to occur in younger patients.

The most important prognostic factors for glioblastoma multiforme are age, histologic grade, and performance status. Even with current surgery, radiotherapy and chemotherapy techniques, the mean survival is very poor, remaining between 9 and 15 months. In a recent review of 22,000 patients in the Surveillance, Epidemiology, and End Results (SEER) data registry, there was a significant decline in survival with age. The median survival time for patients with glioblastoma multiforme diagnoses were 9.9 months for patients under 20 years, 10.8 months for patients 21-64 years, and only 3.5 months for patients greater than 65 years of age.[3]

High-grade gliomas are highly proliferative tumors with both expansive and infiltrating growth. Diffuse astrocytomas are unencapsulated, poorly marginated, and diffusely infiltrate into the surrounding brain. Although they may appear circumscribed on gross and radiographic evaluation, it is difficult to identify the margins of these tumors. Glioblastomas, although originally considered to be tumors of immature precursor cells, or glioblasts, are now generally recognized as poorly differentiated neoplasms arising from transformation of previously normal adult cells. Glioblastomas often contain areas of hemorrhage, necrosis, cyst formation and scarring which provides a varied appearance, thus leading to the term glioblastoma multiforme.

Astrocytomas are predominantly supratentorial involving the cerebral hemispheres. They are diffusely invasive with infiltrating tumor cells that follow white matter tracks accompanied by edema that may facilitate migration. Gray matter involvement is usually minimal outside of cellular foci. Also, subpial spread over the surface of dura, or along blood vessels, is common. Primary brain tumors rarely metastasize outside of the central

nervous system. The malignant manifestations of brain tumors are primarily due to proliferation of the cells within a defined space, that is within the cranium. Morbidity and mortality of glioblastoma can also be significantly influenced by the location of the lesion, which may limit surgical accessibility. Although more extensive extracranial metastases are exceptional, tumor may spread through cerebral spinal fluid with extensive subependymal and subarachnoid dissemination.

The cause of primary astrocytomas is unknown, however glioblastoma multiforme is associated with some specific inherited cancer syndromes including Li-Fraumeni Syndrome, Turcot Syndrome, and Werner Syndrome. Overall, fewer than 5% of patients with brain tumors have such predisposing genetic syndromes.[4]

During the 1980s, the incidence of primary malignant brain tumors was reported to be increasing among all age groups in the United States, while mortality was declining for persons younger than 65 years.[5] An analysis of the SEER data between 1973 and 1985, showed a dramatic rise in the incidence of gliomas in the elderly; 200% for the 75 –79 year age group, 400% for 80-84 year age group, and 500% for those greater than 85.[6]

Figure 1 SEER Data Table [5]

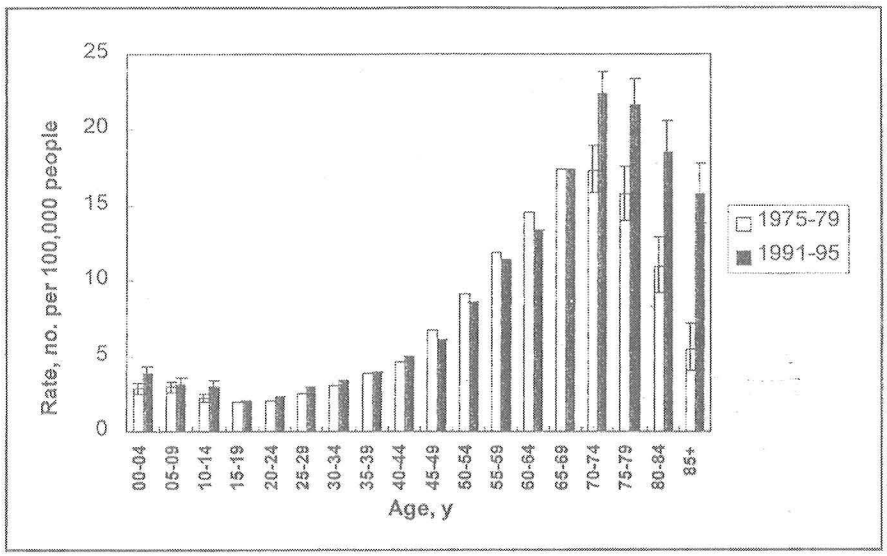


Fig. 1. Age-specific incidence for brain and other central nervous system cancers. Surveillance, Epidemiology, and End Results Program, 1975–1979 versus 1991–1995; 95% confidence intervals are shown for selected age groups.

One commonly cited explanation for the abrupt rise of brain tumor rates among the elderly in the mid-1980s is diagnostic and reporting improvements. Increased use of imaging techniques, including CT, MRI, and stereotactic biopsy, in elderly patients suggests a more aggressive pursuit of diagnosis in these patients. Since the rise in incidence reported in the 1980s, there appears to have been a leveling off of incidence in older patients. An

exception is the over-85 group, which in fact, appears to demonstrate a continued rise in the incidence of high-grade gliomas.

A review of primary malignant brain tumors reported in the Florida Cancer Data System (FCDS) seemed to confirm an increased incidence in primary brain tumors in elderly patients independent of increased case ascertainment.[7] However, one study conducted in Rochester, Minnesota in 1995, did not demonstrate an increased incidence in primary CNS tumors in that population over a 40-year period.[8] Another explanation given for the increasing incidence of primary malignant brain tumors, especially in the elderly, are undefined environmental carcinogens. One author even suggests that the increasing primary malignant brain tumor mortality among elderly in developing countries may be a consequence of differential survival; that is, some intrinsic character of the elderly population has changed and may produce a progressively distinct gene pool with increased susceptibility to cancer.[9]

A firmly established cause of primary brain tumors is ionizing radiation, either after external beam radiation for leukemia, pituitary adenoma, tinea capitis, or meningioma, or with a history of full mouth dental x-rays, particularly at an early age.[10] [11] This is more closely associated with the development of meningiomas and neuromas, and more weakly associated with development of gliomas. Patients who have received external beam radiation therapy for other malignancies, however, have developed aggressive astrocytomas.[12] There are multiple reported cases of patients who have undergone stereotactic radiosurgery for meningiomas or other tumors, who then developed glioblastoma.[13] [14]

One purported etiology of brain tumors is cellular telephone use, causing exposure to low power microwave frequency signals transmitted by antennas on handsets.[15] One case-controlled study attempted to correlate the use of cellular telephones with the incidence of brain tumors, but was not able to demonstrate any increased incidence. However, the authors did indicate that there was limited precision for assessing the risks after a potential induction period of more than several years or among people with very high levels of daily or cumulative use.

Considering other potential etiologies, head injury has not been associated with glioma. Some occupational categories seem to be associated with increased incidence of glioma, such as employment involving high exposure to electric and magnetic fields, polyvinyl chloride, and rubber processing, particularly in the manufacture of tires, as it involves high exposure to N-nitroso compounds. The use of petroleum products did not seem to be associated with increased incidence of glioma.[16] [17]

Epidemiologic studies have tried to establish a link between residential magnetic fields and development of brain tumors. Three of four studies indicated an approximate two-fold increase associated with higher exposure, but the fourth study showed no association.[17]

In one study looking at childhood malignancies related to exposure to extremely low frequency magnetic fields, it was demonstrated that a more accurate association with increased incidence of childhood brain tumors was seen with wire codes, either very low or very high.[18] Much work has been done in the laboratory looking at the effect of extra-cellular electrical gradients exerting their forces on neurons. Pulse repetition rate of radio frequency energies has been shown to be of critical importance in eliciting specific

biological field effects and can cause specific changes seen in animal electroencephalograms.[19]

Weak, extremely low frequency (ELF) magnetic field interactions with neural tumors have been investigated. The potential physical explanation for these interactions, include free radical formation, Faraday induction, ion cyclotron resonance, and magnetite inclusions. Magnetic fields from power lines may affect the general population by producing alternating current magnetic fields that, depending on orientation and ionic species, can be in resonance with the local geomagnetic direct current field.[19] Further studies in the cellular effects of electromagnetic fields, specifically radio frequency electro magnetic radiation, have been well established. There is increasing evidence that electric and/or magnetic fields with frequencies varying from static, or extremely low frequency, to high frequency millimeter wavelength microwaves induced physiologic alterations of unknown causation. There is evidence of direct non-thermal effects of radio frequency radiation on cell proliferation.[20]

One study, which attempted to look at dietary intake and its relationship to gliomas, demonstrated that cholesterol and fat intake actually had an inverse relationship to the incidence of brain tumors. High protein diets seem to have a positive impact, however, the intake of N-nitroso compounds did not have an impact on the incidence of this disease.[21]

Malignant transformation of a cell is a multistage process. it is highly likely that endogenous metabolic processes, rather than environmental factors, are responsible for most brain tumors. With the explosive increase in knowledge of activated oncogenes and inactivated tumor suppressor genes operant in brain tumors, the development of carcinogenesis can be better understood on a molecular level which ultimately can lead to new targets for therapy.

PATHOLOGY

The grading systems currently used for astrocytomas divide astrocytic neoplasms into three or four levels of anaplasia. A greater degree of anaplasia is associated with a worse prognosis. Histologic grading systems for astrocytomas reliably predict tumor behavior. Certain microscopic features, such as hypercellularity, cytologic atypia, mitotic or proliferative activity, microvascular proliferation, and necrosis are associated with tumor behavior and appear sequentially with tumor progression. Gliomas carry a final diagnosis based on the most aggressive histologic component.

Several commonly used grading systems for astrocytomas include the Ringertz/Burger, the St. Ann/Mayo, and the WHO classification schemes as demonstrated below.[22]

Table 1 Comparison of grading systems for astrocytomas[22]

Ringertz/Burger	St. Anne/Mayo	WHO
Grades based on progressive development of anaplasia	Grades based on number of specific histologic features present—atypia, mitoses, microvascular proliferation, and necrosis	Grades based on progressive development of anaplasia. Essentially a modification of St. Anne/Mayo system
Astrocytoma (low grade) Mild atypia and hypercellularity; mitoses rare if present. No microvascular proliferation or necrosis	*Grade 1* No features present (exceptionally rare)	*Grade II/astrocytoma* Atypia and hypercellularity. Mitoses, microvascular proliferation, necrosis absent
	Grade 2 One feature present—atypia	
Anaplastic astrocytoma Moderate atypia and hypercellularity; mitoses usually present and often numerous; microvascular proliferation may be present	*Grade 3* Two features present—usually atypia and mitoses	*Grade III/anaplastic astrocytoma* Increasing atypia. Presence of mitoses required. Microvascular proliferation and necrosis absent
Glioblastoma multiforme Features of anaplastic astrocytoma with tumor necrosis	*Grade 4* Three of four features present—usually atypia and mitoses plus microvascular proliferation and/or necrosis	*Grade IV/glioblastoma multiforme* Increasing atypia and mitotic activity. Presence of necrosis or microvascular proliferation required

WHO = World Health Organization.

Approximately half of GBMs develop as the end stage of a series of genetic changes occurring in astrocytes. Many GBMs arise from progressive transformation from a pre-existing lower grade tumor. Their appearance may be complex because the lesion may be composed of a mixture of different grades of astrocytoma including grade II, grade III, or both. Hallmarks of GBM include microscopic (pseudopalisading) and gross necrosis, frequent mitoses, rich neovascularity, and endothelial proliferation. They may appear localized upon gross inspection but microscopically they are invasive.[23]

The presence or absence of necrosis has been used as a key diagnostic criterion in glioblastoma multiforme. In the past, pathologists have required the presence of tumor necrosis within an astrocytic neoplasm to establish the diagnosis of GBM. According to the new World Health Organization (WHO) system, if a tumor contains endothelial proliferation, the presence of necrosis is not required to establish the diagnosis of glioblastoma multiforme.[23] Similarly, in the St. Ann-Mayo system when the tumor contains nuclear atypia, mitoses and endothelial proliferation, in the absence of necrosis, diagnosis of GBM can be given.[24] One study conducted in San Francisco, reviewed 275 glioblastoma specimens, of which 12% contained tumors diagnosed as glioblastoma multiforme only because of the presence of endothelial hyperplasia. They found that necrosis was a statistically significant prognostic factor in their study group, but the predicted difference in survival was 10.9 months compared to 12.5 months.[25]

The nature of glioblastoma multiforme is to produce neovascularity with vessels that are freely permeable without a blood brain barrier. In many cases, the major component of the tumor mass effect is produced by the surrounding vasogenic edema that develops in the enhancing areas. Infiltration is microscopic so imaging fails to outline the true extent of the tumor. However, most imaging parameters identify the main bulk of the tumor.[22] Two different scenarios account for the frequent finding of neoplastic cells remote from the main

bulk of tumor. First, many glioblastoma mutiformes arise with a pre-existing lower grade diffuse astrocytoma. The field of surrounding neoplastic cells continues to be at risk for transformation to the next highest grade of tumor. Second, even glioblastoma multiformes that arise de novo are noted to send malignant cells streaming into the surrounding brain. Widened extra-cellular spaces created through vasogenic edema facilitate this mode of spread.[23]

MOLECULAR BIOLOGY OF ASTROCYTOMAS

Development of human cancer is believed to result from multiple genetic changes, including the activation of oncogenes and the lack of activation of tumor suppressor genes. Oncogenes encode proteins that stimulate proliferation and mediate biologic activities important for invasion, angiogenesis, immune escape, and other characteristics of malignancy. Tumor suppressor genes encode the genes that function to inhibit cell proliferation and tumor development.[26]

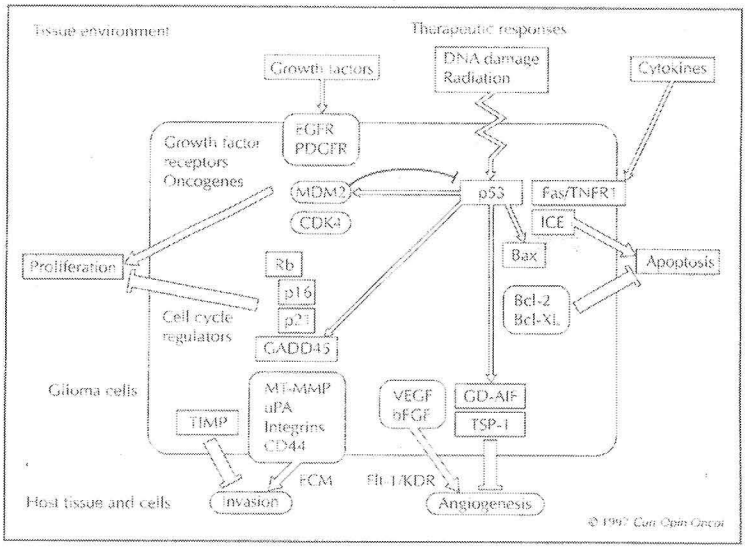


Fig. 2 Functions and interactions of gene products implicated in glioma tumorigenesis is the context of host microenvironment. Factors promoting tumor growth are encircled in rounded boxes, whereas inhibitors are enclosed in quadrilaterals. [28]

There is increased interest in the use of molecular markers as objective standards against which to establish diagnosis and grade. Among many genetic alterations detected in astrocytomas, the loss of loci on chromosome 9p, 10, 17p, and 19q, and amplification of the epidermal growth factor receptor oncogene are the most common. Low-grade astrocytomas have the fewest molecular abnormalities. Loss of heterozygosity on 9p or cyclin dependent kinase CDKN2A occurs more in glioblastoma. Poorer survival has been noted in patients who have a loss of heterozygosity on 9p, deletion of p16, or loss of heterozygosity on 10q. Loss of heterozygosity on chromosome 10 is frequently seen in glioblastoma multiforme, and has been reported to occur in as many as 70% of

glioblastomas.[27] There is evidence for the presence of multiple tumor suppressor genes on chromosome 10.

The p53 tumor suppressor gene located on chromosome 17p has been found to influence multiple cellular functions including progression through the cell cycle, DNA repair after damage, genomic instability, and the tendency for the cell to undergo apoptosis following treatment. It also acts as a transcription factor to induce or repress the transcription of multiple genes through sequence specific interaction with DNA. p53 mutations also mediate the response of tumors to irradiation.

Among transcriptional targets of p53 are p21, which inhibits the formation of cyclin dependent kinase CDK, which promotes cell cycle arrest, and BCL2 associated x protein, which promotes apoptosis.[28]

Another tumor suppressor gene frequently inactivated in astrocytic neoplasms is CDKN2A, which encodes p16. p16 binds and inhibits the function of cyclin dependent kinase CDK4 complexed with cyclin D protein. CDK4 inhibits pRB, the protein product of tumor suppressor gene RB1 located on chromosome 13q, and results in loss of RB1 mediated growth suppression. Inactivation of DKN2 gene located on chromosome 9 appears most commonly to result from deletions of both copies of the gene. This mutation occurs frequently in high-grade astrocytomas. Both p16 and pRB appear to inhibit cell cycle progression through the same pathway. It is possible that defects in both p16 and RB may convey no additional growth advantage to a cell over that resulting from mutation of p16 or RB alone. This is compatible with the observation that most glioblastomas have a defect in either p16 or RB, although both genes are rarely inactivated in the same tumor.[29] Those glioblastomas that do not have mutated p16 or RB have been found to have amplification of CDK4, a third mechanism by which the function of this pathway for cell growth regulation can be inhibited.

MMAC, a tumor suppressor gene also known as p10, or TEP1 and located at 10q, has been found to be mutated in 40% of glioblastomas. These mutations are important in cell cycle regulation, and are seen to arise without evidence of a precursor lesion, as well as arising in those glioblastomas, which occur through changes from a lower grade astrocytoma. Mutation of MMAC is rarely seen in low-grade astrocytomas.[30]

In contrast to tumor suppressor mutations that result in a loss of function of proteins key for the inhibition of cell proliferation, oncogenes result in enhanced function leading to increased cell proliferation. Epidermal growth factor receptor (EGFR) gene is the most frequently found to be amplified in malignant astrocytomas. It encodes a protein that functions as a receptor for epidermal growth factor, which acts as an important growth stimulant for astrocytes.[28]

One model of progression of malignancy suggests that p53 mutations might be linked to the initiation of neoplasia. p16 and RB abnormalities are associated with malignant progression. Alterations of genes located on chromosome 10, might be related to neovascularization.[26]

In looking at the molecular changes that affect de novo, or primary glioblastoma multiforme, versus secondary glioblastoma multiforme, de novo tumors frequently have gene amplification of EGFR and lack p53 mutations. Secondary glioblastoma multiforme show the reverse pattern with no gene amplification, but possessing the p53 gene

mutation, which tends to occur in younger patients. Progressive glioblastoma multiforme arise in patients typically aged less than 50 years whereas the de novo tumors arise in patients greater than 50 years. Primary glioblastoma multiforme are characterized by amplification, or over-expression, of EGFR, homozygous deletion of p16, amplification or over-expression of murine double minute 2 (MDM2), and the entire loss of chromosome 10. In contradistinction, secondary glioblastoma multiforme are characterized by functional loss of TP53 mainly caused by the gene mutations and partial or complete loss of chromosome 10q. Tumor cells carrying p53 mutations are resistant to apoptosis induced by DNA damage, while an over-expression of wild type p53 enhances the radiosensitivity of glioma cells.[31 32]

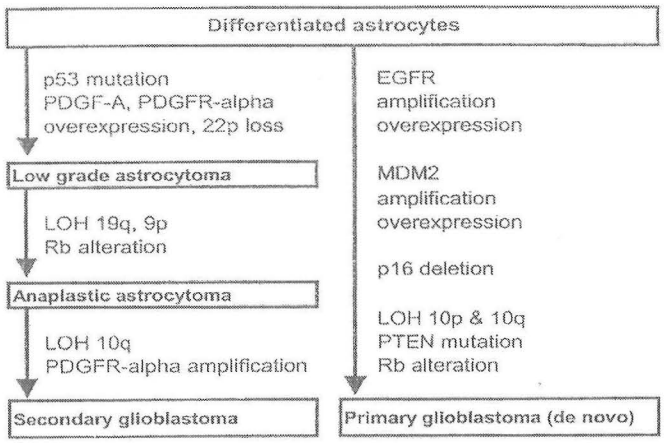


Fig. 3 Summary of major genetic alterations observed at stages of glioblastoma development (modified from ref. 32) PDGFA = platelet -derived growth factor A; PDGFR-alpha = platelet-derived growth factor receptor-alpha; EGFR = epidermal growth factor receptor; LOH = loss of heterozygosity; Rb = retinoblastoma; MDM2 = mouse double minute 2; PTEN = phosphatase and tensin homologue

In one study reported by Shiraishi, there appears to be no marked effect of the p53 gene mutation on glioblastoma multiforme patient survival.[33] Of note, the MDM2 oncogene, whose product degrades and inactivates p53, is amplified and over-expressed in approximately 10% of glioblastomas, particularly in primary glioblastoma multiforme with intact p53. Amplification of MDM2 leads to the inhibition of the tumor suppressor effects of p53.

Loss of heterozygosity on chromosome 10 is the most frequent genetic abnormality in human gliomas. Several possible genetic abnormalities on chromosome 10 may be most responsible. In a report from Tada, et al. (2001) looking at this specific question, it appears as though loss of heterozygosity on Heparin/MMACI is a significantly unfavorable prognostic factor for patients with glioblastoma multiforme.[27]

Phosphatidyl inositol 3-kinase (PI-3K), and the phosphatase and tensin homologue (PTEN), regulate tumor cell invasiveness.[34] These lipid signaling molecules might play a role in regulating matrix metalloproteinases (MMPs). Astrocytes must go through multiple

steps of cellular and molecular alteration before glioblastoma develops. Astrocytomas are capable of moving throughout the brain by a complex process of modulation of the surrounding extra-cellular matrix. This involves the secretion of various proteinases, including metalloproteinases, which allow for invasion of surrounding tissue.[35] Expression of matrix metalloproteinases have been postulated to play a central role in brain tumor invasion, with activation of p38 MAP kinase, MMP, and tissue inhibitors of metalloproteinases.

Gliobastoma is characterized by a marked proliferation of neovascularization. The profusion of blood vessels is a histologic hallmark of disease. This process is promoted by induction of angiogenesis. The molecular mechanisms underlying angiogenesis have only partly been understood. Vascular growth factors released by tumor cells act on endothelial cells in a paracrine manner to promote tumor neovascularization.[36]

Goldman, et al (Molecular Biology of the Cell), reported a relationship between the presence of vascular endothelial growth factor (VEGF) and EGFR in human glioma cell lines and glioblastoma tumor surgical specimens.[37] EGF stimulation of glioma cells increases the secretion of bioactive VEGF. This helps to explain glioblastoma tumor angiogenesis, increased vascular permeability, and cellular proliferation. During glioblastoma multiforme development, there is a 40-fold up regulation in angiogenesis in the brain and a 50-fold overexpression of VEGF with an endothelial cell proliferation rate of 12.5%. VEGF released by glioma cells in situ most likely accounts for pathonomonic, histopathologic, and clinical features, including striking tumor angiogenesis, increased cerebral edema, and hypercoaguability manifested as focal tumor necrosis, deep vein thrombosis, or pulmonary embolism. Other growth factors thought to be involved with angiogenesis in glioblastoma include platelet derived growth factor B (PDGFB), and transforming growth factor B (TGFB).[38]

The neoplastic conversion of human endothelial cells is a multi-step process in which reactivation of the enzyme telomerase (usually inactivated in somatic cells) is a key factor in the establishment of immortalization. Human gliomas are known to express telomerase, which is frequently associated with malignant tumor progression. Telomerase is a ribonucleoprotein containing an RNA template that synthesizes telomeric DNA. Cells with this activated enzyme appear to escape from progressive telomeric shortening and acquire an indefinite growth potential. In a study by Nakatoni, et al (1997), telomerase activity was examined in brain tumor cases, 20 of which were glioblastoma multiforme.[39] Twelve of these cases demonstrated telomerase activity, which tended to correlate with the patient's prognosis, suggesting that it may be an important marker in brain tumor malignancy.

In looking at other markers of tumor aggressiveness, another molecule that has been looked at is metallothionein (MT), from a family of low molecular weight intracellular metalloproteins. Metallothionein has a high affinity for heavy metal ions such as zinc, cadmium, copper, mercury, and platinum that play a role in the absorption, metabolism, and storage of essential trace elements and detoxification of toxic metals. The members of this family are predominately intracellular protein thiols, which appear to be involved in cisplatin resistance. Hiura, et al, identified MT immunohistochemical staining in 66% of astrocytic tumors with a significantly higher MT positivity rate in high-grade astrocytomas compared with low-grade astrocytomas.[40] In fact, glioblastomas show the highest MT expression.

BRAIN TUMOR IMAGING

On radiographic evaluation, enhancement is defined as the increased difference in an imaging characteristic between a lesion and surrounding normal tissue after the administration of a contrast agent. This facilitates the detection of many brain tumors and can help distinguish some tumors from adjacent normal brain. On computerized assisted tomography (CT), the imaging characteristic evaluated is x-ray attenuation, and on magentic resonance imaging, MRI, it is electromagnetic signal intensity. In the case of both CT and MRI imaging, the main mechanism of enhancement of brain tumors is passive contrast material into the extra-cellular spaces of the tumor. The passage is permitted by a defective blood brain barrier of the tumor vasculature. In normal brain, the contrast material cannot move from intravascular compartment to extra-cellular spaces, because the blood brain barrier is intact.[41]

On imaging studies, glioblastoma often presents with characteristic appearance. There is usually a solitary, deep heterogenous ring-enhancing lesion with extensive surrounding vasogenic edema. The central necrosis that is so common in these tumors does not enhance. It is surrounded by living tumor, thus the prominent bright enhancement on MRI and CT. Hemorrhage, which is more common in glioblastoma multiforme than in other glial tumors, also contributes to the heterogeneous appearance. Imaging features that suggest a neoplastic cause of cerebral hemorrhage include the presence of an incomplete hemosiderin rim around the hematoma, vasogenic edema that is out of proportion to the volume and age of the hemorrhage, and edema that does not decrease in volume over time.[42]

The most common feature of the enhancing ring is the irregularity, with a wide rind that varies in thickness and has an irregular or shaggy inner margin. These lesions extend into or through the commisure of the corpus callosum in almost 75% of the cases. Glioblastomas characteristically cross the corpus callosum resulting in a butterfly distribution with bihemispheric involvement. Tumor can spread along the leptomeningial endura, the subpial space, across white matter pathways, and along the ependyma.[42]

The goals of pre-therapy imaging include determining the extent of the lesion, identifying the structures involved, and identifying complications resulting from the tumor. Three common complications are hemorrhage, herniation and hydrocephalus. Imaging also helps to establish a differential diagnosis in providing preliminary non-invasive assessment of lesion grade. As a rule, non-enhancing lesions tend to be low-grade whereas enhancing lesions are commonly malignant. Notable exceptions exist such as pilocytic astrocytoma, which typically has an enhancing mural nodule.[43]

The goals of imaging related to therapy are to provide sufficient anatomic information so the safest operative approach can be selected, and to help define treatment margins for surgery and radiotherapy. Regardless of the protocol used for treatment planning, it is important to recognize that the infiltrative growth of many glial tumors makes determining the true extent of the tumor nearly impossible with anatomic imaging techniques. The enhancing margin does not define the edge of the tumor infiltration. Enhancement is almost universal, and it is also commonly heterogeneous. Approximately 5% of GBMs are multicentric. Subarachnoid seeding has also been described in 5% of cases.[44]

CT contrast agents have a high concentration of the element iodine, which has a much higher atomic number and therefore higher electron density than elements composing soft tissues in the body. The tumors appear as a conspicuous bright lesion after contrast administration.

CT imaging usually is more rapid than MRI. It is a faster modality and therefore is the study of choice for scanning critically ill or medically unstable patients. It is also superior for detecting areas of calcification in a hyperacute hemorrhage and for evaluating calvarial skull base changes related to a tumor. In contrast, MR imaging has superior contrast resolution making it better for detecting small lesions. Overall, the advantages of MRI outweigh those of CT for brain tumor imaging in most patients. Contrast enhanced MRI is the imaging method of choice for the diagnosis and follow-up of brain tumors in the absence of such contraindications as pacemakers, ferrimagnetic aneurysm clips, metallic foreign bodies in the eyes, or cochlear implants. MRI imaging is more sensitive than CT in the detection of gliomas, in the assessment of tumor extent, and for identification of potential complications including herniation syndrome, venous thromboses, leptomeningial and ependymal spread.[42]

Gadolinium based MR contrast agents, in addition to shortening the longitudinal relaxation time, T1, also facilitate proton relaxation in the transverse plane with the result in shortening of the transverse relaxation time, T2. This T2 shortening theoretically may result in a perceptible decrease in signal intensity as opposed to the increased intensity created by T1 shortening on T1 weighted images. Evaluation of fluid attenuated inversion recovery, or flair sequences, for the demonstration of enhancement initially failed to show any benefit over T1 weighted sequences for lesion detection. A more recent investigation has shown that the degree of enhancement on post-gadolinium flare images is often equal or greater than on T1 weighted images. On MR studies, GBMs are typically heterogeneous in signal intensity on both T1 weighted images and T2 weighted images and usually contain both regions of solid tumor and regions of necrosis.[45]

In general, the presence of contrast enhancement and hemorrhage correlate with increasing grade of tumor, however, the presence or pattern of contrast enhancement or degree of T2 prolongation cannot be used to grade these lesions. It has been well recognized that regions of "normal appearing brain" in patients with infiltrative or anaplastic astrocytomas in glioblastoma multiformes can harbor malignancy.

POST-THERAPY IMAGING

Post surgical imaging is used to identify any complications and to determine whether residual of recurrent tumor is present, although such images may be difficult to interpret. Gadolinium enhanced MR imaging is the evaluation of choice for monitoring brain tumor status after therapy. A general principle of the interpretation of post treatment studies is that newer increasing areas of contrast enhancement are worrisome for recurrent tumor, particularly if tumor enhancement was present before tumor treatment.

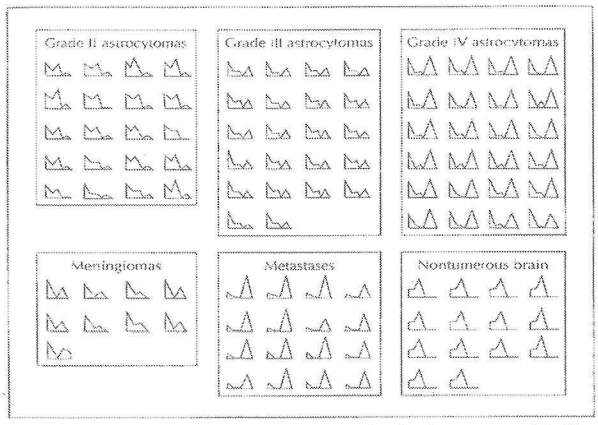
After surgery, however, highly vascularized scar tissue develops at surgical margins. This scar tissue enhances making clear differentiation from tumor very difficult if not impossible.

Immediate postoperative contrast MR imaging is not routinely performed, because of potential confounding factors. Radiation therapy including external beam radiation therapy, interstitial brachytherapy, and stereotactic radiosurgery have been key components in the treatment of brain neoplasms. Differentiation of viable tumor versus radiation necrosis is impossible on the basis of contrast enhancement alone, because both clearly enhance in most circumstances. Radiation therapies inevitably result in delivery of radiation to normal brain tissue. This non-target radiation may lead to deleterious effects on normal brain, including gliosis, demyelination, and radiation necrosis.[46] Enhancing lesions representing radiation necrosis appear 3-30 months after radiotherapy. The volume and number of enhancing lesions may be increased over a period from 3-23 months.

Various methods have been used in association with contrast enhanced MRI in an attempt to increase specificity. PET with flurodeoxyglucose, used for enhancing lesions on MRI imaging, requires a precise special localization technique. Dynamic enhanced contrast MRI has been used to evaluate patients with malignant gliomas to predict outcome. Progression of contrast enhancement was traced by imaging the tumor every 15 seconds during and after bolus contrast injection. Maximal rate of uptake and delayed rate of uptake were calculated.[43]

Radiographic imaging evaluation for determining response to treatment can be misleading in that post-radiation changes can be difficult to distinguish from tumor growth in both CT scanning and MRI evaluation. More accurate measures of disease response are being evaluated. Magnetic resonance spectroscopy (MRS) is a technique that does not require the administration of a contrast agent. It is based on the biochemical aspect of cell metabolism and can distinguish normal brain cells from tumor cells and from necrosis. There is a series of chemical markers that are measured, including choline, NAA (N-acetyl aspartate), creatinine, lipid, and lactate. In normal cells NAA provides the largest signal. This metabolite is localized in neurons and absent in glial cells.[47] In brain tumors, malignant cells replace normal cells and do not contain NAA. Thus, there is an abnormal MRS signal. Malignant lesions tend to have an elevation in the choline signal. Lipid peaks are seen in areas of necrosis. Another modality used in the study of brain tumors is photon emission computed tomography, SPECT.[48]

Fig. 4 Resonance profiles for each of the individual MRS scans classified according to tissue type



The six possible peaks in each pattern correspond to the six average metabolite resonance intensity ratios for each tissue sample. From left to right, the peaks refer to the relative amount of Cho, Cr, NAA, Ala, Lac and Lip. Not all metabolites are present in each tissue type.
From [14], with permission.

CLINICAL ASPECTS OF GLIOBLASTOMA MULTIFORME

Brain tumors, including associated edema and hemorrhage, alter the structure and function of the normal brain. The location, growth pattern and growth rates determine the effects on the brain. Brain tumors produce clinical change through either regional parenchymal, or diffuse intracranial effects. Regional parenchymal effects of brain tumors include compression, invasion, and destruction of surrounding brain; hypoxia, either arterial or venous, competition for nutrients, altered transmitter metabolism, electrolyte concentration, and spread of cytokines and free radicals alter the cellular environment disrupting normal neural and glial cell functions. Irritation, for example, focal seizures or depression, and neurologic defects of neuronal function may result. Tumors may occlude arteries with the resulting infarction causing neurologic dysfunction. Elevated intracranial pressure mediates many clinical effects on brain tumors. A rise in intracranial pressure results from the volume of the tumor or from blockage, cerebro-spinal fluid,CSF, or venous flow. Tumor induced vasogenic edema increases the volume of the affected brain. Tumors can block CSF flow when they extend intraventricularly or when the extrinsically distort the contours of the ventricles. They can block the CSF pathways at the fourth ventricular foramina, the basal cisterns, or the cerebral convexities. [49]

Clinical presentation typically involves a focal neurologic sign or generalized symptoms related to increased intracranial pressure. Signs and symptoms of intracranial pressure include headache, typically more severe in the morning, nausea, vomiting, and visual disturbances, including 6th nerve palsy. In glioblastoma multiforme, these signs can develop rapidly and are progressive. Most of the neoplasms tend to develop and grow in the deep white matter, and can be clinically silent until achieving a relatively large size. Patients who present with focal neurologic signs or seizures tend to have a somewhat better prognosis due to an earlier presentation, as compared to those who present with signs of increased intracranial pressure.

The mean age for a patient presenting with glioblastoma multiforme is 54 years. The most common initial imaging finding is a single large supratentorial contrast enhanced lesion seen on MRI. A short history of neurologic findings is usually noted before diagnosis. High-grade gliomas tend to have more rapidly progressive symptoms. Focal signs and symptoms such as hemiparesis and aphasia reflect intracranial location of the tumor. Frequency and duration of symptoms vary with the type of tumor.[50]

Headache is a common symptom in brain tumor patients, occurring in approximately half of patients. Headaches are usually diffuse, but can accurately indicate the hemisphere in which the tumor is located. They are commonly most significant on awakening in the morning and usually resolve in several hours. They can be unilateral and throbbing and can mimic migraines or cluster headaches.[51]

It is estimated that 10-40 percent of brain tumor patients present with seizures. The seizures are usually focal but may become generalized and cause a loss of consciousness. "Todd's paralysis" is post-ictal hemiparesis or aphasia and may indicate the location of the tumor. Hemiparesis or aphasia not associated with seizures typically have a subacute onset and are progressive. In cases of slowly progressive onset, patients may have an accident before recognizing the presence of a visual field defect.[50]

Peritumoral edema contributes significantly to symptoms associated with the progression of the disease. The edema results from absence of tight endothelial junctions in tumor

blood vessels and the production of factors such as endothelial growth factor, which increase the permeability of those vessels. Corticosteroids are an important part of therapy for brain tumors. They cause decrease in swelling typically in 24-48 hours and usually peak within a week. They provide a significant reduction in brain tumor volume and an even greater reduction in the peritumoral edema. Reversal of increases in permeability of the capillary endothelial cell junctions of the blood brain barrier and stabilization of lysosomal membranes are proposed mechanisms. When there is significant intracranial pressure and mass effect, other measures may be required until corticosteriods have had a chance to take effect or until the patient can undergo a debulking procedure. These include elevation of the head of the bed, fluid restriction, and hyperventilation. Mannitol, a diuretic, given as a bolus 0.25 – 0.5 grams per kilogram every 4-6 hours can also acutely reduce symptoms associated with peritumoral edema.[52]

The most commonly used corticosteroid is dexamethasone because it has little mineralocorticoid activity. It may also be associated with a lower risk of infection and cognitive impairment. The loading dose is usually 10 mg followed by 16 mg a day in two to four divided doses. If lower doses are inadequate, the dose may be increased up to 100 mg a day. Multiple toxicities can be associated with the persistent use of corticosteroids. These toxicities are minimized by reducing the dose as much as possible. Gastrointestinal complications, steroid myopathy, and pneumocystis carinii pneumonia are the most commonly reported toxicities.[53]

The risk of gastrointestinal bleeding or ulceration appears to be low when corticosteroids alone are used. However, the incidence increases significantly when they are used in conjunction with nonsteroidal anti-inflammatory drugs.[54] Steroid myopathy is a significant complication of steroid therapy, with the largest series indicating an incidence of 10.6%. It is more common after prolonged use of high doses of corticosteroids, and in the elderly. It is characterized by subacute onset of proximal muscle weakness and wasting. Serum creatine phosphokinase (CPK) levels are usually normal. It is unclear how steroids exert their effects, although it may be through the inhibition of protein synthesis and increased protein metabolism, or possibly through the induction of glutamine synthetase activity. It appears to be less common in patients taking phenytoin possibly as a result of induction of hepatic metabolism of dexamethasone. Myopathy can be prevented only by using the lowest possible dose of corticosteroids. Treatment of myopathy is difficult and limited to physical therapy and reduction in steroid dose.[55]

Patients who are on prolonged steroid therapy because of brain tumor treatment are at some increased risk of developing pneumocystis carinii pneumonia (PCP). In one review of 587 brain tumor patients at Sloan Kettering hospital between 1981 and 1989, 11 cases of PCP were seen, giving an incidence of PCP of at least 1.7%. One needs a high level of suspicion if any pulmonary symptoms develop in such patients.[56] In light of the toxicities associated with steroids, alternative therapies for peritumoral edema are being explored, such as corticotropin releasing factor, and cyclooxygenase 2 inhibitors. It is possible that inhibitors of VEGF may be helpful in reducing edema.

Between 20 and 62 percent of patients will experience seizures during the course of their illness. These patients need to be treated. Commonly used antiepileptic drugs are appropriate in the treatment of brain tumor induced seizures. Patients with primary brain tumors also seem to have a higher incidence of adverse affects related to the antiseizure medication. Up to 23.8% have been estimated to do so. They appear to be more

susceptible to drug-induced rashes from phenytoin and carbamazepine. They also have a higher incidence of shoulder-hand syndrome in association with phenobarbital.[57]

There is some controversy in the literature regarding use of antiepileptic drugs in brain tumor patients who have not had seizures. Often it is thought that because they have had a craniotomy, antiseizure medication needs to be given. Although, in at least one small prospective randomized study, using prophylactic antiseizure drugs in patients with brain tumors, the seizure rate (26%) between those taking antiseizure drugs and those not taking medication suggested that prophylactic antiseizure drug therapy was not beneficial. This issue has been studied more thoroughly in patients with metastatic brain lesions. It appears as though antiepileptic therapy does not change the seizure rate in patients who had not had seizure activity.[58]

Venous thromboembolism is another common problem seen in patients with brain tumors. The incidence of symptomatic deep venous thrombosis or pulmonary emboli in patients with high-grade gliomas, outside the perioperative period, is approximately 20%. It is generally increased in patients in the post-operative period or in patients with hemiplegia, although the incidence varies between different studies from 3-60%.[59] The etiology of this event is uncertain. It may be that the release of procoagulants and fibrinolytic inhibitors from tumor and surrounding cerebral tissue resulting in a state of low grade DIC. Other contributing factors would include venous stasis and hemiparetic limbs, older age, larger tumor size, chemotherapy, and hormonal therapy. High-dose corticosteroid therapy may also have a prothrombotic effect. With the high risk of developing venous thromboembolism, brain tumor patients undergoing surgery require adequate prophylaxis. Currently low molecular weight heparins appear to be the most commonly used intervention. Compressive stockings are also required.

Most current studies suggest that both low molecular weight heparin and unfractionated heparin are safe to use in such settings. In the past, concerns about intracranial hemorrhage led to most patients with venous thromboembolism to be managed with inferior vena cava filters rather than anticoagulation. Several retrospective studies have suggested the risk of intracranial hemorrhage in anticoagulated patients, outside the immediate post-operative period, may not be significantly increased over patients with other malignancies. One study demonstrated a 2% frequency of intratumoral hemorrhage in patients with high-grade glioma receiving anticoagulation for venous thrombosis.[60] This is similar to frequency of hemorrhage events in patients without venous thrombosis who are not receiving anticoagulants.

Brain tumor patients with venous thromboembolism treated with inferior vena cava filters also experience a high rate of complications. In 42 patients with placement of high inferior vena cava filters, 12% experienced recurrent pulmonary emboli and 57% developed either inferior vena cava or filter thrombosis, recurrent deep venous thrombosis, or post-phlebitic syndrome. Filter complications also occur frequently in patients with other neoplasms if concomitant anticoagulation is not used. Initially, inferior vena cava filters reduce the risk of pulmonary emboli, but ultimately patients are still at risk of developing recurrent deep venous thrombosis.[61]

There is currently no data available to indicate if long-term use of low molecular weight heparin is more effective or safer than oral anticoagulant therapy in patients with brain tumors. Patients with malignant brain tumors probably require treatment indefinitely.

SURGERY

Surgical resection has been a mainstay of therapy in the treatment of patients with glioblastoma multiforme, and generally it has been believed that the greater the size of the resection the better the outcomes. However, several reviews of the literature have revealed that there is little evidence to suggest that aggressive surgical management significantly prolongs survival. In part, this may be related to the ways in which surgical extirpations have been described, that is, gross total resection, sub-total resection, partial resection, and biopsy sampling. It is the lack of precise measurement of these quantities that limits clarification of the data.[62]

The goal of surgery for most brain tumors is maximum removal of tumor without inducing new neurologic defects. Many, but not all, retrospective series suggest that extensive resection can improve neurological condition and prolong survival of anaplastic astrocytomas. Brain tumors often arise in or involve eloquent tissue and because they frequently grow in an infiltrative fashion many brain tumors cannot be completely resected without leaving the patient with significant functional impairment.

A recent review of 416 consecutive glioblastoma multiforme patients, examined both pre- and post-surgical MRIs and looked at survival based upon the percentage of tumor removed. It was noted that if patients had a gross total resection of 98% or more of tumor, the median survival time was longer than those who had resections of less than 98%. In patients who had a 98% resection of their tumor volume, the mean survival was 13 months, and in those who had a less than 98% resection of tumor volume, the mean survival was 8.8 months. It was also found that in addition to age and preoperative Karnofsky performance status score, the degree of necrosis and enhancement on preoperative MR studies were significantly associated with survival in patients with glioblastoma multiforme.[63]

Stereotactic biopsy is employed to provide a histologic diagnosis in cases in which the benefits of surgical resection or debulking are uncertain or outweighed by the risks of these procedures. Frame base biopsy allows a histologic diagnosis of most neoplastic intracranial lesions. Accuracy is limited by neuroimaging because the mechanical accuracy of frame-based stereotactic surgery is often less than a millimeter.[49]

The choice of open operation versus biopsy depends on the location, size, gross characteristics, extent of demarcation, consistency in vascularilty, probable histology, and radiosensitivity of the tumors, as well as on the neurologic and general medical condition of the patient. The role of surgery is to provide tissue for histologic diagnosis, guiding further therapy and predicting prognosis, and to remove tumor mass. The rationale for open craniotomy depends on the need for immediate palliation of symptoms by reduction of intracranial pressure or focal mass effect or improved oncologic control. For rapidly growing tumors unresponsive to anticonvulsants or corticosteroids, surgery represents an important option. Improved tumor control arises from tumor removal, which may delay recurrence or enhance adjuvant therapy when total resection is not possible because of local or diffuse tumor infiltration. Partial resection may still provide a survival advantage relative to biopsy alone.

Generally in the past, patients who were referred for stereotactic biopsy as opposed to more extensive resection, either had deep-seated tumors that were inaccessible to surgery, were older, or had a poor Karnofsky performance status.

Kreth, in a retrospective review of cases treated between 1986 and 1991, compared the results of stereotactic biopsy followed by radiation therapy in 58 patients with those of surgical resection plus radiation therapy in 57 patients.[64] The stereotactic biopsy patients were inoperable. The median survival time for the resection group was 39.5 weeks as compared to 32 weeks for the biopsy group. This difference was not significant. The most important factor was patient age. In patients with midline shift who underwent biopsy, there was a greater decline in performance status. Clinical status six weeks after surgery, however, showed no significant differences between the two groups. The author concluded that decompressive surgery followed by radiation therapy should be performed whenever necessary for severe space occupying lesions and midline shift when it will not cause new neurologic deficits.

Reoperation is often undertaken in patients who have recurrent brain tumors. The rationale is the same as that of the original operation, reduction of tumor mass without causing a new neurologic deficit. It may also confirm tumor histology demonstrating a progression of aggressiveness of tumors. When considering repeat surgery, quality of life is an important endpoint. In one study of 70 patients, the median survival after reoperation was 36 weeks in patients with glioblastoma multiforme and 88 weeks with those with anaplastic astrocytoma. The median duration of high quality survival after reoperation was 10 weeks for patients with glioblastoma multiforme and 83 weeks for patients with anaplastic astrocytoma. The operative mortality rate was 5.7% and the six-week post-operative mortality was 4.3%.[65]

RADIATION THERAPY

Classically, malignant brain tumors have been irradiated with external photon beams delivered in various fractionation schemes designed to limit damage to normal cells while maximizing tumor cell kill. Photons are cytotoxic because of ion-induced damage to cellular enzymes. Late G1 and early S phases of the cell cycle are when DNA is most radiosensitive. Synchronization of the cell cycle maximizes radiation damage. An important part of this process is the generation of oxygen-dependent free radicals. Oxygen also inhibits cellular repair of radiation-induced damage.[66]

The value of conventional radiation therapy as an adjuvant to surgery in the treatment of malignant glioma was established conclusively by a randomized study reported in 1978. This showed a median survival of 14 weeks with operative treatment alone, and of 35 weeks with operative treatment followed by tumor irradiation in a dose of 50 to 60 gray (Gy).[Walker, 1978 #90 The dose dependency of the effective radiation therapy has also been established by randomized study: 56 patients irradiated with 50 Gy to the whole brain had a median survival of 28 weeks; 270 patients receiving 60 Gy had a median survival of 42 weeks.[67]

Typically in the radiation therapy of malignant gliomas, a single dose fraction will be used each day until a dose of approximately 60 Gy is delivered. Various strategies have been used to increase the effective biologic dose, including hyperfractionated and accelerated fractionation techniques which include more frequent dosing schedules. However, alteration of dose delivering schedule has not produced any significant improvements in disease control or survival in most patients in Phase III trials. It appears that cone down therapy is as successful as whole brain therapy. There is active investigation of more

focused types of therapies including peacock systems and radiosurgery techniques. Placement of the radioactive source directly in the tumor bed, i.e. interstitial brachytherapy, is also being studied.[49]

Another focus of investigation in patients treated primarily with radiation therapy is possibly shortening course of radiotherapy so that a patient does not require many weeks of treatment, especially when the life expectancy is short. Most patients receive approximately 60 Gy of radiotherapy over six weeks. A shorter course of treatment such as 37.5 Gy of radiotherapy in 5 fractions for 5 days a week or 36 gray in 12 fractions over the course of three weeks have been studied. In older patients with poor prognosis, it appears as though the overall impact on survival is comparable to that for the more extended course of treatment. However, this is not recommended for patients who could have a more prolonged survival and are patients with a better prognosis. There is very little data regarding long-term adverse neuropsychiatric effects of the radiation therapy in patients with glioblastoma.

Local tumor control is the major problem with recurrence and progression of glioblastoma multiforme. Therefore, ways of delivering more intense therapy to the local regional site has been evaluated in many centers. Once such report from Massachusetts General Hospital looked at dose escalation to 90 Gy equivalent after conventional doses of 55-65 Gy of fractionated irradiation.[68] Twenty-three (23) patients were enrolled in this trial. Actuarial survival rates of two and three years were 34% and 18%, respectively. The median survival time was 20 months with four patients alive at 22-60 months post-diagnosis. Radiation necrosis was only demonstrated in seven patients who had new areas of gadolinium enhancement during follow up. Tumors seem to recur in areas immediately peripheral to the 90 Gy volume, but attempts to extend local control by enlarging the volume are likely to be limited by difficulties with radiation necrosis.

Barker, et al (1996) tried to determine the value of radiographically assessed response to radiation therapy as a predictor of survival in patients with glioblastoma multiforme.[69] Radiation response and survival rates were assessable in 222 patients. The extent of resection and the immediate response to radiation therapy were highly correlated with survival.

Neurologic deficits that occur independently of tumor progression during or after radiation therapy include acute edema during or within two weeks of completion of radiation therapy. Steroids and delay of therapy readily control the acute edema. Subacute demyelination occurs from 6 to 12 weeks after completion of radiation and delayed necrosis occurs 4 to 40 months after completion of irradiation. The delayed necrosis may be progressive, irreversible and often fatal. It is caused by the progressive enlargement of a necrotic mass arising within the tumor and surrounding irradiated brain.

Stereotactic radiosurgery (SRS) has been used in the treatment of gliomas although improved outcomes have not definitively been demonstrated for radio surgery as primary therapy or in the adjuvant setting. In one study evaluating the additional benefit of stereotactic radiosurgery in patients with glioblastoma multiforme, conducted by Shrieve, et al, 78 patients were evaluated who had previously undergone surgery or biopsy and then received conventional external beam radiation therapy.[70] Subsequently patients received SRS as a radiographic boost. The median length of actuarial survival for all patients was 19.9 months. Twelve (12) and 24 month survivals were 88.5% and 35.9%. Twenty-three (23) patients aged younger than 40 years had a median survival of 48.6 months compared

with 55 older patients who had 18.2 months. Of these patients, 50% underwent reoperation. It may be that younger patients derive the greatest benefit, but also patients who are in good performance status might have a superior survival if SRS is given in addition to best surgical extirpation followed by conventional radiation therapy.

Maschipinto, looked at 31 patients who underwent tumor debulking or biopsy, stereotactic radiosurgery, and standard radiation therapy as part of their primary treatment to determine the value of radiosurgery in glioblastoma multiforme.[71] The actuarial 12-month survival was 37% with a median survival of 9.5 months. There were 31 patients with a median age of 57 years, 22 men and 9 women. The values were similar to previous results for surgery and standard radiation therapy alone suggesting that the additional value of radiosurgery upfront was limited.

Highly focal delivery of high doses of radiation can be achieved with brachytherapy which involves the placement of radioactive implants or the installation of radioactive solutions or suspensions into preexisting cavities. The concept of brachytherapy is not new. Harvey Cushing, who performed over 800 operations for gliomas, implanted radioactive radium needles, known as a "radium bomb," in a small number of those patients. He was not impressed with the results and did not pursue this course of action in a serious way.[72] Interstitial brachytherapy is possible with stereotactic procedures, however, the late radiation injury may cause symptomatic necrosis with 40-55% of patients requiring surgical removal of necrotic brain tissue from 6 months to 2 years later.

A brachytherapy trial from Amsterdam examined patients treated with iodine 125 or iridium 92 seeds in addition to conventional radiotherapy. Those who received iodiine 125 seeds received 50 Gy, whereas those who received iridium 192 received 60 Gy, of radiation before implantation. The median survival was 16 months for both types of therapy, which was six months longer than in a matched control group.[73] Another study looking at neutron brachytherapy, conducted by Patchell, et al (1997), utilized californium 252 as the sole source of radiation, but patients developed severe radiation necrosis of the brain, as well as scalp necrosis when the dose was escalated to 1300 Gy.[74]

The boron neutron capture therapy (BNCT) reported by Perks, et al, describes the technique involving preferentially attaching B10 atoms to tumor cells and irradiating them with thermal neutrons.[75] The thermal neutron capture products of B10 are short range and highly damaging, so they kill the tumor cells, but healthy tissue is relatively undamaged. Early trials required extensive neurosurgery to expose the tumor to thermal neutrons and were unsuccessful. It was thought that intermediate energy neutrons would overcome many of the problems encountered in the early trials because they have greater penetration prior to thermalization. BNCT has also been reported in a recent article from Japan. Intraoperative therapy requiring neutrons from a nuclear reactor was delivered during craniotomy. This neutron could not be adequately shielded and it caused damage to electrical and metal devices in the operating room.[76]

CHEMOTHERAPY

The goal of adjuvant chemotherapy in malignant gliomas is different from that of adjuvant chemotherapy in other types of malignancies. In most cases, adjuvant therapy is delivered after all evidence of disease has been eradicated, and it is an attempt to prevent the

development of metastatic disease. In dealing with brain tumors, typically there is residual disease and the intention is to try to improve local control.[77] One of the main obstacles to the successful eradication of gliomas is the fact that the boundaries of the tumors are difficult to define pathologically. These are diffuse diseases of the nervous system and malignant cells can be present distally from the edematous brain. Local failure is the problem in the vast majority of cases, and most people die from local recurrence even with the most intensive therapies.

The role of chemotherapy in the treatment of glioblastoma multiforme is not well established. These tumors are often resistant to the chemotherapy agents currently available. A large analysis of more than 3,000 patients looking at 16 studies linking chemotherapy in astrocytomas demonstrated that very few patients with glioblastoma received any benefit from chemotherapy. Those patients who benefited were generally younger with good performance status and minimal residual disease post-surgically. Anaplastic astrocytomas, primary brain tumors with an oligodendroglioma component, mixed oligodendroglial tumors, and anaplastic oligodendrogliomas are very often chemosensitive with the most commonly used regimen for oligodendrogliomas being procarbazine, lomustine (CCNU), and vincristine.[78]

A study conducted by the Northern California Oncology Group looked at post radiation chemotherapy in 133 patients, 73 with anaplastic astrocytoma and 69 with glioblastoma multiforme, using either single agent carmustime (BCNU) or the drug combination of procarbazine, CCNU, and vincristine (PCV). The study reported that patients with anaplastic astrocytomas treated with PCV chemotherapy after radiation had a median survival of 157 weeks as opposed to 87 weeks for those using BCNU alone. In patients with gliobastoma multiforme, however, there was no statistical difference found in the two chemotherapy groups.[79]

The use of nitrosoureas, possibly in combination with vincristine and procarbazine, have demonstrated very limited benefit in the treatment of malignant gliomas. Nitrosoureas cause damage by DNA alkylation, particularly at the 06 position of deoxyguanine residues. These adducts then crosslink DNA, producing a lethal lesion. Often the response is short lived or inapparent, likely due to either de novo resistance or rapid generation of acquired resistance of tumor cells. Two mechanisms of resistance have been identified. The first is the repair of alkylater based DNA crosslinks by O6 alkylguanine DNA alkyltransferase (AGT), a protein present in tumor cells that mediates the repair process.[80] Pretreatment with O6 methylguanine, which inactivates the enzyme, may overcome resistance. The second mechanism involves the failure of the cell to conduct DNA mismatch repair due to deficiencies in the complex of proteins that comprise the DNA mismatch repair system. [35] [81]

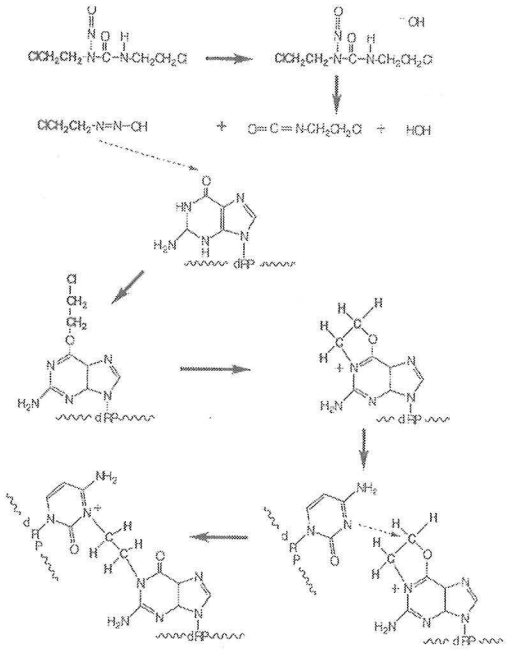


Fig. 5 Reaction of BCNU with DNA to produce a G-C interstrand cross-link.

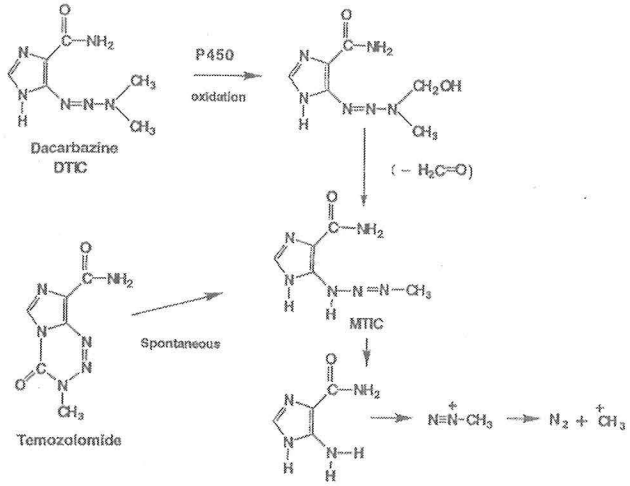


Fig. 6 Generation of methyl diazonium from the triazenes dacarbazine and temozolomide.

Temozolomide is a chemotherapy agent newly approved for use in the United States in patients with refractory anaplastic astrocytoma, and approved in Europe for refractory glioblastoma multiforme and anaplastic astrocytoma. The standard dosage of temozolomide is 150-200 mg per meter squared per day for 5 days in a 28-day cycle. Other dose schedules, such as a daily dose of 75 mg per meter squared, have been utilized.

Temozolomide is a second generation alkylating agent and an imidazotetrazine derivative of dacarbazine (DTIC). It differs from DTIC in that it can be taken orally, degrades spontaneously into an active metabolite, and readily penetrates the blood brain barrier. It has nearly 100% bioavailability and results in central nervous system concentrations that are approximately 40% of those observed in the plasma. It is well tolerated. Myelosuppression is the primary toxicity, with non-hematologic toxicities being infrequent. The most common non-hematologic toxicity is vomiting which can be effectively managed with antiemetics.[82]

The mechanism of action of temozolomide is via the production of 06 methylguanine DNA adducts, which initiate a futile recycling of the mismatch repair (MMR) pathway. This causes DNA strand breaks and apoptotic cell death in cells with proficient MMR systems. The DNA repair protein 06 alkylguanine DNA alkyltransferase (AGT) repairs these adducts in a suicide manner and reduces the cytotoxic action of temolzolomide. When sufficient adducts are formed by temozolomide to inactivate AGT, an anti-tumor threshold is reached. Depletion of AGT by O6 benzylguanine significantly increases the cytotoxicity of temozolomide.[83]

In a study by Taverna, looking at the DNA MMR pathways, the two MMR proteins most commonly mutated in human cancers were MLH1 and MSH2.[84] Absence of MLH1 protein

was always linked to resistance to the methylating chemotherapeutic agent temozolomide. This was independent of cellular levels of AGT.

Other mechanisms of resistance to temozolomide include the reduction in the amount of proapoptotic proteins including Bad, Bax, Bcl-X which were reduced 2-4 fold in resistant cell lines, whereas the antiapoptotic proteins including Bcl-2 and Bcl-Xl were expressed at similar levels in both cell lines.[85]

An important trial published recently from Switzerland looked at the combination of temozolomide and concomitant radiation therapy followed by temozolomide monotherapy in patients with newly diagnosed glioblastoma multiforme. The primary endpoints were safety and tolerability, and the second endpoint was overall survival. The results demonstrated that temozolomide was safe and well tolerated. Non-hematologic toxicities were rare during the concomitant treatment phase. Grade 3 or 4 neutropenia, thrombocytopenia, or both were observed in 6% of patients including 2 severe infections with pneumocystis carinii. During adjuvant temozolomide, 2% and 6% of cycles were associated with grades III and IV neutropenia or thrombocytopenia. Median survival was 16 months and the one and two year survival rates were 58% and 31% respectively. Patients younger than 50 years old, and patients who had undergone debulking surgery, had the best surgical outcome.[86]

One trial conducted at UT Southwestern Medical Center looked at the combination of BCNU and temozolomide in 45 patients, 25 of whom were diagnosed with glioblastoma. The toxicity was primarily hematological, although there were three instances of pulmonary toxicity, which likely represented potentiation of nitrosourea-induced pulmonary fibrosis. There were 5 partial responses in the 25 patients with glioblastoma multiforme.[87]

In a multicenter phase II trial of 138 patients, those patients with glioblastoma multiforme who had good performance status at first relapse were treated with temozolomide. Progression-free survival at six months was 18%; median progression-free survival and median overall survival were 21 months and 5.4 months respectively, with six-month survival rate of 46%. The objective response and partial response was 8% with an additional 45% having stable disease.[88]

A large meta-analysis of the role of adjuvant chemotherapy in addition to radiation therapy for malignant gliomas in adults, looked at results from 16 randomized clinical trials involving more than 3,000 patients.[78] The authors compared the survival rates of patients who received radiation therapy alone or radiation therapy with chemotherapy. The estimated increase in survival for patients treated with combination radiation/chemotherapy was 10.1% at one year and 8.6% at two years. These absolute increases in survival in patients treated with chemotherapy represented relative increases of 23.4% at one year and 52.4% at two years. When prognostic variables of age and histology were factored into the analysis, the data suggested the survival benefit from chemotherapy occurred earlier in patients with anaplastic astrocytoma than in patients with glioblastoma.

The Brain Tumor Study Group (BTSG) in 1978 reported the results of a clinical trial comparing the best conventional supportive care with carmustine and radiotherapy either alone or in combination. The median survival of the patients in the support only group was 14 weeks as compared to 19 weeks in the BCNU alone group, 36 weeks in the RT alone group, and 35 weeks in the RT/BCNU group. Although survival distribution curves were the same for the first 12 months, there was a greater survival rate at 18 months among the

patients receiving a combination of carmustine plus radiotherapy, with 10% still alive at that time as compared to only 4% of patients in the radiation therapy alone group.[89]

BTSG Trial 7201 confirmed the survival advantage of the combination of RT and nitrosourea based chemotherapy offered at 18 months. In that study, semustine alone proved to be significantly worse compared to RT, RT plus BCNU, and RT plus semustine. However, the difference between nitrosourea plus RT arms and radiation therapy alone was not statistically significant.[67] A follow up trial, BTSG Trial 7501, randomized patients to receive in addition to 60 Gy rounds of radiotherapy, either carmustine, high dose methylprednisolone, procarbazine, or BCNU plus high dose methylprednisolone. The RT high dose methylprednisolone arm was worse than the procarbazine, BCNU, or BCNU plus high dose methylprednisolone arms.[90] A Phase III comparison of BCNU and the combination of procarbazine, CCNU, and vincristine administered after radiotherapy with hydroxyurea showed that out of 133 patients (60 of whom had a glioblastoma multiforme),.the PCV combination produced longer overall survival and time to tumor progression than BCNU. The difference was statistically significant only for the anaplastic gliomas.[77]

When RT is combined with nitrosoureas in these trials, there is either no difference in survival, or there is only a statistically significant difference in long-term survival.

Post-operative adjuvant therapies for glioblastoma multiforme patients have not yielded impressive results. A trial recently published by Prados, et al looked at hydroxyurea followed by 6TG and BCNU, and although this seemed to improve survival in patients with anaplastic glioma, the patients with glioblastoma multiforme had no improvement in survival over other reported series, including BCNU alone, PCV, 6TG followed by BCNU or BCNU plus hydroxyurea.[91] Another trial looking at sequential preradiation chemotherapy with cisplatin BCNU followed by radiation versus concomitant chemotherapy and radiation therapy, demonstrated no improvement in survival but the concurrent therapy arm had more profound myelosuppression. There is a larger intergroup trial now being undertaken looking at the sequential course of chemotherapy followed by radiation therapy for high-grade astrocytomas.[92]

A randomized multicenter open label phase II study compared temozolomide to procarbazine in 225 patients with glioblastoma at first relapse. The six month progression-free survival rate for patients with temozolomide was 21%; six month progression-free survival rate for those who received procarbazine was 8%. Overall progression-free survival rate was significantly improved with temozolomide, with a median progression-free survival rate of 12.4 weeks compared to 8.32 weeks in the procarbazine group. Overall six month survival for temozolomide patients was 60% versus 44% in the procarbazine group. The increase in overall survival of 1.5 months demonstrated in the randomized controlled trial was not found to be significant. Although there is very little overall survival benefit, there appear to be some health related quality of life improvements due to temozolomide.[93]

In an example of an attempt to try to improve drug delivery for the improvement of chemotherapy effects in glioblastoma multiforme, liposomal doxorubicin was evaluated in six patients with glioblastoma.[94] The liposomal encapsulation of doxorubicin using polyethylene glycol liposomes has been shown to significantly improve the penetration of doxorubicin across the blood/brain barrier.

In an effort to find effective new chemotherapy agents, a trial reported by Weller, et al looked at patients receiving preirradiation gemcitabine chemotherapy followed by radiotherapy.[95] Their conclusion was that although the drug was safe, it did not confer survival advantage over radiation therapy alone.

Matrix metalloproteinase inhibitors (MMPI) have also been looked at in an effort to find new agents for the treatment of glioblastoma multiforme, as elevated levels of matrix metalloproteinases are seen during tumor growth. Marimastat is one drug in the class of MMP inhibitors which is being tested in glioblastoma multiforme. In a trial recently published from MD Anderson, temozolomide was combined with a matrix metalloproteinase inhibitor, marimastat, in recurrent and progressive glioblastoma multiforme. There were 44 patients, 25 of whom had previously received chemotherapy with the remaining 19 receiving their first chemotherapy after tumor progression and radiation. The temozolomide was given days 1-5 at a 28-day interval in combination with marimastat. Joint and tendon pain was the major therapy related toxicity in 47% of patients. For all patients, the progression free survival at six months was 39%, median progression free survival was 17 weeks, median overall survival was 45 weeks and 12 month progression free survival was 16%.[96]

Another group of drugs that are being investigated for possible therapeutic benefit inglioblastoma multiforme are the angiogenesis inhibitors. Vascular endothelial growth factor (VEGF) in the family of protein tyrosine kinase receptors and ligands is thought to play its role in tumorigenesis by promoting neovascularization. Thalidomide is an agent that has been shown to block angiogenesis induced by VEGF, and as a single agent, has modest effect in glioblastoma. A phase II trial was conducted using thalidomide at a high dose of 1200 mg a day for patients with recurrent high-grade gliomas. Of 32 patients accrued, two had radiographic response, a total of four patients had objective responses, and an additional 12 patients achieved stabilization of disease lasting at least two months.[97]

Another field of investigation is a modulation of mechanisms of resistance to standard drugs such as BCNU. One such mechanism of resistance involves the DNA repair protein, alkyltransferase. A new drug in clinical trial testing, O6 benzylguanine, is capable of temporarily inhibiting the alkyltranferase. Therefore, one strategy is to use the O6 benzylguanine (O6BG) before alkylator-based therapy. One of the potential problems of O6BG in inhibiting the DNA repair protein alkyltransferase, which then provides resistance to nitrosoureas, is that if it is successful, it could actually increase the effective dose of carmustine causing increased toxicity. If O6BG crosses the blood/brain barrier and is administered systemically, whereas the carmustine is administered locally, that would reduce the risk of systemic toxicities.[98]

Most patients with glioblastomas have tumor recurrences within 2 cm of the original resection field. As a consequence, some efforts have focused on treating the local regional area, and a unique mechanism of drug delivery has been developed for glioblastoma specifically. One strategy is to use implantable biodegrable polymers that release high concentrations of chemotherapeutic agents directly into the CNS. This approach minimizes systemic toxicity and bypasses the restrictions of the blood/brain barrier. In 1993, 3.85% carmustine-impregnated polymers (Gliadel) received FDA approval for the treatment of recurrent malignant brain tumors. Higher doses of carmustine impregnated polymers have been evaluated, and it appears as though there may be a dose response curve and that higher doses may have acceptable toxicity.[99] One

complication that can ensue from placement of BCNU-impregnated wafers is cyst formation in the tumor resection cavity. This can cause acute neurologic deterioration with significant mass effect of the previous resection site.[100]

Another drug delivery system that is being evaluated for the treatment of glioblastoma multiforme is a bioerodable polymer, which has a successful history of use as a suture material. Microspheres can be delivered stereotactically to deep-seated lesions in the brain not readily accessible by surgery.[101] Drug impregnated microspheres can also be injected into the walls of surgical resection cavities.

The combination of polymer and microchip technology has resulted in a new approach to complex controlled drug delivery with the development of the solid state silicone microchip that can provide controlled release of single and multiple chemical substances at varying time points. Therapeutic agents whether solid, liquid, or gel are released after the electrochemical dissolution of the thin anode membrane covering the microresevoir. A microbattery, multiplexing circuitry, and memory can be integrated directly into the device.[76]

Intra-arterial chemotherapy has been tried in an effort to circumvent problems with drug delivery, however, one trial by the Brain Tumor Cooperative Group in which intra-arterial versus intravenous BCNU with or without intravenous 5FU demonstrated that those patients who received intra-arterial carmustine fared worse than those given carmustine intravenously. Furthermore, intra-arterial chemotherapy produced much greater brain damage than had been anticipated by early Phase II studies.[102] Neuwelt, et al has forcibly opened the blood brain barrier using hyperosmotic mannitol to treat gliomas, but 50% of treatment through this technique resulted in seizures, and it has failed to produce substantial improvement in patient outcome.[103]

Another novel approach to local therapy uses diphtheria toxin conjugated to transferrin administered by a microinfusion. Transferrin receptors are highly expressed on rapidly dividing cells including glioblastoma multiforme and other tumors. The toxin depends on transferrin to enter the cell by binding the transferrin receptor, which is then internalized by endocytosis.[104]

Regarding the immunologic mechanisms in the brain, the mammalian brain has been considered to be an immunologically privileged organ, in that allografts of carcinomas in embryonic tissue are not very successfully grown in the brain. The brain lacks defined lymphatic drainage, and the expression of major histocompatability complex class 1 and 2 molecules in the brain is low with only activated T-lymphocytes crossing the blood brain barrier. Whereas the brain may be incapable of priming or initiating an immune response, T-cells activated in the periphery carry effector functions into the central nervous system. The immunologic privileges of the brain are not complete. Microglia have recently emerged as capable of presenting antigens and initiating an immune response when properly activated. Thus, the brain needs to be stimulated to evolve into an immunologically active organ.[105]

Cytokines such as interleukin 2 and, in some cases, interleukin 4 may have an antitumor effect, however most of these cytokine agents work in a paracrine fashion and only work on the local regional effects. Thus, because these do not cross the blood/brain barrier successfully, it is necessary to consider a local regional approach to administration of these drugs should they achieve any effect in this disease process.[106]

Some gliomas secrete TGF-beta which still exerts potent immunosuppressive effects including the inhibition of cytotoxic T-lymphocytes. Successful induction of primary antitumor immunity in the brain is demonstrated by experiments in which malignant gliomas are genetically modified to secrete interferon gamma.[107]

Tamoxifen appears to have an inhibitory activity against glial derived cell lines. This effect is independent of the antiestrogenic activity. It is mediated by the inhibition of the intracellular enzyme protein kinase C. Protein kinase C constitutes a family of proteins that play an important role in growth factor mediated signal transduction and are overexpressed in malignant gliomas. One study conducted by Couldwell, treated 32 adult patients with recurrent high-grade gliomas with tamoxifen.[108] They reported an objective radiologic partial response of 25% and a significant improvement in survival. Another tamoxifen-based trial from Broniscer, looked at brain stem gliomas in children using tamoxifen concomitant with radiation therapy.[109] There was no significant change in the overall poor prognosis of those patients, although a minority of patients seemed to benefit from the extended use of tamoxifen. It was generally well tolerated, but the results were poor overall. Another tamoxifen trial looked at high dose tamoxifen as salvage chemotherapy for recurrent anaplastic astrocytomas. Twenty-four (24) patients heavily pretreated with chemotherapy, radiation therapy, and surgery, were administered a median of four cycles of tamoxifen. Treatment was well tolerated; 17% demonstrated a neuroradiographic partial response, 46% stable disease, and 38% progressive disease, following a single cycle. Time to progression ranged from 3-25 months, median 12 months, survival range from 3-27 months, median 13 months. This was felt to demonstrate modest activity in this group of patients.[110]

Other foci of new therapeutic agents include inhibitors of signal transduction and angiogenesis as well as anti-invasion agents. Amplification of genes encoding for proteins that stimulate cell growth are known to be overexpressed in a portion of malignant gliomas. This is particularly true for protein tyrasine kinase receptors such as platelet derived growth factor (PDGF) receptor. There are both autocrine and paracrine mechanisms for PDGF mediated tumor growth in glioblastomas. Inhibitors of the PDGF receptor signaling pathway have been tested in glioblastomas.[111]

NABTC TRIALS AT UT SOUTHWESTERN

It is essential that we find superior therapies in improving the outcome of this disease. A better understanding of the molecular basis and etiology of this disease will lead to novel targets for targeted therapy which may provide better therapeutic options in the future. Among agents currently in preclinical or early stages of clinical development are rapamicin analogs that inhibit the action of protein kinase m-TOR or FRAP, farnesyl transferase inhibitors, and tyrosine kinase inhibitors. Tyrosine kinases may play an important role in pathogenesis of malignant gliomas, for example, epidermal growth factor receptor has tyrosine kinase activity.

UT Southwestern Medical Center through the Annette Strauss Center for Neuro-oncology is a member of the North American Brain Tumor Consortium (NABTC), a group of research facilities through out the U.S. dedicated to finding new treatments for brain tumors. There are several trials currently ongoing for the treatment of malignant gliomas. These drug

trials utilize agents on a molecular level to interfere with specific cell processes by interfering with protein activation.

One trial, NABTC 99-01, is looking at the use of R115777, which is a farnesyl transferase inhibitor. The compound is a competitive inhibitor of the CAAX peptide binding site of the farnesyl transferase enzyme. R115777 is part of a new class of agents specifically designed to inhibit the initial step in RAS protein activation. It prevents activation of oncogenic RAS genes via suppression of their post-translational farnesylation. Their anti-cancer activity appears to stem from their ability to inhibit farnesylation of various proteins that mediate signal transduction, growth, apoptosis, and angiogenesis. One of the major effects of, farnesyl transferase inhibitors (FTI), is to alter cell cycle progression.[112]

Another trial NABTC 01-01 is looking at CCI-779, the mammalian target of rapamicin, or m-TOR. It is a rapamicin ester cell cycle inhibitor. This molecule binds to FKBP-12, intracellularly forming a complex that inhibits the kinase activity of the mammalian target of rapamicin. This interferes with key signal transduction pathways including those regulated by p70s6 kinase and PHAS-I protein resulting in inefficient translation of proteins involved in cell cycle progression. m-TOR, also known as FRAP and RAFT1, controls the translation machinery via activation of S6 kinases 1 and 2, an inhibition of the eukaryotic initiation factor 4E binding proteins 1,2, and 3. m-TOR controls cell growth in part by regulating the aforementioned S6 kinase and eukaryotic initiation factor 4E.[113]

Two other trials NABTC 00-01 and NABTC 01-02 both involve Iressa, the latter study involving Iressa plus temozolomide. Iressa, or ZD1839, is a signal transduction inhibitor. It inhibits epidermal growth factor (EGF) from attaching to the receptor, which causes tyrosine kinase to trigger chemical processes inside the cell to grow and divide. Iressa attaches itself to the EGF receptor inside the cell, which blocks the activation of tyrosine kinase and switches off the signals from the EGF receptor.[114]

NABTC99-08 is a trial looking at the role of Gleevec, STI571, a signal transduction inhibitor, which has had impressive responses in the treatment of chronic myeloid leukemia. These small molecule inhibitors, which show some promise in the treatment of brain tumors, generally can cross the blood brain barrier readily and may be administered orally or intravenously. They have well tolerated toxicity profiles. It does appear that when these small molecule inhibitors are active, they need to be administered chronically because a discontinuation of the drug will cause release of the inhibition.

Gliobastoma multiforme remains one of the most aggressive of all tumors. Our ability to battle this disease has clearly been limited despite extensive trials and new approaches in surgery, radiation therapy, and chemotherapy. Much is currently being learned about the molecular basis for the development and progression of this disease. With a greater understanding of these mechanisms, we will be able to select targeted therapies which will limit toxicity and eventually lead to improved survival for our patients.

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