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Large, Single Institution Review of Prognostic Factors in Oligodendroglioma

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LARGE, SINGLE INSTITUTION REVIEW OF PROGNOSTIC FACTORS IN OLIGODENDROGLIOMA

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Abstract

Studies have demonstrated an association between loss of heterozygosity on chromosome 1p and chromosome 19q in oligodendrogliomas with both chemosensitivity and prolonged survival. This represents the first time genetic mutations have been utilized to guide clinical decision making. Studies have also found these genetic mutations to be associated with magnetic resonance imaging (MRI) features including indistinct tumor borders on T1-weighted imaging, susceptibility effect, and mixed signal intensity. However, no study has yet demonstrated an association between imaging features and survival. We seek to confirm the clinical utility of known prognostic factors such as age and tumor grade while investigating the potential importance of imaging characteristics in predicting survival.

We conducted a large, single-institution retrospective chart review of patients with tissue diagnoses of oligodendroglioma. Pathology reports, allelic status studies, MR imaging, and survival information were reviewed. Survival curves, Two-sided chi-square tests, and generalized linear models failed to reveal an association between survival and gender, age, tumor grade, allelic status, or imaging characteristics. We found no association between imaging characteristics and allelic status. The failure to confirm even well-accepted prognostic factors suggests limitations in the study largely attributable to small sample size. This limitation was due to availability of necessary information, rarity of the tumor, and only recent availability of genetic testing. Further studies with larger populations need to be conducted to fully determine the prognostic utility of MRI features.

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Introduction

Epidemiology and Clinical characteristics.

Primary brain tumors are traditionally subdivided into tumors of neuroepithelial tissue composed predominantly of gliomas, tumors of the meninges, germ cell tumors, tumors of the sellar region, lymphoma, and hemopoietic neoplasms (See Table A).[1]

Gliomas, including astrocytomas, oligodendrogliomas, and ependymomas, comprise 70% of primary brain tumors with an incidence of 5.27/100,000 persons.[2] Incidence rates have been similar in recent population-based studies with the Central Brain Tumor Registry of the United States reporting rates of primary brain tumors where oligodendrogliomas represented 2.7%, anaplastic oligodendrogliomas 1.3%, and oligoastrocytomas 1.1%. Trends reveal an increasing incidence of these tumors although this is difficult to determine with changing histopathologic criteria and increasing use of MR imagining.[3, 4]

Oligodendrogliomas are currently defined by the World Health Organization (WHO) as "well-differentiated, diffusely infiltrating tumors of adults that are typically located in the cerebral hemispheres and composed predominantly of cells morphologically resembling oligodendroglia."[1] While the exact wording of this definition has changed, the basic classification and grading of these tumors has changed little since the 1993 WHO classifications.[1] Oligodendrogliomas are classified as either oligodendrogliomas (WHO Grade II) or anaplastic oligodendrogliomas (WHO Grade III). While anaplastic tumors are technically defined as having "focal or diffuse histological features of malignancy and a less favorable prognosis," they are histologically defined by

the presence of increased cellularity, increased mitotic activity, marked atypia, microvascular proliferation, and necrosis.[1]

Oligodendrogliomas can develop anywhere in the neuroaxis where oligodendrocytes are located. Ninety percent of these tumors are supratentorial, predominantly in the frontal and frontotemporal cortex.[4, 5] Tumors are commonly found in the frontal (55%), temporal (47%), parietal (20%), and occipital (4%) lobes.[6] This distribution is age dependent, however, as supratentorial oligodendrogliomas are not observed in children. In patients under the age of 18 years, 67% of oligodendrogliomas occur in the cerebellum.[2] Interestingly, the tumor's location correlates with the presence of specific genetic mutations to be discussed later.[7] Oligodendrogliomas have a predilection for subcortical white matter with extension to the cortex.[8] Their pattern of spread can result in tumor extension through the corpus callosum, across the ependyma, into deeper brainstem structures and even into the leptomeninges inducing a desmoplastic reaction.[8, 9]

Gliomas present at slightly different ages with oligodendrogliomas WHO grade II presenting at 40.9 + 15.1 years, anaplastic oligodendrogliomas at 50.4 + 13.9 years, anaplastic oligoastrocytoma at 48.2 + 17.4 years and glioblastoma at 62.2 + 13.4 years.[2] Oligodendrogliomas have a bimodal age distribution with the first peak from 6-12 years and then a second peak from 26-46 years.[4] However, only 7.5% of oligodendrogliomas are diagnosed in children.[4] There is a slightly gender dimorphic distribution of disease with a male-to-female ratio ranging from 1.2 to 3.0.[6] The age of presentation peaks differently according to gender with males peaking at 45-49 years and women peaking at 55-59 years.[10] Clinical symptoms at presentation are similar to other intracranial tumors

including seizures and symptoms of increased intracranial pressure such as headache, visual changes, paralysis, vomiting, papilledema, ataxia, and mental status change.[4, 6, 11] No single symptom has been found to correlate with tumor grade.[6] The median duration of symptoms before diagnosis has been described as 20.5 months (mean 43 months)[12] but can range from 2.9 months to 16 years.[4, 6]

Pathology

The pathogenesis of oligodendrogliomas remains elusive. Candidate progenitor cells include dedifferentiated mature cells or glial progenitor cells. While stem cell-like cells have been isolated from glial tumors, neoplastic cells have such marked genetic hetrogenicity and epigenic alterations that the critical event in tumor development has not been determined.[3]

Macroscopically, oligodendrogliomas are well defined masses of "fleshy to pinkish-gray color" with a soft, gelatinous texture. They may have a gritty texture due to microcalcifications. Hypervascularity may be noted but edema is uncommon. Grossly cystic necrosis is only present in malignant forms although zones of cystic degeneration can be detected in more benign tumors. Due to their tendency to invade the cortex, oligodendrogliomas may obliterate the gray-white junction and focal areas of leptomeningeal infiltration may be present. [8, 9] In some cases, this infiltration can produce a desmoplastic reaction that resembles metastatic carcinoma.[9]

Microscopic examination of oligodendrogliomas reveals uniform, round nuclei with clear cytoplasm. The presence of oligodendroglia suggests an oligdendroglioma, astrocytoma, or mixed glioma. Fibrillary astocytoma is an infiltrative astocytoma that most closely resembles oligodendroglioma however exhibits irregular, elongated,

hyperchromatic nuclei that differ from the appearance of oligodendrogliomas.[13] The distinction between these can be challenging due to interobserver and intratumor variability. However, this differentiation is an important one as patients with astrocytomas or mixed gliomas have a shorter life expectancy than patients with oligodendrogliomas.[14-17] Another entity that closely resembles oligodendroglioma is dysembryoplastic neuroepithelial tumor (DNT). DNTs are extremely slow growing, superficial neoplasms found during the first decades of life that presents with long-standing seizures.[5]

Cellular characteristics of oligodendrogliomas are best appreciated on tissue smears. With this preparation, samples appear moderately cellular with individual cells being uniformly round with homogeneous nuclei, swollen clear cytoplasm and a lack of cohesion. Cells are typically enmeshed in a fibrillary, eosinophilic matrix and lack fibrillary processes however there is variability in the cell density and relationship with surrounding tissue.[8, 9] A classification system has been proposed that classifies oligodendrogliomas according to these characteristics. In this system oligodendrogliomas are classified into structure type II or structural type III. Structural type II is defined by solid tumor tissue components and destruction of surrounding neurons. Structural type III is defined by isolated tumor cells closely resembling oligodendroglia. While this classification system is not universally accepted, it has been shown to correlate with biological behavior of tumors.[18]

Tumor cells are particularly susceptible to processing which can result in characteristic perinuclear vacuolization.[9] On formalin-fixed, paraffin embedded material, cells appear to have perinuclear halos that create the classic “fried-egg” appearance. This

appearance is due to artifact but still represents a useful diagnostic feature.[5, 8] Other microscopic characteristics include microcalcifications, mucoid/cystic degeneration,[8] and intratumoral hemorrhage.[5] Characteristic changes in vascularity include a "chicken-wire" appearance created by increased branching of local capillaries.[8] Varying frequency of astrocytes within oligodendrogliomas causes challenges in determining if they represent reactive astrocytes within an invasive tumor, transitional forms of oligodendroglial cells, or independently neoplastic cells.[8] Also present could be sub-populations of minigemistocytes [8] characterized by eccentric cytoplasm that is GFAP positive. Characteristics typical of anaplastic degeneration include increased nuclear size, hyperchromasia and pleomorphism.[9] Focal necrosis and endothelial proliferation are also ominous changes.

Few immunohistochemical markers have proven helpful in confirming the diagnosis of oligodendroglioma. Glial markers include GFAP, an intermediate filament, and S-100 protein. Neither marker is specific for oligodendrogliomas.[13] Other candidate markers have included overexpression of PDGF and its receptor,[3] expression of galactocerebroside (GalC),[9] elevated myelin basic protein,[9] p53 positive cells,[19, 20] presence of Olig2,[21] and expression of YLK-40 in the tissue and serum.[22, 23] All of these have insufficient specificity to be routinely used in clinical practice. Oligodendrogliomas, more frequently than astrocytomas, overexpress a variety of genes including DES, TDGF1, TGF-β, GABA-BR1A, Histone H4, CDKN1A, PCDH43, Rho7 and Jun-D while underexpressing JNK2, ITGB4, JNK3A2, RgoC, IFI-56K, AAD14, and EGFR.[24] However, none of these markers have proven to be sufficiently sensitive or specific. Markers of cellular proliferation have been useful in prognosis but not diagnosis. The

most frequently used marker is Ki-67, a nuclear protein that is expressed only when cells are in the M phase of the cell cycle[13, 25] and can be marked with MIB-1 antibody.[26] Expression of TP53 and LOH at 17p are uncommon.[24, 27] TP53 overexpression is noted in 88% of gemistocytic astrocytomas, 53% fibrillary astrocytomas, and 44% of oligoastrocytomas but only 13% of pure oligodendrogliomas.[2] Other genetic alterations have remained elusive with promising yet ultimately unrevealing characteristics including deletions on 4q, 9p, 13q, and 17p along with amplification of 7q, 8q, and 11q.[14, 28] Loss of heterozygosity (LOH), where one of two copies of a specific gene is lost, on chromosome 10 and gain of genes on chromosome 7 have been shown to be associated with glioblastoma multiforme but not oligodendroglioma.[2, 28] Homozygous deletion or epigenetic silencing of *CDKN2A/p14*^{ARF}, overexpression of EGFR, loss of RB1, and amplification of CDK4 have been shown to be useful with identifying anaplastic oligodendrogliomas [3],[29]

Although immunohistochemistry has limited usefulness in differentiating oligodendrogliomas from other CNS malignancies, detecting LOH on chromosome 1p with or without LOH on chromosome 19q has revolutionized diagnosis, treatment decisions, and prognosis of these tumors. [8, 15, 27] Various genetic mutations harbored within neoplastic cells have been defined and subsequently used for diagnosis and therapy decisions. With two copies of each chromosome, loss or gain of genetic material on one or both copies can profoundly influence the behavior of that cell. Loss of a tumor suppressor gene or gain of an oncogene has been associated with neoplastic growth. These two types of mutations are thought to be the underlying mechanism of tumorgenesis in these tumors. With oligodendrogliomas, however, the link between

genetic mutation and phenotype remains elusive as the characteristic loss of one of the two copies of the short arm of chromosome 1 (1p) with or without the loss of the long arm on chromosome 19 (19q) lack known oncogenic mechanism. These mutations are unique as it is not just a specific gene that is gained or lost but typically an entire arm of the chromosome leading to homozygosity.

Various techniques are used in the detection of these mutations including loss of heterozygosity (LOH), quantitative polymerase chain reaction (PCR), and fluorescence in situ hybridization (FISH).[30] Briefly, LOH testing is conducted by grossly detecting restricted fragment length on the chromosomes of interest. As this technique requires large quantities of tumor and is very time-consuming it is rarely used today.[30] PCR testing involves detecting the presence or absence of microsatellite gene sequences in tumor cells verses constitutional tissue (frequently peripheral lymphocytes) from the same patient. If the chromosome of interest from the tumor cells has markers for only one set of microsattelite markers whereas the peripheral cells exhibit two copies, the sample is deemed LOH.[30] Finally, FISH utilizes fluorescent markers of intranuclear signals reflecting a heterozygous state. Thus, these signals are lost when genetic deletions are present. Advantages of this technique include the ability to use paraffin-embedded samples and not requiring constitutional tissue for comparison. No standard means of detection has been established as the sensitivity and specificity of these tests have not yet been determined. Thus, different studies utilize different techniques and are considered equivalent.

Many studies have demonstrated the importance of 1p with or without 19q deletions (1p/19q LOH) in oligodendrogliomas. The original study demonstrated that of

21 oligodendrogliomas, 81% had LOH on 19q and 67% had LOH on 1p.[27] Subsequent studies with larger sample sizes have suggested the actual proportion is closer to LOH on 1p occurring in 83% (40-92%), 19q in 66% (50-80%)[8], and combined loss in 66% of oligodendrogliomas.[31] Loss of 1p is highly associated with loss of 19q, together constituting the earliest known molecular change in 50-70% of oligodendrogliomas.[32] Only 20% of mixed tumors have LOH at 1p/19q and studies have shown the deletions to be significantly more common in oligodendrogliomas than astrocytomas[31] LOH on 1p/19q is also mutually exclusive to other markers found in intracranial tumors including TP53 overexpression, LOH on 17p,[24, 27] LOH on 10, gain on 7.[2, 28] Thus, the presence of any of these mutations makes the diagnosis of oligodendroglioma extremely unlikely. The presence of epigenetic silencing of *CDKN2A/p14*^{ARF}, overexpression of EGFR, loss of RB1, and amplification of CDK4 would also make the diagnosis of oligodendroglioma extremely unlikely.[29]

The significance of 1p/19q LOH in oligodendroglioms has raised questions regarding contents of the deleted genes, particularly as this deletion is seen in numerous other solid tumors.[31] Many groups have attempted to identify a tumor suppressor gene on these chromosomes but a definitive gene has not been identified.[3] One group determined that a deletion of 1p36 within the *CAMTA1* gene to be present in all oligodendrogliomas with LOH on 1p. This deletion is associated with reduced *CAMTA1* expression by half suggesting it could represent a tumor suppressor gene.[31] Another group identified a potential 19q locus to be 19q13.3 and have suggested *P190RhoGAP*, a protein known to be involved in oligodendrocyte differentiation located in that area, as a candidate gene. The suggested mechanism is that this deletion leads to decreased Rho activity thus

reducing PDGF levels and oncogenesis.[33] However, other studies have demonstrated that PDGF levels are actually elevated in oligodendrogliomas making this explanation questionable.

Imaging

The evolution of imaging intracranial tumors has changed profoundly over the previous 50 years. Early articles on oligodendrogliomas noted frequent calcifications seen on cranial X-ray.[12] Computer tomography provided a major advancement in determining tumor location and characteristics. A CT finding that can suggest a mass is an oligodengroglioma is the presence of linear or nodular tumoral calcifications. Oligodendrogliomas have the highest frequency of calcification among intracranial tumors and such calcifications are reported in 50-90% of these tumors.[5]

The widespread availability and use of magnetic resonance imaging (MRI) has revolutionized the diagnosis, surgical management, radiation planning, chemotherapeutic evaluation, and monitoring of intracranial neoplasms.[32] However, traditional criteria such as location in the neuroaxis and patient age are still relied upon in determining the most likely type of tumor prior to tissue diagnosis. MRI provides unprecedented details of the characteristics of the tumor. As discussed earlier, oligodendrogliomas tend to be found in patients in their fourth and fifth decades and located in the frontal lobes. T1 weighted imaging typically reveals a hypointense mass unless paramagnetic effects from hemorrhage, necrosis, or calcification are present.[5] Enhancement after administration of gadolinium-DTPA on T1 weighted imaging has been linked to more aggressive tumor behavior, however this observation remains controversial.[4, 18, 34] T2 weighted imaging reveals a heterogeneous mass that may be isointense or hypterintense compared with gray

matter. T2 imaging also reveals characteristic cortical thickening that can help distinguish the mass from astrocytic tumors which arise within the white matter. The distinction of a large intratumoral hemorrhage from a large intracranial hemorrhage in the absence of a structural lesion can be difficult, especially in the acute setting. Often repeat imaging is needed after an interval of 4-6 weeks. Since most oligodendrogliomas are of low grade, typically, minimal edema is seen. To better detect intratumoral calcification, gradient echo MR imaging can be performed as it is more sensitive to calcium products than is conventional spin echo MR imaging .[5]

A number of MRI characteristics have been statistically associated with LOH on 1p/19q. Tumors located in the frontal, parietal, or occipital lobe are likely to harbor these mutations while tumors of the temporal lobe, insula, and diencephalon are more likely to lack these genetic alterations.[7] Tumors growing across the midline are also likely to have 1p/19q LOH.[7] Tumors that have LOH on 1p/19q are more likely to display indistinct borders on T1 pre-gadolinium images whereas those with intact chromosomes 1 and 19 are more likely to have sharply demarcated borders.[32] Additionally, oligodendrogliomas with LOH on 1p/19q are more likely to display mixed signal intensity on T1 and T2 and magnetic susceptibility change.[32] Independent of genetic mutations, anaplastic tumors frequently have ring enhancement on post-gadolinium T1 weighted images.

Positron emission tomography (PET) scans have proven a useful supplement to conventional MR imaging. Tumors with LOH at 1p/19q display relatively increased metabolism of 18-fluorodeoxyglucose in PET.[35] While PET scans are not in widespread clinical use, they have numerous potential uses including determining tumor type, degree

of malignancy, discriminating tumor vs. radiation necrosis, guiding biopsy, and assessing post-op tumor burden.[4]

Therapy

Surgery remains the mainstay of treatment as it allows physicians to obtain tissue for diagnosis while reducing mass effect causing symptoms and neurological deficits.[4] The surgical goal is gross total removal[4] as it has been shown to prolong survival.[3] However, it is currently impossible to determine extent of tumor infiltration into surrounding tissue and thus patients are rarely cured by surgery alone.[4] Due to the inability to guarantee gross total resection most patients ultimately undergo both radiation and chemotherapy. With low-grade tumors, adjuvant therapy is given at the time of radiographic progression or if clinical symptoms remain uncontrolled. For anaplastic tumors, adjuvant therapy is usually administered at initial diagnosis.

The radiation field includes the area of T2 signal abnormality[3] and has been shown to prolong survival, especially when gross total resection is not accomplished;[4] however the timing and effectiveness remains controversial.[16, 36, 37] Response to RT has been associated with 1p/19q LOH. Of patients who received radiation, those with 1p LOH have significantly longer survival such that WHO Grade II tumors with LOH on 1p having a mean progression-free survival of 55.0 months versus 6.2 months for those with retained heterozygosity. For Grade III tumors, patients with LOH had progression free survival of 49.8 months versus 5.7 months in those with retained heterozygosity. In fact, in that study LOH on 1p was of greater prognostic value than age or Karnofsky performance score.[38]

The importance of determining if there is LOH at 1p/19q is best appreciated in the dramatic effect on chemosensitivity. LOH on 1p has been strongly associated with radiographic response to chemotherapy and long survival times.[15] In one study, all chemosensitive tumors harbored LOH on 1p while 89% of chemosensitive tumors had LOH on 1p.[15] With this discovery, chemotherapy is now able to be used in a more selective fashion. It is frequently used when radiotherapy would have exposed more that 50% of the hemispheres.[39] The current mainstay of therapy is the use of temozolomide or a combination of procarbazine, lomustine (CCNU), and vincristine (PCV) before and/or after radiotherapy.[4] Prospective studies investigating efficacy or incidence of secondary malignancy have not yet been done.[3]

Recurrent or anaplastic oligodendrogliomas are treated with some combination of resection, radiation, and chemotherapy. Roughly 75% of these patients respond to PCV,[40-42] although monotherapy with temozolomide might be efficacious and better tolerated.[43] Regardless, patients invariably relapse if chemotherapy is given without radiation.[3] Salvage regimens include using carboplatin or high does thiotepa with stem cell rescue.[3]

Prognosis

The natural history of gliomas can range from slow-growing to rapidly fatal. The mean survival time differs for the subtype of glioma and age of the patient. Oligodendroglioma WHO grade II mean survival averages 11.6 years while anaplastic oligodendroglioma survival averages 3.5 years. Importantly, astrocytomas mean survival is only 5.9 years.[2] Oligodendrogliomas tend to be progressive with a recurrence rate of

43%, 68% of which are a more malignant grade.[2] The rate of progression from oligodendroglioma to anaplastic oligodendroglioma ranges from 5.0 to 6.6 years.[2, 16]

Prognostic factors used to predict patient survival remains controversial as, with the exception of age, few factors have reproducibly been associated with survival (For summary, see Table B).

However, there have been a number of prognostic factors that have shown promise and, while only in a limited number of studies, have been associated with patient outcome. Age, tumor location, presence of neurological deficits, presenting symptom, degree of surgical intervention, and ABO blood type have been correlated with outcome while gender has not.[12] Age is one of the strongest predictors of survival with one study demonstrating that patients who were less than 20 years old had a median survival of 17.5 years whereas patients over 60 years old had a median survival of 13 months.[36] Another study demonstrated patient survival to be correlated to age with 75% of patients under 30, 43% patients age 30-50, and only 21% of patients over 50 survive 10 years after diagnosis.[44] The importance of age has been verified by a number of studies.[15, 20, 25, 26, 45, 46] Tumor location has been strongly associated with survival such that patients with frontal lobe tumors survive longer than those patients with tumors in any other location.[25] Interestingly, this location is associated with LOH on 1p/19q. Tumors that cross the midline have been associated with poorer survival.[46] The presence of neurological deficits is a negative prognostic factor; patients with deficits had a median survival of 2.5 years whereas those without had a median survival of 11 years.[46] Another study noted a 5 year survival discrepancy such that 5% of patients with neurological deficits versus 43% of those without were still alive.[36] One study found presenting symptom to be of

prognostic significance such that patients who presented with seizures had better survival.[45] Patient survival has been correlated to degree of surgical intervention with 57% of patients with gross total resection, 48% of patients who underwent biopsy only, and 33% of patients with sub-total resection surviving 10 years post-operatively.[44] However, other studies have found no correlation between surgical intervention and survival.[45] Interestingly, ABO blood type has been shown to be associated with differences in outcome such that patients had consistently shorter survival with blood type A vs. B or O.[12]

Some studies have demonstrated histological grade to be associated with survival[20, 25, 45] while others have addressed individual characteristics. Degree of nuclear atypia and presence or absence of necrosis have not been associated with survival.[34] One study noted that for patients with grade II tumors, 46% were alive after 5 years whereas patients with grade III tumors only 10% were alive.[36] Presence of mitoses, endothelial hypertrophy, endothelial proliferation, and necrosis have been associated with survival although poorly reproducible between observers.[44] The same study demonstrated the presence of endothelial proliferation to be independently associated with survival having a hazard ratio of 2.7 (95% confidence interval 1.5-4.8, $p<.001$) even when calculated by consensus scores and not individual scores. "Endothelial hyperplasia" has been demonstrated to significantly correlate with survival such that median survival was 3.5 year for patients with hyperplasia versus 11 years for patients with no hyperplasia. However, that study failed to associate endothelial proliferation or glomeruloid vessels with survival.[34] Another study used vascular endothelial surface area index (VESI) as defined by the CD-34 immunostained endothelial area in μm^2 per 1000 tumor cells as a

prognostic marker. They noted patients with VESI scores of less than 15 had longer survival than patients whose tumors scored over 15.[47]

Mitotically active tumors are thought to be more malignant and thus have a poorer prognosis than inactive tumors. Interestingly, the number of mitotic figures has such variability between pathologists that this marker has been shown to not predict survival in some studies[25, 44] but does in others.[20] Markers of cellular proliferation have been shown to have prognostic value. Ki-67 labeling index (LI) reflects the percentage of MIB-1 labeled cells and is a commonly used marker due to its ability to be used in paraffin-embedded tissue and minimal technical concerns.[26] It has been shown to more accurately reflect mitotic activity than counting mitoses[44] but has to be validated within each individual institution.[3] Disease-free survival and overall survival correlate with lower Ki-67 labeling indices[20] and Ki-67 index has been shown to have prognostic significance independent of patient age, tumor site, and histology.[25, 26] Conversely, higher Ki-67 labeling indices have been associated with decreased survival.[15] Ki-67 labeling indices have also been associated with tumor grade such that in one study the mean proliferation rate of WHO grade II oligodendrogliomas was 7.7% versus WHO grade III tumors with 16.9%.[38] Another marker of proliferation is topoisomerase IIα, a molecular target for certain chemotherapeutic drugs that has been shown to have prognostic value in a variety of cancers.[48] Higher portion of topoisomerase IIα positive cells (>3.3%) has been associated with higher grade tumors and higher tumor proliferation rate but not with gender or age.[48] A lower portion of topoisomerase IIα positive cells is strongly associated with improved survival at 5-year follow up such that of patients with <3.3% nuclei positive for topoisomease IIα, 88% were alive after 5 years versus only 69% of

those with higher rates.[38] Older means of determining mitotic activity, such as using antibodies to detect the presence of cyclin and proliferating cell nuclear antigen (PCNA) have not proven reliable.[49, 50]

Various immunohistochemical markers have been utilized with limited utility. Cyclooxygenase, an enzyme involved in prostaglandin synthesis, has been associated with survival. COX-1a levels, a protein constitutively expressed in microglia, are significantly lower in low grade oligodendrogliomas and are associated with longer progression free and overall survival.[51] Negative prognostic markers have included CDKN2A deletions[15] and increased p53 levels. However, other studies have failed to correlate p53 levels with survival.[20] Patients with CDKN2A deletion or decreased p16 expression have been shown to have significantly shorter overall survival. Interestingly these deletions are inversely correlated with LOH on 1p/19q.[15] High p53 levels such that over 75% of cells are immunoreactive is associated with reduced survival[19] while other studies have shown no relationship.[20]

Finally, in addition to its diagnostic value, tumor features on MRI can also be used for prognosis. Necrosis, a histological feature known to be associated with poorer outcomes, may be seen as higher or lower intensity on T1 weighted imaging as well as on T2 weighted imaging due to paramagnetic cations and free radicals or cystic changes with increased water. FLAIR imaging is an additional useful tool for determining cystic degeneration.[5] However, the only imaging characteristic demonstrated to be significantly associated with outcome is T1 weighted post-gadolinium enhancement's association with shorter survival[15] where one study reported a median survival difference of 3 versus 11 years for patients with and without contrast enhancement.[34]

Statement of hypothesis

We seek to better understand prognostic factors in oligodendrogliomas. Specifically, we will examine the potential association between age, gender, tumor grade, allelic status on 1p/19q and MRI features with survival. Age, a proven prognostic factor, and gender, known to be independent of survival, will be used to validate our test population. Tumor grade will be included due to controversy over its association with survival. Allelic status will be addressed in order to confirm or refute recent studies associating LOH on 1p/19q with chemosensitivity and overall prolonged survival. As there is an established association between 1p/19q LOH and indistinct borders on T1 weighted pre-gadolinium MRI, this feature will be the focus of our study. We seek to confirm the association between indistinct tumor borders on T1 weighted pre-gadolinium MR imaging and 1p/19q LOH while also determining if there is an association between indistinct tumor borders and survival. This finding would suggest that MR can be used as a prognostic factor and determinant of therapy when allelic status of the tumor is unknown.

Methods

Case selection. Cases were chosen from patients seen at the Yale Brain Tumor Center between 1995 and 2005 and selected based on fulfillment of all eligibility criteria. Demographic information including date of diagnosis, age, and gender were collected. Eligibility criteria included availability of original pathology, age over 16 years, pre-operative MR imaging, and survival data. A search of the Department of Pathology database revealed 260 pathology reports with a final diagnosis of "oligodendroglioma." These reports were pulled and reviewed. 43 cases represented tumor recurrences and were therefore excluded. 74 cases had age under 16 years or lack of age data and were therefore excluded. Pre-operative MRI studies were acquired from Yale-New Haven Hospital's online database or original films were reviewed from outside hospitals. 50 patients for whom imaging was not available were excluded from the study. Survival information was obtained from the Yale-New Haven Hospital Tumor Registry and from private clinic notes. Length of survival was calculated as the number of days from the date of tissue diagnosis to death or date of last follow-up. Time of progression free survival could not be included as an alternative outcome as that information was not consistently available. Ultimately 89 patients were included in the study, 36 of whom had known allelic status.

Pathology review. Pathology reports were reviewed. Only those with a final diagnosis of oligodendroglioma or anaplastic oligodendroglioma were included. Histopathologic features evaluated included predominant cell type, presence of endothelial proliferation, presence of necrosis, and Ki-67 labeling index. Histologic grade was determined by pathologist statement. When the pathologist did not make a

definitive comment, tumors were designated grade III if endothelial proliferation and necrosis were present along with a Ki-67 labeling index over 5.0. Genetic analysis reports were amended to the original path reports. Allelic status was determined in one of two ways. The allelic status of one subset was determined using PCR technology at Johns Hopkins University. After the technology became available at Yale-New Haven Hospital, the remainder of patients underwent LOH analysis using FISH. Studies have demonstrated comparable sensitivity and specificity between these two techniques.

Image analysis. MRI scans were assessed by medical student HM and neuro-oncologist JB. Both reviewers were blinded to genetic analysis and survival information. Only T1-weighted pre-gadolinium, T1 weighted post-gadolinium, and T2-weighted MR images were consistently available. Each T1-weighted pre-gadolinium image was evaluated qualitatively for distinct (see Figure 1) vs. indistinct (see Figure 2) tumor borders. Tumors deemed to have "distinct borders" were noted to have sharp tumor edges such that the distinction between tumor and surrounding normal tissue clear. These tumors also tended to also appear more hypointense and homogeneous. Tumors deemed to have "indistinct borders" were noted to lack a clear transition point between tumor and surrounding normal tissue. These tumors also tended to appear more heterogenous and isointense compared to grey matter. T2 weighted images were not used as previous studies indicated T1 weighted imaging characteristics are more likely to be associated with genetic status. CT scans were not consistently available to evaluate for calcium contents. Intra and inter observer correlation rates were not calculated. However, observationally there were few discrepancies.

Statistical considerations. Data were entered in excel spreadsheet and analyzed using SAS (version 9.1 SAS Institute, Cary, NC). Two-sided chi-square tests were used to assess differences in death rate in gender, tumor grade, allelic status and MRI characteristics. In case were the expected cells were small, Fisher's exacts tests were employed. Kaplan-Meier survival curves were used to calculate the estimated cumulative survival rate for gender, tumor grade, allelic status, and MRI characteristics. The differences in the survival rate were tested by means of the two-sided log-rank test. Cox proportional hazard modeling was used to examine covariate effects on survival rate. One way ANOVA and generalized linear modeling (GLM) were used to assess the difference in means of surviving days for each outcome measure defined by specific criteria. In all analysis a two tailed alpha of < 0.05 was considered statistically significant.

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Results

89 patients were included and consisted of 43 males and 46 females. The mean age at diagnosis was 43.3 years with a range of 16 to 85. The average survival was 1573 days with 86% of patients still alive. Clinical, radiographic, and genetic features are summarized in Table C.

Gender, tumor grade, allelic status on 1p/19q and imaging characteristics were not significantly associated with survival (See Table D, E). 36 females (84%) were still alive while 39 males (80%) were still alive (p=.7696). The mean survival for females was 1193.47 days, for males 1909.89 days (p=.0765). The Kaplan-Meier survival analysis for gender likewise failed to reveal a significant difference between groups (see Figure 3). 72 patients were diagnosed with "oligodendrogliomas grade II," 16 with "oligodendroglioma grade III." Of patients with grade II tumors, 64 (89%) were alive with a mean survival of 1606.69 days. Of patients with grade III tumors, 12 (71%) were alive (p=.1185) with a mean survival of 1431.88 days (p=.7338). Likewise, Kaplan-Meier survival analysis failed to reveal a significant difference (see Figure 4).

36 (40%) of patients had know allelic status. Of these, 28% harbored LOH on 1p, 36% had LOH on 19q, 22% LOH on 1p and 19q, while 56% had intact 1p and 19q. Interestingly, of patients with known allelic status, only 2 patients had died making statistical analysis of morbidity data impossible (See Figure 5). Mean survivals days can be analyzed, however they have limited utility in determining long-term survival given the short period of follow-up. Patients with 1p LOH had a mean survival of 676.81 days while those with retained heterozygosity survived a mean of 564.08 days (p=.6871). Patients with 19q LOH had a mean survival of 691.93 days while those with retained

heterozygosity survived a mean of 539.09 days (p=.5628). While there is a large absolute difference in 1p and 19q LOH survival, significant variability and imbalance within the data set contributed to failure to achieve statistical significance.

MRI characteristic analysis revealed 37% of patients had distinct borders while 63% had indistinct borders. 24 (80%) of patients with distinct borders were still alive with a mean survival of 1874.97. 47 (89%) of patients with indistinct borders were still alive (p=.3366) with a mean survival of 1369.47 days (p=.2515). Kaplan-Meier survival analysis failed to demonstrate a significant difference (see Figure 6).

Cox proportional hazard ratio also failed to reveal any of the potential prognostic factors as significant independent predictors of survival. Age, gender, and tumor grade were included in the model while allelic status and imaging characteristics were not due to model distortion.

Finally, no association was found between allelic status and MR imaging characteristics. Of patients with 1p LOH, 8 (80%) had indistinct borders while 2 (20%) had distinct borders. Conversely, of patients with retained heterozygosity on 1p, 14 (64%) had indistinct borders while 8 (46%) had distinct borders (p=.4399). Of patients with 19q LOH, 9 (69%) had indistinct borders while 4 (31%) had distinct borders. Of patients with retained heterozygosity on 19q, 13 (68%) had indistinct borders while 6 (32% had distinct borders (p=1.000)

Discussion

New discoveries in the pathogenesis and therapeutic sensitivity of oligodendrogliomas represent some of the most significant progress within neuro-oncology over the past 20 years. The association between 1p/19q LOH and chemosensitivity has revolutionized therapeutic decision making. While the use of 1p/19q testing has become increasingly widespread for its diagnostic and therapeutic importance, its use remains limited to major academic institutions. Further investigation of prognostic factors for oligodendrogliomas remains important as the significance of genetic mutations continues to unravel.

Our study sought both to confirm established prognostic factors and investigate the utility of MRI as an additional clinical prognostic factor. A single-institution, retrospective study yielded a very limited number of subjects leading to difficulties with statistical power to detect significant differences. This major limitation is manifested by our data departing significantly from previous literature on several key, fundamental points. First, previous descriptive studies have established the expected male-to-female gender ratio to be as much as 3.0.[6] Our studies revealed a departure from this expectation with an almost equal distribution between the genders. This simple inconsistency may reflect the potential biases in our sample due to the relatively small number of cases. We did find gender to be independent of survival as previous studies have shown. In fact, the Kaplan-Meier survival curve for females and males follow almost the exact same path confirming that males and females with olidodendrogliomas do equally well.

The mean age at diagnosis for our patients was 43.3 years. In previous studies, age has persistently proven the strongest prognostic factor in oligodendrogliomas yet

failed to predict survival in our study. Of note, most previous studies have approached the question of age by grouping patients into categories such as those patients under 30, aged 30-50, and those over 50 years.[44] We took a different approach and used age as a continuum in order to decipher if there is a linear, and not just categorical, relationship between age and mean survival. However, we found neither absolute survival nor mean survival days to be associated with age at diagnosis. Thus, it may not be correct to assume older age has a linear relationship to worsening prognosis. Clinically, it may be more useful to risk stratify patients into age categories such that those over 50 years old having expected shorter survival than those younger but not differentiate between two patients who are both over 50 years. However, again our study is limited by statistical power secondary to small sample size.

While multiple studies have correlated tumor grade to survival, others have found no such correlation but relied instead on individual aspects that contribute to grading. While advanced tumor grade should be, a priori, associated with poorer survival, this has been inconsistently demonstrated in the literature. Our study found no association between tumor grade and survival. Although the Kaplan-Meier survival curve looks promising, it did not achieve statistical significance. Factors influenced by histopathological grade such as degree of surgical resection and subsequent treatment decisions may play a stronger role than the aggressiveness of the tumor histopathology itself. We did not address individual characteristics such as Ki-67 labeling index, the presence of necrosis, or endothelial hyperplasia. Addressing these characteristics instead of the tumor grade may prove more revealing.

The frequency of 1p/19q LOH in oligodendrogliomas ranges from LOH on 1p in 40-92% averaging 83%[27], LOH on 19q in 50-80% averaging 66%.[8, 27] Our study found that of the small number of patients with known allelic status, only 28% had LOH on 1p and 36% had LOH on 19q. This frequency more closely approximates the earlier, smaller studies whereas it is the more recent, larger scale studies that reveal higher frequencies. There are many potential reasons for the disparity. First, we had a relatively small sample size with only 36 patients having known allelic status. This small number may lead to a number of potential selection biases. For example, prior to the widespread acceptance of the importance of determining allelic status, genetic testing may have been done only in those patients with questionable histopathology and not on those patients with clear oligodendrogliomas. Additionally, two different tests were used to determine allelic status: some patients were tested using PCR at an outside institution while others were tested using FISH in our own Department of Pathology. While there are no known disparities of significance in the sensitivity and specificity of these two techniques, there is the possibility that this inconsistency biased the results. Even within the small sample size of patients with known allelic status, the rarity of LOH on 1p/19q further compromised our statistical power. In addition, of those patients with know allelic status, only 2 patients had expired. Previous studies have addressed populations with less than 50% of patients still alive. The longevity of these patients made determining an association between 1p/19q LOH and absolute survival impossible. Likewise, addressing mean survival days was also challenging as these numbers reflect time since diagnosis and not necessarily length of survival given the short follow-up time. Longer follow-up times are needed to confirm the association between 1p/19q LOH and longer survival.

The major focus of our study was to address MRI characteristics as a possible independent prognostic factor as previous studies have demonstrated an association between certain features and 1p/19q LOH. Specifically, studies have found that T1 pre-gadolinium indistinct tumor borders, presence of susceptibility effect, heterogeneous signal intensity, and frontal lobe location are all associated with LOH on 1p/19q.[7, 32] Establishing the association of any of these imaging features with improved outcome would benefit areas where genetic testing has not yet come into widespread use and allow the clinician to offer the patient prognostic information even before a tissue diagnosis is made. We chose to address T1 pre-gadolinium tumor border characteristics as T1-weighted images are most consistently available to clinicians. In our study, tumor imaging on T1 weighted, pre-gadolinium MRI was classified as having either a "distinct border" or an "indistinct border." We found no association between border characteristics on MRI and 1p/19q status or survival. While this is an unexpected result given previous studies demonstrating the association between this imaging feature and 1p/19q status, there are multiple reasons why detecting a difference between these two groups proved challenging. First, we found that the difference between distinct and indistinct borders was not always clear. Tumors located in the temporal lobe were particularly challenging due to the size of sulci. When a tumor border abutted a sulcus, the border appeared deceptively sharp in that cut. Tumors directly adjacent to ventricles could also have deceptively distinct borders. Each of these biases was minimized by analyzing the tumor characteristics in both coronal and sagittal cuts. Finally, oligodendrogliomas are notoriously heterogeneous with multiple areas of distinct and indistinct borders within the same tumor. Given these discrepancies, additional characteristics occasionally aided in

determining if the tumor should be designated as having distinct or indistinct borders. While these characteristics have not been addressed in previous studies, they proved subjectively useful in our study. We found that tumors with indistinct borders tended to be more isointense with respect to gray matter while tumors with distinct borders tended to appear hypointense. Tumors with indistinct borders also appeared more heterogeneous while tumors with distinct borders were frequently homogeneous. These challenges would suggest that while MR characteristics can not be used as a prognostic factor, we hesitate to definitively rest on these findings. Our hesitation comes from our limited power to detect a difference and the inconsistencies shown in other factors we addressed. Further study addressing other imaging characteristics and developing additional criteria to distinguish between distinct and indistinct borders may prove useful. If reliably available, cystic changes on FLAIR images may also be a useful radiographic prognostic marker.[5] The presence of calcium, traditionally considered an ominous histopathologic sign, can only be reliably detected through MRI using gradient echo images, a technique that is not yet standard on all MRIs. If these images could be obtained reliably, their prognostic value could prove interesting.

Our data set contains multiple factors that limited our ability to significantly detect the importance of various prognostic factors, including confirming the importance of 1p/19q LOH and determining the significance of MRI characteristics. Having only a small number of patients leads to limited statistical power. This small sample size was due to both the relative rarity of the tumor and the limitations in our ability to obtain pathologic data, pre-operative imaging, allelic status, and survival information on many of the patients. Sample size remained small due to the limited clinical use of 1p/19q data

as this test has been used clinically for less than 5 years. Another interesting point is the survival of the patients. Where other studies have considered samples where approximately 50% of patients had died,[44, 46] our sample had only 15% of patients expired. This may be due to the relatively short amount of follow-up time. Where our mean follow-up time was 1,573 days (approximately 52 months), other studies have averaged 6.6[46] to 8.7 years.[44] This observation proves important in light of the average survival of 11.6 years for patients with Grade II tumors.[2]

There are numerous potential prognostic factors that we did not directly address in this study that should be re-examined in light of new data on allelic status. Demonstrated prognostic factors such as presenting symptom, presence of neurological deficits at presentation, frontal lobe location, blood type, and Ki-67 labeling indices require further study and were unfortunately largely unavailable for the current project. Likewise, growth across the midline has been associated with poorer survival.[46] In other studies, this growth pattern has been associated with the presence of 1p/19q LOH which would thus indicate increased chemosensitivity and improved survival.[7] This discrepancy requires further investigation. Finally, PET technology has great potential as a prognostic factor that has not yet been studied. While this study attempted to further investigate the role of prognostic factors in oligodendrogliomas, further work needs to be done in light of the dramatic molecular advances in tumor diagnosis and therapy.

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Appendix

Table A WHO Classification of Gliomas[1]

Astrocytic tumors	**Ependymal tumors**
Diffuse astrocytoma	Ependymoma
Fibrillary astrocytoma	Cellular
Protoplasmic astrocytoma	Papillary
Gemistocytic astrocytoma	Clear cell
Anaplastic astrocytoma	Tanycytic
Glioblastoma	Anaplastic ependymoma
Giant cell glioblastoma	Myxopapillary ependymoma
Gliosarcoma	Subependymoma
Pilocytic astrocytoma	
Pleomorphic xanthroastrocytoma	
Subependymal giant cell astrocytoma	
Oligodendroglial tumors	
Oligodendroglioma	
Anaplastic oligodendroglioma	

Table B Prognostic factors in oligodendrogliomas

Unfavorable	*Favorable*	*Not demonstrated*
Older age, especially >50 years old	Younger age, especially <30 years old	
		Gender
Contrast enhancement		
Tumor crosses the midline	Frontal location	
Neurological deficits at diagnosis	No Neurological deficits at diagnosis	
	Presenting symptom seizures	
Subtotal resection	Gross total resection	
WHO Grade III	WHO Grade II	
Vascular changes	No vascular changes	
Higher Ki-67 index, especially	Lower Ki-67 index, especially	
10q deletion, 8q gain	Chromosome 1p and/or 19q deletion	
P16/CDKN2A deletion		EGFr expression
Increased p53		
Type A Blood		

Table C Clinical, radiographic, and genetic features

Total number of patients	89	
Patients without genetic studies	53	(60%)
Patients with genetic studies	36	(40%)
Mean age at diagnosis (years)	43.3	(16-85)
M/F (*n*)	43/45	
Tumor grade		
Grade II Tumors	72	(82%)
Grade III Tumors	16	(18%)
Imaging		
Tumor with distinct border on T1 MRI	30	(37%)
Tumor with indistinct border on T1 MRI	51	(63%)
Genetics		
Patients with known allelic status	36	
1p LOH	10	28%
19q LOH	13	36%
1p and 19q LOH	8	22%
1p and 19q intact	20	56%
Survival		
Alive	76	(86%)
Dead	12	(14%)
Mean survival (days)	1573	(30-8897)

[a] LOH, loss of heterozygosity

Table D Statistical analysis of morbidity results

Variable	Alive	Dead	P-value*
Gender			.7696
F	36	7	
M	39	6	
Tumor grade			.1185
WHO Grade II	64	8	
WHO Grade III	12	5	
MRI			.3366
Distinct border	24	6	
Indistinct border	47	6	
Allelic status on 1p			.5238
1p LOH	10	1	
1p heterozygosity	24	1	
Allelic status on 19q			.5111
19q LOH	14	0	
19q heterozygosity	20	2	

* Fisher's exact test

Table E Statistical analysis of mean survival days

Variable	Mean survival	SD	P-value*
Gender			.0765
Male	1909.89	2361.35 (30-8897)	
Female	1193.47	1160.55 (186-4867)	
Tumor grade			.7338
WHO Grade II	1606.69	1825.51 (32-8897)	
WHO Grade III	1431.88	2202.21 (30-7333)	
MRI			.2515
Distinct border	1874.97	2246.33 (32-8733)	
Indistinct border	1369.47	1720.21 (30-8897)	
Allelic status on 1p			.6871
1p LOH	676.81	874.13 (41-2523)	
1p heterozygosity	564.08	717.51 (30-3545)	
Allelic status on 19q			.5628
19q LOH	691.93	796.03 (41-2523)	
19q heterozygosity	539.09	745.03 (30-3545)	

* Generalized linear model

Table F **Cox proportional Hazard Analysis**

Variable	Hazard ratio	Pr > Chi-square
Age	1.012	0.4920
Gender	0.930	0.9088
Tumor grade	1.897	0.2977

Table G **Statistical analysis of relationship between allelic status and imaging characteristics**

Variable	Distinct borders	Indistinct Borders	P-value*
Allelic status: 1p			.4399
LOH	2	8	
Heterozygous	8	14	
Allelic status: 19q			1.000
LOH	4	9	
Heterozygous	6	13	

Table H Abbreviations

1p	Short arm of chromosome 1
19q	Long arm of chromosome 19
1p/19q LOH	LOH on 1p and/or 19q
AAD14	Gene encoding AAD14 protein
CAMTA1	Calmodulin binding transcription activator 1
CDK4	Cyclin-dependent kinase 4
CDKN1A	Cyclin-dependent kinase inhibitor 1
CDKN2A/p14^{ARF}	Cyclin-dependent kinase inhibitor 2A and encoded protein p14 or p16
COX, COX-1a	Cyclooxygenase, Cyclooxygenase subtype 1a
CT	Computer tomography
DNT	Dysembryoplastic neuroepithelial tumor
EGFR	Endothelial growth factor receptor
FISH	Fluorescence in situ hybridization
GABA-BR1A	GABA-B receptor 1A subunit
GalC	Galactocerebroside
GBM	Glioblastoma multiforme
GFAP	Glial fibrillary acidic protein
Histone H4	Histone subtype H4
IFI-56K	Interferon-induced 56-kDa protein
ITGB4	Integrin beta 4
JNK2, JNK3 A2	c-jun N-terminal kinase 2, 3 alpha 2

Jun-D	Gene encoding protein Jun-D
Ki-67	Nuclear protein marker expressed only when cells are actively dividing
LOH	Loss of heterozygosity
MIB-1 Ab	Antibody marker of Ki-67
MRI	Magnetic Resonance Imaging
Olig2	Oligodendroglial lineage gene number two
P190RhoGAP	RhoA-specific GAP
PCDH43	Protocadherin 43 precursor
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
RB1	Retinoblastoma gene 1
Rho7	Gene encoding protein Rho7
RhoC	Gene encoding for protein RhoC
TDGF1	Teratocarcinoma-derived growth factor 1
TGF-β	Transforming growth factor-beta
WHO	World Health Organization
YLK-40	A secreted glycoprotein

Figure 1 **48 year old man with tissue diagnosis of oligodendroglioma with retained heterozygosity on 1p and 19q demonstrating distinct borders on axial T1 pre-gadolinium magnetic resonance imaging.**

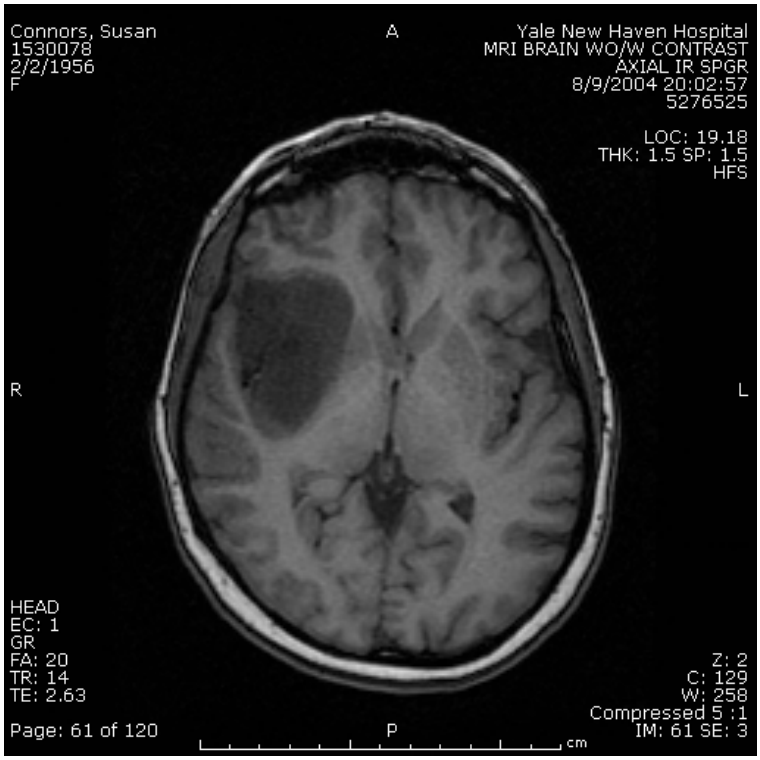

Figure 2 **46 year old man with tissue diagnosis of oligodendroglioma with loss of heterozygosity on 1p and 19q demonstrating indistinct borders on axial T1 pre-gadolinium magnetic resonance imaging.**

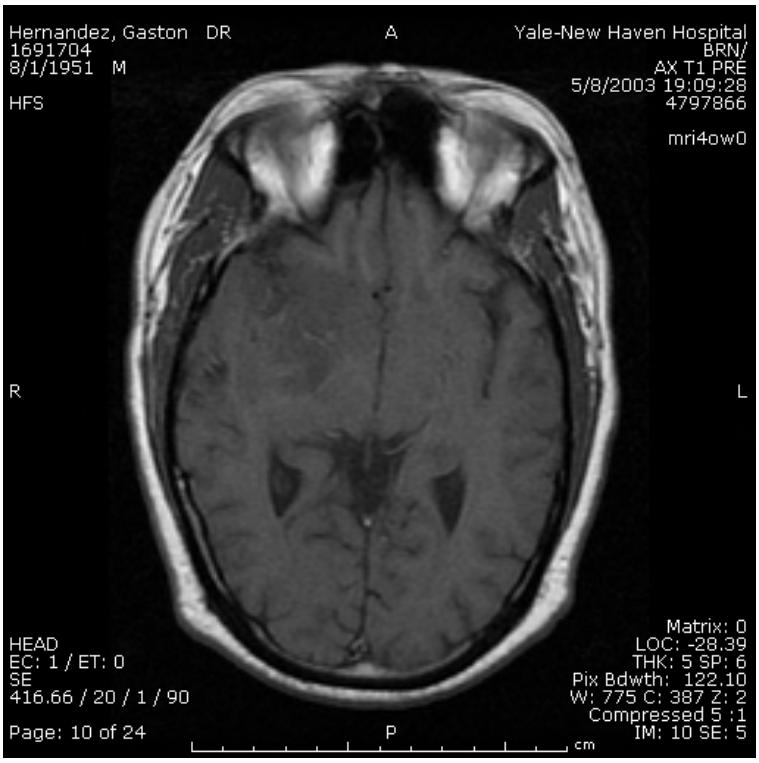

Figure 3 **Kaplan-Meier survival analysis for gender. The two groups did not have significantly different survival.**

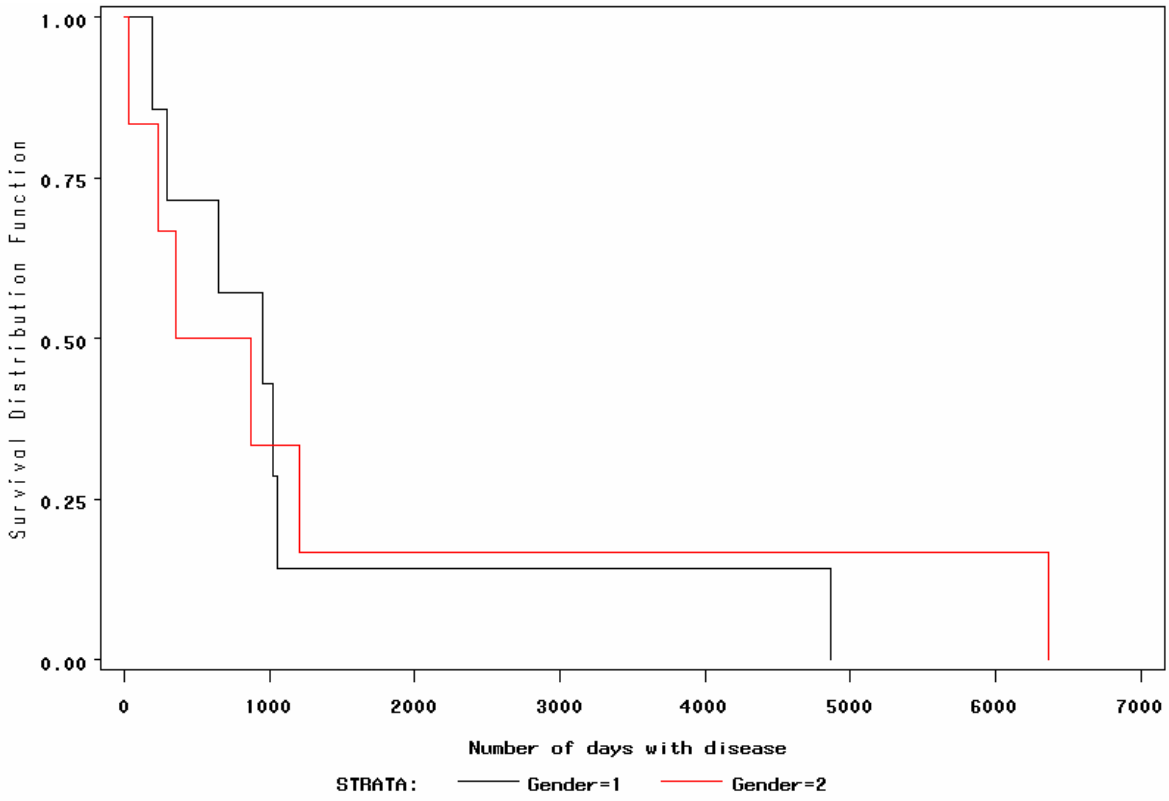

Figure 4 **Kaplan-Meier survival analysis for tumor grade. The two groups did not have significantly different survival.**

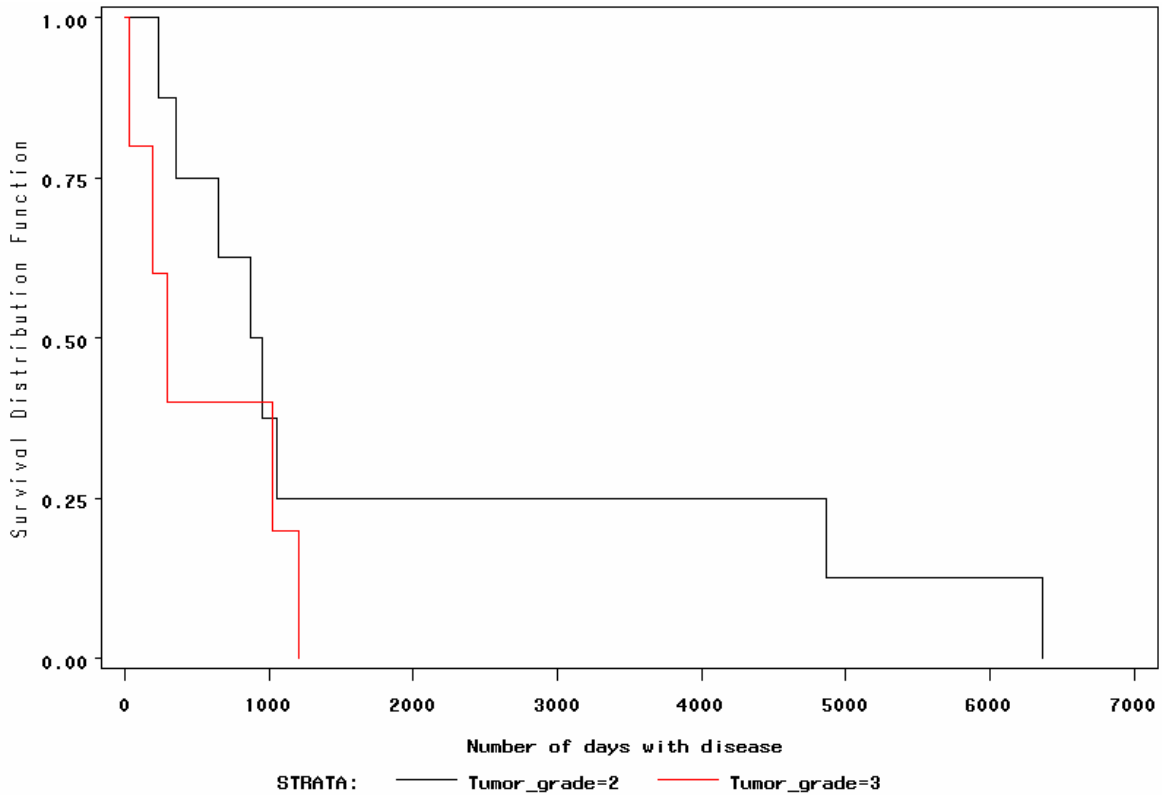

Figure 5 **Kaplan-Meier survival analysis for allelic status on chromosome 1p. The two groups did not have significantly different survival.**

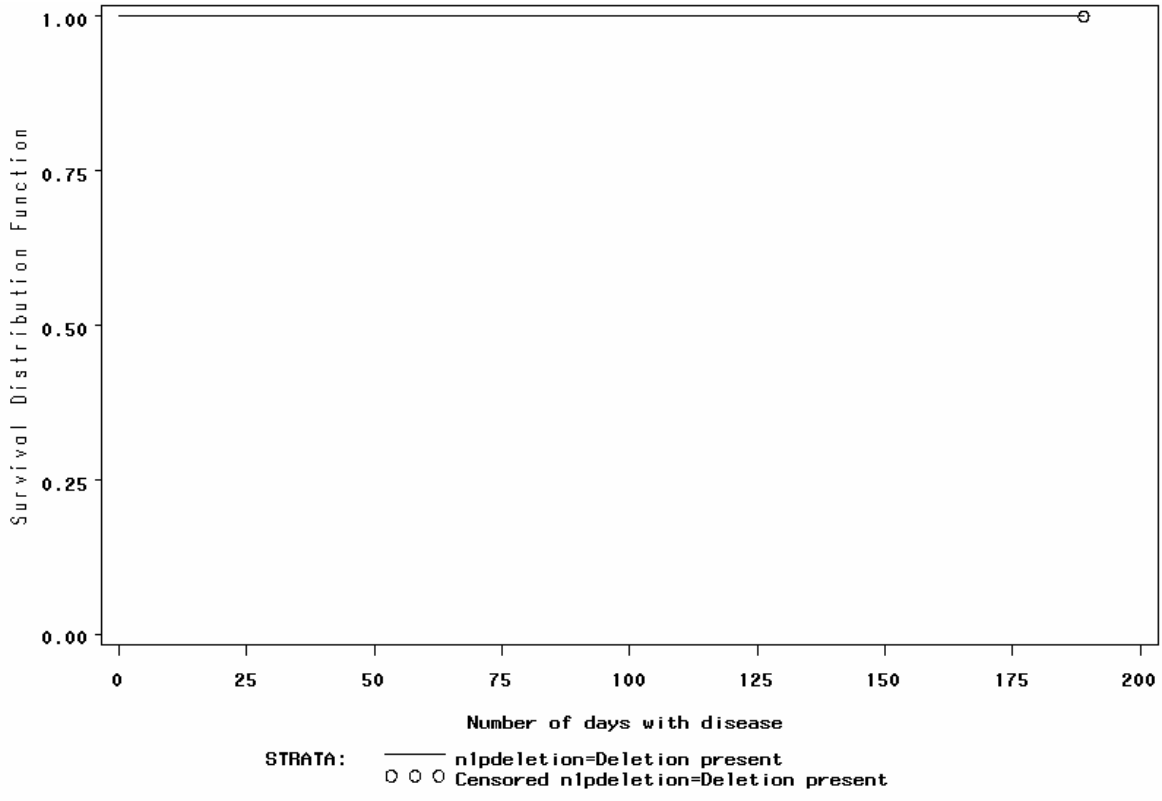

Figure 6 **Kaplan-Meier survival analysis for tumor border characteristics on T1 pre-gadolinium MRI. The two groups did not have significantly different survival.**