[Frontiers in Bioscience 5, d213-231 January 1, 2000]

GENETIC ALTERATIONS IN ADULT DIFFUSE GLIOMA: OCCURRENCE, SIGNIFICANCE, AND PROGNOSTIC IMPLICATIONS

Justin S. Smith and Robert B. Jenkins

Department of Laboratory Medicine and Pathology, Mayo Clinic and Foundation, 200 First Street Southwest, Rochester, Minnesota, 55905

TABLE OF CONTENTS

1. ABSTRACT

Our understanding of diffuse glioma development and progression has expanded remarkably over the past decade. As the genetic alterations responsible for these tumors are identified, molecular models of glioma pathogenesis are emerging and hold great promise to explain the biologic mechanisms of these neoplasms. Although these models continue to evolve and remain highly simplified, some of the genetic alterations that they encompass appear to be prognostically useful. Among the astrocytic gliomas, age and tumor grade are the most powerful indicators of patient survival, however, a wide range of variability remains, particularly among the low-grade and anaplastic astrocytomas. Recent reports indicate that alterations of the PTEN tumor suppressor gene are independent predictors of overall survival for anaplastic astrocytoma patients, helping to distinguish the cases with behavior resembling their more malignant counterparts, the glioblastomas. Among the oligodendroglial tumors, alterations of the 1p and 19q chromosome arms have emerged as potentially powerful predictors of overall patient survival and *in vivo* chemotherapeutic response, while alterations of the p16/CDKN2A tumor suppressor gene suggest shorter overall survival. As our molecular models continue to improve, through functional analyses and the identification of additional genetic contributors, we will expand our capacity to more effectively prognose these patients and to design rational therapeutic strategies.

2. INTRODUCTION

Diffuse gliomas exhibit an inherent tendency to progress to more malignant phenotypes and a broad range of clinical behavior. Classification of diffuse gliomas by histologic subtype (figure 1) and malignancy grade (figure 2),

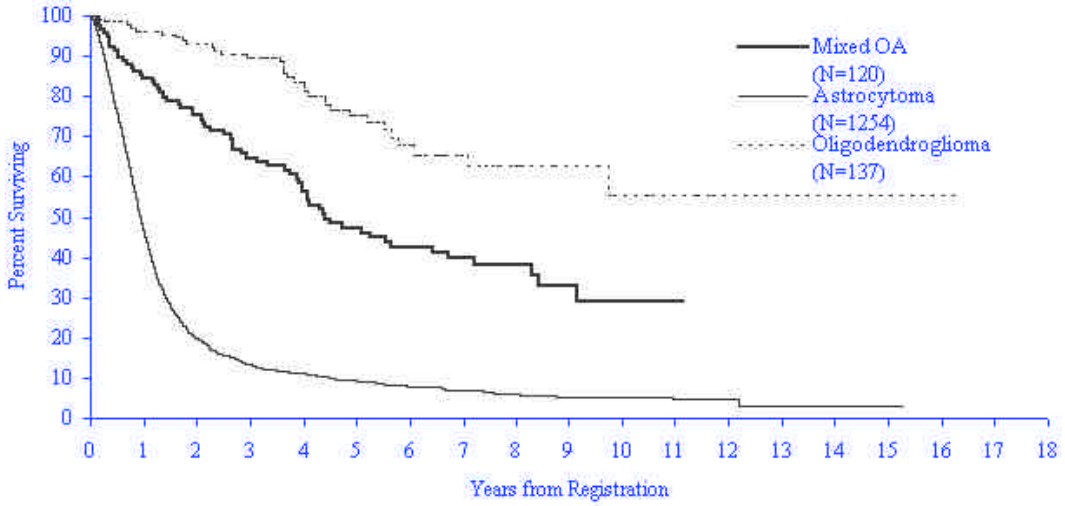

Figure 1. Patient survival by glioma histologic subtype. Data represent 1,511 glioma patients entered on North Central Cancer Treatment Group (NCCTG) therapy trials. Subtype is based on World Health Organization (WHO) guidelines. Log rank p-value <0.0001.

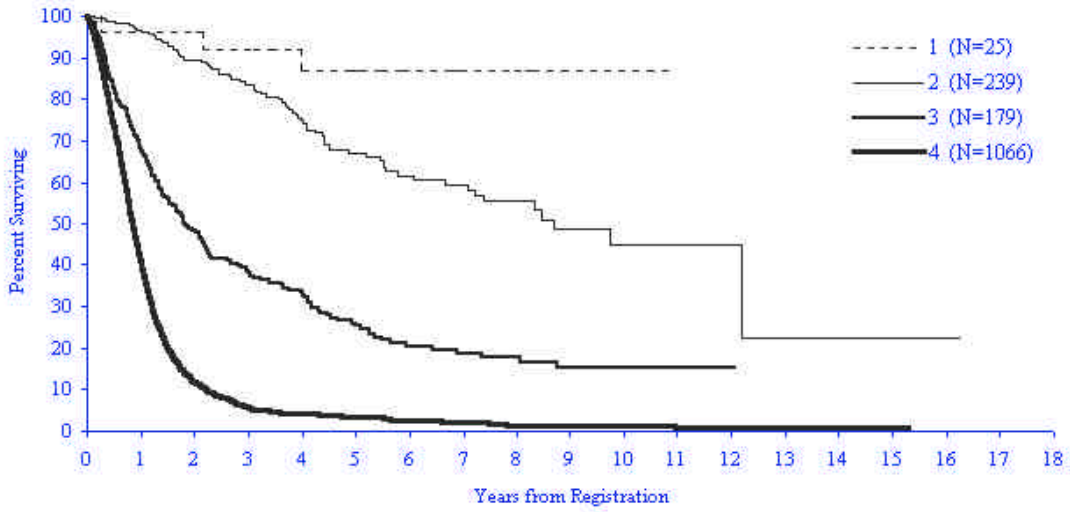

Figure 2. Patient survival by malignancy grade. Data represent 1,509 glioma patients entered on North Central Cancer Treatment Group (NCCTG) therapy trials. Grade is based on World Health Organization (WHO) guidelines. Log rank p-value <0.0001.

in addition to simple clinical variables such as patient age and performance score, remain the gold standards for determining prognosis and treating these patients. However, our abilities to effectively determine the prognosis and to meaningfully stratify diffuse glioma patients for therapy remain limited. Molecular genetic analyses are beginning to provide clinically useful information and promise to extend the capacities of our current approaches. This review provides an overview of the known genetic alterations associated with adult diffuse gliomas and provides discussion of their occurrence, significance, and prognostic implications.

3. ASTROCYTOMAS

Diffuse astrocytic gliomas are defined as tumors consisting primarily of neoplastic astrocytes and comprise approximately 90% of the diffuse gliomas. These tumors represent a continuum from well-differentiated to highly anaplastic lesions and are graded according to histologic features. The current World Health Organization (WHO) criteria (1) for grading astrocytic gliomas notes the presence of nuclear atypia, mitoses, endothelial proliferation, and necrosis, without placing emphasis on any particular feature. According to this system, astrocytomas

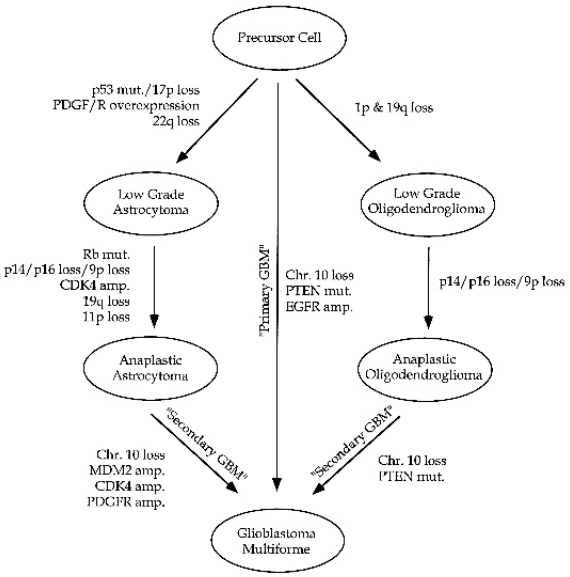

Figure 3. Possible genetic pathways of glioma progression. Histologic subtype and malignancy classifications are based on current WHO guidelines (1).

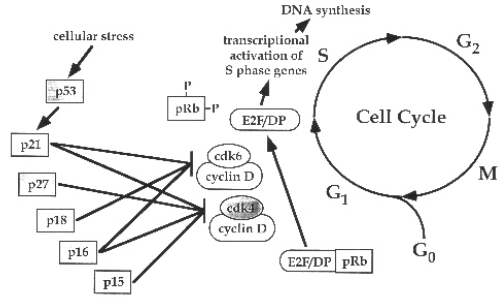

Figure 4. Cell cycle and proteins involved in the regulation of the G_1-to-S transition. Cell cylce inhibitors are squared, and activators are circled. See text for discussion of shaded componenets. (Modified from De Beeck AO and Caillet-Fauquet P: Viruses and the cell cycle. *Prog Cell Cycle Res* 3:1-19, 1997).

with one of these features are classified as low-grade astrocytomas (grade II), those with two features are termed anaplastic astrocytomas (grade III), and those with either three or four features are termed glioblastomas multiforme (grade IV). Notably, WHO grade I is reserved for special histologic variants of astrocytoma with excellent prognoses following surgical resection. These variants include juvenile pilocytic astrocytoma, subependymal giant cell astrocytoma, and pleomorphic xanthoastrocytoma. Based on their lack of diffusely infiltrating growth and rare capacity for malignant progression, these tumors are not considered among the diffuse gliomas.

The multi-step process of tumorigenesis involves initiation, proliferation, and eventually invasion of the surrounding environment. Astrocytic gliomas, with their inherent tendency to progress to more malignant phenotypes, have served as powerful substrates for the modeling of these processes, and in this regard, their study has been remarkably productive. Several investigators have studied the genetic alterations associated with the various stages of astrocytoma development and progression and have proposed models to account for these associations (2-7). Possible genetic pathways of glioma progression in relation to known genetic alterations are depicted in figure 3.

3.1. Clinical features of diffuse astrocytic glioma patients

3.1.1. Low-grade astrocytomas

Low-grade diffuse astrocytomas may occur at any site in the central nervous system but favor the cerebral hemispheres and generally afflict patients in the fourth or fifth decades of life. These patients frequently present with seizures and/or symptoms of increased intracranial pressure, and treatment typically involves surgical resection of the symptomatic lesion (8-10). Since the effectiveness of postoperative radiation therapy remains uncertain, some clinicians prefer to reserve its use for recurrent lesions (11-13), and no effective role for chemotherapy has been defined for these patients. The mean survival time following surgical resection is approximately 6-8 years, though there is considerable individual variation, with some cases remaining relatively latent for several years and others progressing rapidly to anaplastic astrocytoma or glioblastoma multiforme (14). Our current capacity to distinguish these latter subsets of patients and to determine the prognosis of low-grade astrocytoma patients in general remains limited and primarily based on clinical features, including patient age, functional status, and extent of surgical resection (15-19). Additional indicators are needed in order to provide more effective prognostic information to these patients and to better design treatment strategies.

3.1.2. Anaplastic astrocytomas

As with low-grade diffuse astrocytomas, anaplastic astrocytomas may occur at any site throughout the central nervous system but most often occur in the cerebral hemispheres. Also similar to the patients with low-grade lesions, anaplastic astrocytoma patients most frequently present with seizures or symptoms resulting from increased intracranial pressure (9, 10). While these tumors may result from progression of a low-grade astrocytoma, they are often diagnosed on first biopsy without indication of a precursor. Surgical resection is generally indicated for these patients, however, since neoplastic cells from these tumors typically migrate away from the main tumor mass, total resection is not possible. Postoperative radiation appears to prolong survival in these patients and is often applied regardless of the extent of resection (20, 21). Recognized overall prognostic indicators include age, functional status, and residual tumor mass (22, 23). The median survival of these patients is two years, and approximately 15% have a five year survival (14). However, providing individual prognosis is difficult, since the latency and rapidity of progression vary considerably, thus affirming the need for additional prognostic markers.

3.1.3. Glioblastoma multiforme

Glioblastomas represent 20-25% of all intracranial tumors and 50% of gliomas (24), most commonly occur in the cerebral hemispheres, and typically afflict patients in the fifth or sixth decade. These tumors may arise from low-grade astrocytomas or anaplastic astrocytomas that have undergone malignant progression, termed "secondary glioblastoma", or they may arise *de novo*, termed "primary glioblastoma" (25) (figure 3). Presenting symptoms of increased intracranial pressure (e.g. headaches, nausea, and vomiting), mental status changes, and motor deficits are common in glioblastoma patients, while seizures are considerably less common (10, 26). Despite their often circumscribed appearance with imaging studies, glioblastomas typically exhibit extensive infiltration of surrounding tissues, making complete resection impossible. Resection is usually indicated, however, for palliative measure, to improve the effectiveness of adjuvant therapies, and to obtain tissue for pathologic diagnosis. Furthermore, retrospective studies suggest that extent of tumor removal correlates with survival (27). Postoperative radiation therapy provides only a modest prolongation of survival, and chemotherapy is only marginally effective and typically used following surgical resection and radiation therapy (20). The effectiveness of newer therapeutic options, such as stereotaxic radiosurgery and interstial brachytherapy, remains under evaluation (28-30). Median survival is approximately 10 months, with fewer than 20% surviving longer than one year and approximately 5% surviving longer than five years (14). Prominent prognostic factors are simple clinical indicators, including patient age and performance status (23, 31, 32).

3.2. Genetic alterations and prognostic implications

3.2.1. p53, MDM2, and p14^{ARF}

The p53 tumor suppressor gene serves as the "guardian of the genome" by coordinating a complex series of responses to DNA damage (33). Following DNA damage, p53 protein levels are upregulated due to increased synthesis and prolonged stabilization of the protein (34-37). Wild type p53 then induces the transcription of genes that promote cell cycle arrest or apoptosis, including the p21 and BAX genes (38-42). Mutated p53 lacks the ability to induce cell cycle arrest or apoptosis, allowing cells to accumulate mutations and chromosome abnormalities (33, 41, 42). In addition, wild-type p53 has been implicated as a suppressor of angiogenesis, and thus, mutated p53 may also promote neovascularization (43-45).

Mutation of p53 is among the earliest and most frequent events in astrocytic glioma development, occurring in approximately 40% of low-grade and anaplastic astrocytomas (46-48). In glioblastomas, p53 mutation is associated with secondary tumors, which evolved from low-grade or anaplastic astrocytomas, and is rarely observed in primary tumors, which developed clinically *de novo* (49) (figure 3). Thus, while the incidence of p53 mutation in unselected series of glioblastoma is approximately 40%, separately, primary and secondary glioblastomas exhibit incidences of approximately 10% and 65%, respectively (49).

Based on the apparent capacity of p53 mutations to distinguish primary and secondary glioblastomas, several investigators have hypothesized that these alterations may be prognostically relevant. However, whether searching for p53 gene mutation or p53 protein accumulation, the distinct preponderance of these studies indicates that alteration of p53 is not an independent predictor of astrocytoma patient survival, regardless of histologic grade (31, 46, 47, 50-64). Tachibana *et al*. (47) identified p53 mutation as a strong univariate predictor of prolonged overall survival in a set of 66 similarly treated anaplastic astrocytoma patients. However, after adjusting for the effects of patient age through multivariate analyses, the predictive value of p53 mutation status did not reach statistical significance.

A limited subset of studies do suggest that p53 alterations are of prognostic relevance (65-67). van Meyel *et al*. (65) searched for p53 mutations in 15 low-grade astrocytic gliomas that had progressed to high-grade gliomas, examining both the initial and recurrent tumors. Patients with p53 mutant tumors survived nearly twice as long as those without mutations, and, while there was no significant difference in recurrence-free survival, there was a significant difference in post-recurrence survival. Furthermore, these authors suggested that low-grade tumors that recurred as anaplastic gliomas exhibited p53 gene mutation, while those that recurred as glioblastomas had intact p53 genes. This limited number of cases, however, was selected for recurrence and progression and may not be representative of all low-grade tumors.

Watanabe *et al*. (66) analyzed 144 biopsies from 67 patients with recurrent astrocytoma for p53 alterations and identified mutations in 46 of 67 patients (69%) in at least one biopsy. Among the 28 low-grade astrocytomas studied, the p53 status remained the same in 95% of the cases, irrespective of whether the recurrent lesion had the same or higher grade of malignancy. In addition, progression of low-grade astrocytomas to anaplastic astrocytomas or glioblastomas occurred at similar frequencies in cases with and without p53 mutations. Thus, these authors suggested that p53 alteration is associated with tumor recurrence but not predictive of progression to a more malignant phenotype, in contrast to the findings of van Meyel *et al*. (65). These cases, similar to those studied by van Meyel *et al*, were selected for recurrence and progression and may not be representative of all low-grade tumors.

Korkolopoulou *et al*. (67) examined p53 expression in 51 astrocytic gliomas, predominantly glioblastomas. These authors reported a statistically significant association between p53 overexpression and disease-free survival on both univariate and multivariate analyses, the latter including correction for age and tumor grade. However, a subsequent study from these authors did not confirm these findings (64).

Loss of p53 function may be mediated through amplification of the MDM2 oncogene (68, 69). MDM2 transcription is induced by p53, and the MDM2 protein binds to and promotes the degradation of p53, thus serving as a negative feedback regulator of p53 (70). MDM2 amplification and overexpression, without p53 mutation, occurs in approximately 10% of glioblastomas and anaplastic astrocytomas (70). Furthermore, both p53 and MDM2 proteins are stabilized through binding of the $p14^{ARF}$ tumor suppressor gene to MDM2 (71). This dual stabilization induces a p53 response, resulting in elevated levels of MDM2 and cell cycle arrest in both G1 and G2/M. MDM2 alterations, as assessed by both gene amplification and overexpression, do not appear to be independent predictors of patient survival (58, 72, 73). The potential prognostic relevance of $p14^{ARF}$ alterations remains to be evaluated.

3.2.2. Platelet-derived growth factor/receptor (PDGF/R)

PDGF, a major mitogen for connective tissue cells and glia, is involved in normal development, especially of the central nervous system, and has been implicated in various pathological processes, including wound healing, inflammation, and neoplasia (74-77). In addition, the PDGF system has been intimately linked to the developing rat central nervous system in which O-2A progenitor cells are stimulated to divide and inhibited from premature differentiation into type-2 astrocytes and oligodendrocytes through PDGF-mediated activation of PDGFRA (78). PDGF occurs as three isoforms of disulfide-linked A- and B-chains (AA, BB, AB) that each bind with different affinities to two different receptors, PDGFRA and PDGFRB (79, 80). Whereas PDGFRA binds all three isoforms with high affinity, PDGFRB only binds the BB isoform with high affinity. These receptors share similar structures and belong to the protein-tyrosine kinase superfamily of growth factor receptors.

Although the mechanism remains unclear, astrocytic gliomas overexpress the components of the PDGF-PDGFR system. Overexpression of PDGFRA mRNA has been identified in all grades of astrocytic gliomas, and overexpression of PDGFRB and PDGFB chain mRNA have been demonstrated in the hyperplastic blood vessels of these tumors (81-83). Thus, these data suggest the presence of both autocrine and paracrine loops in glioma, with activation of A receptors occurring in the glioma cells and activation of B receptors occurring in the intermingled endothelial cells (81). Since elevated levels of PDGFRA mRNA have been found in all malignancy grades of astrocytic gliomas with the highest levels in the most malignant tumors, overexpression of PDGFRA is likely an early event in glial tumor development and related to tumor progression (82, 84). Consistently, amplification of the PDGFRA gene has only been identified in glioblastomas, the most malignant form of astrocytoma (85).

The wide variability in time to progression for low-grade astrocytomas could conceivably be related to the level of activation of growth factors, including those involved in the PDGF-PDGFR system. In addition, amplification of the PDGFRA and EGFR genes appear to occur in distinct subsets of glioblastomas (85), suggesting different developmental pathways and perhaps different prognoses and optimal therapeutic strategies (86-88). However, no reports to date have specifically addressed the prognostic significance of either PDGFRA expression or gene amplification in these patients.

3.2.3. Chromosome 22

Loss of heterozygosity on chromosome arm 22q occurs in approximately 20-30% of diffuse astrocytomas, regardless of malignancy grade, suggesting the presence of a tumor suppressor gene involved in early astrocytic glioma development (89-93). A recent report by Ino *et al*. (89) has defined two minimal deletion regions on 22q. The incidence of allelic loss of the more proximal region was similar among the histologic grades, consistent with an early event in tumor development. The incidence of allelic loss of the more distal region, however, increased with tumor grade, suggesting that a second tumor suppressor gene mapped to this locus may be associated with tumor progression. This potential dual role of tumor initiation and progression is intriguing and suggests that allelic losses of 22q may have prognostic significance, although such an association remains to be evaluated.

3.2.4. p16, Rb, and CDK4

The mammalian cell cycle is divided into four distinct phases: G1, S, G2, and M (figure 4). A complex system of positive and negative regulators governs cell cycle progression, exerting control at various checkpoints. One of the most critical checkpoints, the transition from G1 to S phase, involves the p16, Rb, E2F, cdk4, cdk6, and cyclin D proteins (figure 4). Under non-replicating conditions, the Rb protein binds to and sequesters key transcriptional factors, including E2F. When the decision is made for the cell to replicate, cyclin D binds to cdk4 and cdk6, forming active kinases that phosphorylate Rb. Phosphorylated Rb then releases the factors that transcriptionally activate the S phase genes, permitting transition from G1 to S phase. The p16 protein is one of the most important regulators of the cdk4-cyclin D complex. p16 blocks the binding of cdk4 to cyclin D, preventing phosphorylation of Rb, arresting the cell in the G1 phase. Inactivation of p16 or Rb or gain of cyclin D1 or cdk4/6 activity all have the same consequence, progression to S phase without regard to genomic integrity.

Release of the G1-to-S checkpoint is a critical step for the development of many human cancers (94), including astrocytic gliomas. Approximately half of all anaplastic astrocytomas and nearly all glioblastomas exhibit inactivation of this checkpoint (95-102). In astrocytic gliomas one of the most freqeunt mechanisms of abrogating this control is through p16 inactivation which occurs in 33-68% of glioblastoma tumors and in 75-90% of glioblastoma-derived cell lines (98-104). Typically, p16 inactivation occurs through homozygous deletion, though rarer mechanisms include point mutations and 5' CpG island methylation (96, 105, 106). Rb inactivation is the second most common mechanism of abrogating the p16-

cdk4-cyclin D1-pRb pathway in the formation of astrocytic gliomas, occuring in approximately 20-30% of high-grade astrocytomas (95, 96, 107, 108), and amplification of the CDK4 gene is apparent in approximately 10-15% of high-grade astrocytomas (68, 69, 95, 99).

Notably, alterations of p16, Rb and cdk4 are inversely correlated, suggesting that alteration of any one of these components is adequate to sufficiently abrogate the G1-to-S checkpoint (95, 96, 99). However, the Ki-67 index, a measure of proliferative potential, is significantly elevated in high-grade astrocytomas with p16 alteration when compared to those without p16 alteration (109). These observations suggest alteration of p16 may have additional ramifications not encountered with alteration of Rb or cdk4.

Survival analyses have evaluated the prognostic utility of several alterations involving the G1-to-S checkpoint, although the number of studies is limited and not entirely consistent. Nakamura *et al.* evaluated Rb immunostaining in 50 primary anaplastic astrocytomas and identified lack of immunoreactivity as an independent prognostic variable (110). A study by Korkolopoulou *et al*., however, did not identify Rb immunoreactivity as an independent prognostic indicator when evaluating a set of 48 astrocytic gliomas, predominantly composed of low-grade astrocytomas and glioblastomas (64).

CDK4 gene amplification was examined by Olson *et al*. in 109 astrocytic gliomas, representing all three histologic grades (72). After correcting for the effects of tumor grade and patient age, CDK4 amplification did not emerge as an independent predictor of overall survival. However, expression of $p27^{Kip1}$, a negative regulator of cdk4 (figure 4), has been suggested to correlate with prolonged patient survival, independent of tumor grade and patient age (73, 111, 112).

Expression of p16 has been evaluated and compared with survival in a set of 80 glioblastomas (58) and in 36 diffuse astrocytic gliomas, spanning all three histologic grades (113). Neither of these studies identified p16 expression as an independent prognostic indicator. Whether homozygous deletion or mutation of the p16 gene provides prognostic information has not been reported.

The general lack of correlation between alteration of the G1-to-S cell cycle regulators and diffuse astrocytic glioma patient survival may be because the Rb, CDK4, and p16 gene products are only portions of larger signaling systems or, simply, because it is universally altered. The potential predictive value of $p27^{Kip1}$ warrants further investigation in prospectively collected patient cohorts.

3.2.5. Chromosome arm 19q

Although the presence of a glioma 19q tumor suppressor gene was first suggested nearly a decade ago (114, 115), this putative gene remains unidentified. Alterations of the chromosome 19 q-arm are of particular importance for several reasons. These alterations have not been reported in other common malignancies (116), and they are the only known common genetic alteration shared by all three diffuse glioma subtypes (117-119), with gross deletions occurring in approximately 40% of astrocytomas, 45% of mixed oligoastrocytomas, and 75% of oligodendrogliomas (120). In addition, allelic loss of 19q in astrocytic gliomas has been associated with the frequent progression of low-grade lesions to anaplastic astrocytomas and glioblastomas (3, 7, 121). Collectively, these observations strongly suggest that 19q harbors one or more genes important for the pathogenesis of diffuse gliomas and further suggest that this gene may be of importance for glial development and growth regulation.

Multiple investigations have progressively narrowed the 19q common deletion region to an interval within the 19q13.3 cytogenetic band (120, 122-124), and several positional and/or functional candidates for the 19q tumor suppressor gene have been eliminated based on mutational analyses (125-130). The 19q glioma tumor suppressor gene, however, remains to be identified.

Allelic deletion of 19q was not associated with patient survival in a series of 53 glioblastomas analyzed by Smith *et al*. (131). Whether specific alteration of 19q gene(s) provides prognositically relevant information for astrocytoma patients awaits identification of candidate 19q tumor suppressor gene(s).

3.2.6. Chromosome arm 11p

Allelic deletion of the chromosome 11 p-arm occurs in approximately 30% of high-grade astrocytomas and rarely occurs in low-grade astrocytomas (121, 132-135) , suggesting the presence of a tumor suppressor gene involved in the malignant progression of astrocytic gliomas. Based on a limited number of mapping studies, the common deletion interval has been localized to a 21-cM region on 11p15.5-pter (133, 134). The c-H-ras gene maps to this interval but has been excluded as a candidate based on single-strand conformation polymorphism analysis (133). Thus, the identity and function of the 11p glioma tumor suppressor gene remain unknown. Allelic loss of 11p has been associated with the malignant progression of astrocytic gliomas (121, 132), but whether these alterations provide prognostically useful information has not been reported.

3.2.7. Epidermal growth factor receptor (EGFR)

EGFR is a transmembrane glycoprotein composed of an extracellular ligand-binding domain, a single hydrophobic membrane-spanning domain and a cytoplasmic tyrosine kinase domain (136). Binding of EGF or transforming growth factor (TGF) to EGFR results in receptor dimerization, autophosphorylation of the receptor itself and phosphorylation of cellular substrates leading to cell division and proliferation (136).

Amplification of the EGFR gene is observed in approximately 40% of all glioblastomas, resulting in overexpression of the EFGR transcript (137-139). In addition, approximately one-third of gliomas with EGFR gene amplification express a mutated variant with constitutive activity (140, 141). Notably, with rare

exception, glioblastomas with EGFR gene amplification also exhibit deletion of chromosome 10, especially 10p (139), suggesting that loss of chromosome 10 precedes EGFR gene amplification in glioma development and may be a prerequisite event for the oncogenic effects of EGFR amplification.

EGFR gene amplification and/or overexpression have been evaluated in multiple series of astrocytic gliomas, and the preponderance of these studies suggests that these alterations are not independent predictors of patient survival (56, 57, 59, 73, 142-148). One of the largest series was studied by Galanis *et al.* (73), who examined 121 astrocytic gliomas for EGFR gene amplification by Southern blotting and did not identify an association between amplification and patient survival by univariate or multivariate analyses. A limited number of studies have suggested an association between EGFR expression and patient survival. However, most of these series are either based on small patient populations ($\leq$20 cases) (149, 150) or have studied cases selected based on recurrence or progression (151). Two recent studies have suggested that EGFR amplification may have prognostic relevance in some circumstances. A study from Zhu *et al*. examined EGFR expression in 71 astrocytic gliomas, predominantly anaplastic astrocytomas and glioblastomas and suggested that the percentage of cells positive for EGFR is predictive of patient survival on univariate analysis and after correcting for the effects of patient age and tumor grade (152). While this study is potentially interesting, it stands in contrast to the general consensus of other reports and may simply reflect differences in immunohistochemical approaches.

A recent report by Tachibana *et al*. (47) suggested that EGFR gene amplification may be of prognostic significance after stratifying patients based on age. A collection of 196 high-grade astrocytomas, derived from a series of North Central Cancer Treatment Group (NCCTG) prospective phase III trials, was analyzed for EGFR gene amplification using FISH and PCR. The cohort included 66 anaplastic astrocytomas, 19 mixed oligoastrocytomas, and 111 glioblastomas. Classification and regression tree (CART) analysis revealed that EGFR gene amplification was associated with shorter overall survival among patients less than 40 years of age and was associated with longer overall survival among patients older than 60 years. These opposing effects were negated when CART analyses were performed without age as a parameter. Thus, these data suggest that EGFR gene amplification may have prognostic utility and further suggest that the biological significance of EGFR gene amplification in astrocytic gliomas may be related to patient age.

3.2.8. Chromosome 10, PTEN/MMAC (PTEN), and DMBT1

Deletions of chromosome 10 are the most frequent genetic alteration in glioblastomas, occurring in 60-93% of these neoplasms (99, 114, 139, 153-157). Most cases exhibit loss of an entire chromosome 10, and distinct patterns of deletion on both the long and short arms suggest the involvement of multiple tumor suppressor genes (157). Recently, a putative tumor suppressor gene, PTEN, was cloned from 10q23 (158, 159) and has proven altered in approximately 30% of glioblastoma tumors and 50-60% of glioblastoma-derived cell lines (158-161), with loss of function occurring through homozygous deletion, point mutation, or loss of expression.

Although PTEN is a dual lipid and protein phosphatase, recent evidence suggests that the biologically relevant targets are probably not proteins, but rather a subset of inositol phospholipids (reviewed in 162). In the phosphorylated state, these phospholipids facilitate the activity of AKT, a serine/threonine-kinase that promotes cell survival and cell cycle entry. Thus, by dephosphorylating the lipids that promote AKT activity, PTEN functions as an indirect inhibitor of AKT.

DMBT1, a member of the scavenger receptor cysteine-rich (SRCR) superfamily, was recently identified from a homozygous deletion at 10q25.3-26.1 in a medulloblastoma cell line (163). Intragenic deletions of DMBT1 have been identified in approximately 25% of glioblastomas and in multiple brain tumor cell lines (163). Members of the SRCR superfamily have been linked to diverse functions, including the initiation of proliferation or differentiation in the immune system or in other tissues (164) and switching of polarity of epithelial cells through mediation of contacts between the extracellular matrix and cell surface proteins (165).

Although two putative glioma tumor suppressor genes, PTEN and DMBT1, have been cloned from chromosome 10, there is evidence for additional contributors. Nearly all glioblastomas with EGFR amplification also exhibit chromosome 10 deletion (139), strongly suggesting a biologic relationship between these alterations. Intuitively, the lost phosphatase activity conferred by PTEN alterations could be prerequisite to enable EGFR amplification to have oncogenic significance, thereby accounting for the close tie between EFGR amplification and chromosome 10 loss. However, EGFR amplification has been demonstrated to occur at similar frequencies among cases with or without PTEN inactivation, suggesting that there may be additional chromosome 10 glioma tumor suppressor genes (161). Mounting evidence suggests that 10p may harbor one of these additional contributors (154-157).

Regarding the prognostic significance of PTEN alterations, Tachibana *et al*. have examined the PTEN coding region by direct sequencing in 196 similarly treated high-grade astrocytic gliomas from patients on NCCTG trials (47). Mutation of PTEN was a strong predictor of shorter overall patient survival in anaplastic astrocytoma patients, independent of patient age. In contrast, mutation of PTEN was neither a univariate nor a multivariate predictor of survival in glioblastoma patients.

Sano *et al*. examined PTEN expression by RT-PCR in 135 diffuse gliomas, including both high- and low-grade astrocytic gliomas and oligodendrogliomas (166).

Multivariate analyses, adjusting for patient age and tumor grade, showed a significantly better prognosis for patients whose tumors expressed high levels of PTEN.

Lin *et al*. (167) examined the PTEN and DMBT1 loci by loss of heterozygosity (LOH) analyses in 26 anaplastic oligodendrogliomas, 31 anaplastic astrocytomas, and 53 glioblastomas. Multivariate analyses, adjusting for patient age and histologic grade (GBM versus non-GBM), showed that LOH at the PTEN locus was a significant predictor of shorter survival. Similar analyses for the DMBT1 locus did not reveal a significant association with survival.

4. Oligodendrogliomas

Oligodendogliomas are defined as tumors consisting primarily of neoplastic oligodendrocytes and comprise approximately 10-17% of all intracranial gliomas (168, 169). Four-tiered classification systems have been proposed for oligodendrogliomas, including the Kernohan system (170) and one based on the degree of anaplasia proposed by Ludwig *et al*. (171). Neither of these systems, however, consistently produces distinct survival curves (171, 172). The WHO system, a simpler approach, has been advocated as a replacement of the four-tiered systems. WHO guidelines distinguish two types of "pure" oligodendroglioma with distinct survival curves, a low-grade phenotype (grade II) and a more malignant phenotype (grade III), termed anaplastic oligodendroglioma (1). Histologic features that distinguish the latter include high nuclear/cytoplasmic ratios, nuclear atypia, markedly increased mitotic activity, and prominent endothelial proliferation.

It is important to distinguish histologically between oligodendroglial and astrocytic gliomas, since these two tumor subtypes have considerably different clinical courses and optimal therapeutic approaches. Specifically, oligodendrogliomas tend to have a more benign course and are more responsive to treatment than astrocytomas. In addition, genetic and immunohistochemical analyses suggest that these two tumors likely have very different mechanisms of development (114, 173-175), and thus, in order to design more meaningful prognostic markers for these patients and to design more effective and specific treatments, it is critical that these patients are appropriately classified.

4.1. Clinical features of oligodendroglioma patients

4.1.1. Low-grade oligodendrogliomas

Oligodendrogliomas most commonly arise in the fronto-temporal region, though they may develop at any location throughout the neuraxis, in relative proportion to the volume of white matter. These tumors most frequently afflict patients in the fourth or fifth decade of life and often present with seizures, headaches, mental status changes, visual complaints, or focal weaknesses (168-171). For the majority of cases, these tumors grow slowly with an average interval prior to clinical signs of more than one year, regardless of age (176, 177). Although optimal management of low-grade oligodendroglioma has not been defined, many of these tumors are amendable to complete surgical resection. In addition, a subset of these cases responds to systemic combination chemotherapy with procarbazine, lomustine (CCNU), and vincrsitine (termed PCV) (178). This chemotherapy may be used as initial treatment followed by surgical resection of remaining tumor or treatment with stereotaxic radiosurgery. Alternatively, surgical resection may be followed by chemotherapy and stereotaxic radiosurgery or external beam radiation (179). Length of postsurgical survival is variable and ranges from five years (50%) to nearly a decade (25-34%) (168, 180-184). Our capacity to provide prognostic information for these patients is limited and primarily relies upon clinical and histologic features, including patient age, preoperative functional status, presence of calcification, and presence of anaplasia (131, 184-189). These factors, however, do not fully account for the considerable variation in clinical behavior observed among these patients.

4.1.2. Anaplastic oligodendrogliomas

As with the low-grade oligodendrogliomas, anaplastic oligodendrogliomas favor the frontotemporal region and have a peak incidence in the fourth and fifth decades. These tumors may develop from a low-grade oligodendroglioma that has progressed to a more maliganant lesion, or they may arise de novo. The presenting symptoms and imaging studies of an anaplastic oligodendroglioma typically resemble those of the more differentiated oligodendrogliomas, thus requiring tissue for histologic diagnosis. Approximately two-thirds of patients with anaplastic oligodendroglioma respond predictably, durably, and often completely to PCV chemotherapy (190-192). No histologic or clinical parameters have been identified to distinguish the remaining one-third that either responds incompletely or not all. As with low-grade oligodendroglioma, PCV chemotherapy may precede surgical resection and sterotaxic radiosurgery, or it may be administered after resection and then followed by stereotaxic radiosurgery (179). Overall survival time for anaplastic oligodendroglioma patients is less than 30% of that for low-grade oligodendrogliomas (193). Favorable clinical and histologic prognostic factors include younger patient age, high preoperative functional status, lack of ring enhancement on imaging, and presence of calcification (131, 184-186, 194).

4.2. Genetic alterations and prognostic implications

Oligodendrogliomas are generally thought to develop from molecular pathways distinct from those of astrocytomas (figure 3). While multiple genetic alterations have been identified in astrocytic gliomas and chained together in a logical progression of events, only a limited number of alterations have been defined in oligodendrogliomas, and most of these are observed in both low- and high-grade cases (132). This may reflect the fewer analyses of oligodendrogliomas as compared to astrocytomas, or it may simply indicate that fewer alterations are necessary for the development of oligodendrogliomas.

4.2.1. Chromosome arm 1p and 19q

Allelic loss of 19q occurs in approximately 50-80% of oligodendroglial tumors and with rare exception

involves an entire copy of 19q (117, 118, 120, 195, 196). The incidence of 19q deletion is not significantly different between low- and high-grade oligodendrogliomas, suggesting that this alteration is an early event in neoplastic development (117, 195, 196), in contrast to astrocytic gliomas which typically feature 19q deletion only in high- grade cases (120). Deletion mapping of 19q in oligodendrogliomas is complicated by the rare occurrence of tumors with only partial deletion of 19q. Thus, hypothesizing that astrocytic and oligodendroglial tumors share at least one 19q tumor suppressor gene in common, deletion mapping studies of the oligodendroglioma 19q gene have primarily relied upon astrocytoma samples (120, 122-124).

Deletion of chromosome arm 1p is another frequent event in oligodendrogliomas, occurring in 40-90% of these tumors (120, 195-197). Interestingly, nearly all cases of oligodendroglioma studied with deletion of 1p also exhibit deletion of 19q, suggesting that inactivation of one or more genes on each of these arms is an important event in oligodendroglioma oncogenesis. Deletion mapping of the oligodendroglioma 1p tumor suppressor gene, however, has been complicated by the limited number of cases with only partial 1p deletion (117, 120). A recent report by Husemann et al. identified two distinct deletion regions on 1p, D1S76-D1S253 at 1p36.3 and D1S482-D1S2743 at 1p34-35 (198). Notably, the 1p36.3 deletion interval overlaps the D1S468-D1S1612 deletion interval defined by Smith et al. (120), and narrows the interval to the approximately 5 cM between D1S468 and D1S253.

Deletions of 1p and 19q are of remarkable prognostic utility. Cairncross et al. examined 39 anaplastic oligodendroglioma patients, 37 of which had received PCV chemotherapy (194). Allelic loss of 1p was a statistically significant predictor of chemosensitivity, and combined loss of 1p and 19q was statistically significantly associated with both chemosensitivity and longer recurrence-free survival following chemotherapy. Moreover, Smith et al. have recently demonstrated that the association of 1p and 19q loss with prolonged survival is also evident in low- grade oligodendroglioma patients and that this association may be independent of PCV chemotherapy (131). Importantly, these data need to be confirmed in a larger cohort of prospectively collected patients.

4.2.2. p16

Deletion of the cell cycle regulator p16 is an uncommon event in oligodendrogliomas (199), however, it has recently surfaced as an important prognostic indicator in oligodendroglioma patients. In a study by Cairncross et al. (194), approximately 15% of anaplastic oligodendrogliomas had p16 gene deletion and these patients exhibited significantly shorter overall survival when compared to those patients without this alteration. Furthermore, combined loss of 1p and 19q was inversely correlated with p16 gene deletion, suggesting that oligodendrogliomas may be subgrouped based on these genetic alterations (194).

4.2.3. Chromosome 10

Approximately 25% of anaplastic oligodendrogliomas exhibit allelic loss of 10q while maintaining intact 1p and 19q chromosome arms, a genetic profile more consistent with an astrocytic, rather than an oligodendrial tumor (194, 200). Indeed, this may hint that these tumors are actually glioblastomas with prominent oligodendroglial features (200). Allelic loss of 10q, however, is not predictive of survival in anaplastic oligodendroglioma patients (194), contrary to the expected finding if these tumors actually are glioblastomas masquerading as oligodendrogliomas.

4.2.4. EGFR

Although EGFR overexpression is primarily associated with high-grade astrocytomas, several reports implicate its importance in oligodendroglial tumors as well. Increased levels of EGFR expression have been demonstrated in the majority of oligodendrogliomas and are rarely due to EGFR gene amplification (137, 201-204). Based on the approximately equal incidence of EGFR overexpression in both low- and high-grade oligodendrogliomas, this alteration is most likely an early event in oligodendroglial tumor development and not associated with malignant progression of these tumors (201). Whether EGFR overexpression is of prognostic significance in these patients has not been reported.

5. Mixed oligoastrocytomas

Although mixed oligoastrocytomas were first identified by Cooper in 1935 (205), definitive criteria for their diagnosis and classification remain elusive (206). Tumors classified as mixed oligoastrocytomas consist of an eclectic assortment of tumors, ranging from those with distinct regions of oligodendroglial and astrocytic differentiation, to those with an admixture of the two elements. In addition, some cases feature oligodendroglial and astrocytic phenotypic features in the same cells, termed "hybrid" oligoastrocytomas (207). While the classical "pure" astrocytic and "pure" oligodendroglial tumors may be readily distinguished by the trained observer, effective classification of mixed oligoastrocytic tumors is, at best, subjective and remains controversial, even among the most experienced neuropathologists (120). These distinctions are of more than passing academic interest, since diffuse glioma classification has very real implications for patient prognosis and treatment planning. The incidence of mixed oligoastrocytomas has not been well defined, but probably does not exceed 10-20% of supratentorial gliomas (192, 208). Similar to the oligodendrogliomas, WHO guidelines distinguish low-grade and anaplastic subtypes of mixed oligoastrocytomas (1). These two subtypes of mixed glioma are distinguished by the features that separate anaplastic astrocytomas and anaplastic oligodendrogliomas from their respective low-grade counterparts.

5.1. Clinical features of mixed oligoastrocytoma patients

5.1.1. Low-grade mixed oligoastrocytomas

Mixed oligoastrocytomas typically afflict middle-aged patients, with a slight male predominance, and the great majority of these lesions are supratentorial, favoring involvement of the frontal lobe (209, 210). Similar to oligodendroglioma patients, seizures are a frequent presenting symptom for mixed glioma patients (211).

Though optimal management has not been defined for mixed oligoastrocytoma patients, at least a subset appears to be sensitive to the combined PCV chemotherapy regimen (212). Using the WHO classification system, Shaw *et al.* reported a median survival of 6.3 years for low-grade mixed oligoastrocytoma patients (209), although there is considerable variation among studies due to widely differing diagnostic criteria. It is generally accepted, however, that the greater the oligodendroglial component, the more benign the clinical course, since oligodendroglial tumors are typically less infiltrative and thus more amenable to surgical resection (178, 213). Other prognostically favorable clinical and histologic criteria are similar to those of the low-grade astrocytomas and oligodendrogliomas.

5.1.2. Anaplastic mixed oligoastrocytomas

Similar to their low-grade counterparts, anaplastic oligoastrocytomas most commonly affect the middle-aged, favor supratentorial locations, and frequently present with seizures. Optimal treatment remains to be clarified, though study of a limited patient population suggests that these tumors are sensitive to PCV chemotherapy (212). The median survival time is considerable shorter than for the lower grade variant, with one report of 2.8 years and survival rates at 5 and 10 years of 36% and 9%, respectively (209). Another study reported a median survival of only 1.1 years (22). Other prognostic criteria resemble those of anaplastic astrocytomas and oligodendrogliomas.

5.2. Genetic alterations and prognostic implications

The lack of unambiguous criteria for the definition of mixed oligoastrocytomas provides considerable latitude for classifying these tumors and incorporates a heterogeneous collection ranging from those with distinct regions of astrocytic and oligodendroglial differentiation to those with intermingled cells of each type. Furthermore, the widely variable extent of oligodendroglial and astrocytic differentiation in these tumors is not accounted for through current classification schemes (1). This variability, coupled with the relative rarity of these mixed tumors, may account for the paucity of studies focusing on the genetics of mixed oligoastrocytomas.

Most reports indicate that similar genetic alterations are found in mixed oligoastrocytomas as are found in pure astrocytomas and oligodendrogliomas, however, they tend to occur at lower frequencies in mixed gliomas (114, 118, 119, 195, 196). This observation suggests that the mixed oligoastrocytoma histologic type may include at least two subtypes of tumors, those with developmental origins resembling astrocytomas and those with development origins resembling oligodendrogliomas. For example, Maintz *et at*. (119) investigated a large collection of astrocytomas, oligodendrogliomas, and mixed oligoastrocytomas for genetic alterations that occur disproportionately between astrocytic and oligodendroglial tumors, including p53 mutations and allelic loss of chromosomes 1p and 19q. p53 mutations and allelic losses on 1p and 19q were inversely correlated in oligoastrocytomas, suggesting the existence of at least two different subtypes of oligoastrocytomas based on these alterations, one with p53 point mutations genetically resembling astrocytomas and the other with allelic losses of 1p and 19q genetically resembling oligodendrogliomas.

6. SUMMARY

Our understanding of glioma development and progression on the molecular genetic level has expanded remarkably over the past decade and our ability to use this information for patient prognosis is surfacing. Although age and tumor grade remain the most powerful predictors of patient survival among astrocytic glioma patients, recent reports indicate that alterations of the PTEN tumor suppressor gene are independent predictors of overall patient survival for anaplastic astrocytoma patients, helping to distinguish those cases with behavior resembling their more malignant counterparts, the glioblastomas. The potential prognostic value of EGFR gene amplification in malignant astrocytoma patients stratified by age is intriguing and warrants further investigation. Among the oligodendroglial tumors, alterations of the 1p and 19q chromosome arms have emerged as potentially powerful predictors of overall patient survival and *in vivo* chemotherapeutic response, while alterations of the p16/CDKN2A tumor suppressor gene suggest shorter overall survival. As our molecular models continue to improve, through functional analyses and the identification of additional genetic contributors, we will expand our capacity to more effectively prognose these patients and to design rational therapeutic strategies.

7. REFERENCES

1. Kleihues P, P. C. Burger & B. W. Scheithauer: The new WHO classification of brain tumors. Brain Pathol 3, 255-268 (1993)

2. Louis D N: A molecular genetic model of astrocytoma histopathology. Brain Pathol 7, 755-764 (1997)

3. Louis D N & J. F. Gusella: A tiger behind many doors: multiple genetic pathways to malignant glioma. Trends Genet 10, 412-415 (1995)

4. von Deimling A, D. N. Louis & O. D. Wiestler: Molecular pathways in the formation of gliomas. Glia 15, 328-338 (1995)

5. Furnari F B, H. J. Huang & W. K. Cavenee: Molecular biology of malignant degeneration of astrocytoma. Pediatr Neurosurg 24, 41-49 (1996)

6. Collins V P & C. D. James: Gene and chromosomal alterations associated with the development of human gliomas. FASEB 7, 926-930 (1993)

7. Ohgaki H, B. Schauble, A. zur Hausen, K. von Ammon & P. Kleihues: Genetic alterations associated with the evolution and progression of astrocytic brain tumours. Virchows Arch 427, 113-118 (1995)

8. Cascino G D: Epilepsy and brain tumors: implications for treatment. Epilepsia 31, 537-544 (1990)

9. K L Kink, E J Rushing & S C Schold, Jr: Neuro-oncology. In: Atlas of clinical neurology. Ed: Rosenberg R N, Butterworth-Heinemann, Boston 8.1-8.44 (1990)

10. J D McDonald & M L Rosenblum: Gliomas. In: Principles of Neurosurgery. Eds: Rengachary S S & Wilkins R H, Mosby, London 26.1-26.32 (1994)

11. Morantz RA: Radiation therapy in the treatment of cerebral astrocytoma. Neurosurgery 20, 975-982 (1987)

12. Soffietti R, A. Chio, M. T. Giordana, E. Vasario & D. Schiffer: Prognostic factors in well-differentiated cerebral astrocytomas in the adult. Neurosurgery 24, 686-692 (1989)

13. Leibel S A & G. E. Sheline: Radiation therapy for neoplasms of the brain. Neurosurgery 66, 1-22 (1987)

14. Daumas-Duport C, B. W. Scheithauer, J. O'Fallon & P. Kelly: Grading of astrocytoma: a simple and reproducible method. Cancer 62, 2152-2165 (1988)

15. Leighton C, B. Fisher, G. Bauman, S. Depiero, L. Stitt, D. Macdonald & G. Cairncross: Supratentorial low-grade glioma in adults: an analysis of prognostic factors and timing of radiation. J Clin Oncol 15, 1294-1301 (1997)

16. Knut L,T. Egeland, B. Hager, B. Stenwig, K. Skullerud, J. Berg-Johnsen, I. Storm-Mathisen & H. Hirschberg: Survival, prognostic factors, and therapeutic efficacy in low-grade glioma: a retrospective study in 379 patients. J Clin Oncol 15, 3129-3140 (1997)

17. Nicolato A, M. A. Gerosa, P. Fina, P. Iuzzolino, F. Giorgiutti & A. Bricolo: Prognostic factors in low-grade supratentorial astrocytomas: a uni-multivariate statistical analysis of 76 surgically treated adult patients. Surg Neurol 44, 208-223 (1995)

18. Loiseau H, J.-F. Dartigues & F. Cohadon: Low-grade astrocytomas: prognosis factors and elements of management. Surg Neurol 44, 224-227 (1995)

19. Iwabuchi S, S. Bishara, P. Herbison, A. Erasmus & H. Samejima: Prognostic factors for supratentorial low grade astrocytomas in adults. Neurol Med Chir 39, 273-281 (1999)

20. Fine H A: The basis for current treatment recommendations for malignant gliomas. J Neuro-Oncol 20, 111-120 (1994)

21. Laperriere N J & M. Berstein: Radiotherapy for brain tumors. Ca Cancer J Clin 44, 96-108 (1994)

22. Winger M J, D. R. Macdonald & J. G. Cairncross: Supratentorial anaplastic gliomas in adults. J Neurosurg 71, 487-493 (1989)

23. Salminen E, J. M. Nuutinen & S. Huhtala: Multivariate analysis of prognostic factors in 106 patients with malignant glioma. Eur J Cancer 32A, 1918-1923 (1996)

24. Surawicz T S, B. J. McCarthy, V. Kupelian, P. J. Jukich, J. M. Bruner, F. G. Davis & the collaborating registries of the Central Brain Tumor Registry of the United States: Descriptive epidemiology of primary brain and CNS tumors: results from the central brain tumor registry of the United States, 1990-1994. Neuro-Oncol 1, 14-25 (1999)

25. Kleihues P & H. Ohgaki: Primary and secondary glioblastomas: from concept to clinical diagnosis. Neuro-Oncol 1, 44-51 (1999)

26. Frankel S A & W. J. German: Glioblastoma multiforme: review of 219 cases with regard to natural history, pathology, diagnostic methods, and treatment. J Neurosurg 15, 489-503 (1958)

27. Curran W J, C. B. Scott, J. Horton, J. S. Nelson, A. S. Weinstein, A. J. Fischbach, C. H. Chang, M. Rotman, S. O. Asbell, R. E. Krisch, et al.: Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cacner Inst 85, 690-691 (1993)

28. Larson D A, P. H. Gutin, M. McDermott, K. Lamborn, P. K. Sneed, W. M. Wara, J. C. Flickinger, D. Kondziolka, L. D. Lunsford, W. R. Hudgins, G. M. Friehs, K. Haselsberger, K. Leber, G. Pendl, S. S. Chung, R. J. Coffey, R. Dinapoli, E. G. Shaw, S. Vermeulen, R. F. Young, M. Hirato, H. K. Inoue, C. Ohye & T. Shibazaki: Gamma knife for glioma: selection factors and survival. Int J Radiat Oncol Biol Phys 36, 1045-1053 (1996)

29. McDermott M W, P. K. Sneed & P. H. Gutin: Interstitial brachytherapy for malignant brain tumors. Sem Surg Oncol 14, 79-87 (1998)

30. Voges J, K. Herholz, T. Holzer, M. Wurker, B. Bauer, U. Pietrzyk, H. Treuer, R. Schroder, V. Sturm & W. D. Heiss: 11C-methionine and 18F-2-fluorodeoxyglucose positron emission tomography: a tool for diagnosis of cerebral glioma and monitoring after brachytherapy with 125I seeds. Stereotact Funct Neurosurg 69, 129-135 (1997)

31. Cunningham J M, D. W. Kimmel, B. W. Scheithauer, J. R. O'Fallon, P. J. Novotny & R. B. Jenkins: Analysis of proliferation markers and p53 expression in gliomas of astrocytic origin: relationships and prognostic value. J Neurosurg 86, 121-130 (1997)

32. Shrieve D C, E. Alexander, P. McL Black, P. Y. Wen, H. A. Fine, H. M. Kooy & J. S. Loeffler: Treatment of patients with primary glioblastoma multiforme with standard postoperative radiotherapy and radiosurgical boost: prognostic factors and long-term outcome. J Neurosurg 90, 72-77 (1999)

33. Lane D P: p53. guardian of the genome. Nature 358, 15-16 (1992)

34. Hensey C & J. Gautier: Regulation of cell cycle progression following DNA damage. Prog Cell Cycle Res 1, 149-162 (1995)

35. Maltzman W & L. Czyzyk: UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. Mol Cell Biol 4, 1689-1694 (1984)

36. Kastan M B, Q. Zhan, W. S. El-Deiry, F. Carrier, T. Jacks, W. V. Walsh, B. S. Plunkett, B. Vogelstein & A. J. Fornace:

A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71, 587-597 (1992)

37. Sun X, H. Shimizu & K. Yamamoto: Identification of a novel p53 promoter element involved in genotoxic stress-inducible p53 gene expression. Mol Cell Biol 15, 4489-4496 (1995)

38. Mercer W E: Checking on the cell cycle. J Cell Biochem 30, 50-54 (1998)

39. Bates S & K. H. Vousden: Mechanisms of p53-mediated apoptosis. Cell Mol Life Sci 55, 28-37 (1999)

40. Agarway M L, W. R. Taylor, M. V. Chernov, O. B. Chernova & G. R. Stark: The p53 network. J Biol Chem 273, 1-4 (1998)

41. Kastan M B, O. Onyekwere, B. Sidransky, B. Vogelstein & R. W. Craig: Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51, 6304-6311 (1991)

42. Kuerbitz S J, B. S. Plunkett, W. V. Walsh & M. B. Kastan: Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 89, 7491-7495 (1992)

43. Folkman J & R. Cotran: Relation of vascular proliferation to tumor growth. Int Rev Exp Pathol 16, 207-248 (1976)

44. Dameron K M, O. V. Volpert, M. A. Tainsky & N. Bouck: Control of angiogenesis in fibroblasts by p53 regulation of thombospondin-1. Science 265, 1582-1584 (1994)

45. Van Meir E G, P. J. Polverini, V. R. Chazin, H. J. S. Huang, N. de Tribolet & W. K. Cavenee: Release of an inhibitor of angiogenesis upon induction of wild-type p53 expression in glioblastoma cells. Nature Genet 8, 171-176 (1994)

46. Kraus J, C. Bollin, H. K. Wolf, J. Neumann, D. Kindermann, R. Fimmers, F. Forster, A. Baumann & U. Schlegel: TP53 alterations and clinical outcome in low grade astrocytomas. Genes Chrom Cancer 10, 143-149 (1994)

47. Tachibana I, J. S. Smith, B. K. Huntley, S. M. Hosek, T. J. Borell, J. R. O'Fallon, M. R. Mahoney, B. W. Scheithauer, C. D. James, J. C. Buckner & R. B. Jenkins: Analysis of EGFR amplification, TP53 mutation, and PTEN alteration in similarly-treated cohorts of anaplastic astrocytoma and glioblastoma patients: relationships and prognostic value. (submitted)

48. Louis D N: The p53 gene and protein in human brain tumors. J Neuropathol Exp Neurol 53, 11-21 (1994)

49. Watanabe K, O. Tachibana, K. Sato, Y. Yonekawa, P. Kleihues & H. Ohgaki: Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol 6, 217-223 (1996)

50. Perry A, R. B. Jenkins, J. R. O'Fallon, P. L. Schaefer, D. W. Kimmel, M. R. Mahoney, B. W. Scheithauer, S. M. Smith, E. M. Hill, T. J. Sebo, R. Levitt, J. Mrook, L. K. Tschetter, R. F. Morton & J. C. Buckner: Clinicopathologic study of 85 similarly treated patients with anaplastic astrocytoma. Cancer 86, 672-683 (1999)

51. Kyritsis A P, M. L. Bondy, K. R. Hess, J. E. Cunningham, D. Zhu, C. J. Amos, W. K. A. Yung, V. A. Levin & J. M. Bruner: Prognostic significance of p53 immunoreactivity in patients with glioma. Clin Cancer Res 1, 1617-1622 (1995)

52. Hilton D A, S. Love, R. Barber, D. Ellison & D. R. Sandeman: Accumulation of p53 and Ki-67 expression do not predict survival in patients with fibrillary astrocytomas or the response of these tumors to radiotherapy. Neurosurgery 42, 724-729 (1998)

53. Danks R A, G. Chopra, M. F. Gonzales, J. M. Orian & A. H. Kaye: Aberrant p53 expression does not correlate with the prognosis in anaplastic astrocytoma. Neurosurgery 37, 246-254 (1995)

54. Vital A, H. Loiseau, G. Kantor, V. Daucourt, G. Chene, F. Cohadon, A. Rougier, J. Rivel & C. Vital: p53 protein expression in grade II astrocytomas: immunohistochemical study of 100 cases with long-term follow-up. Pathol Res Pract 194, 831-836 (1998)

55. Iuzzolino P, C. Ghimenton, A. Nicolato, F. Giorgiutti, P. Fina, C. Doglioni & M. Barbareschi: p53 protein in low-grade astrocytomas: a study with long-term follow-up. Br J Cancer 69, 586-591 (1994)

56. Jaros E, R. H. Perry, L. Adam, P. J. Kelly, P. J. Crawford, R. M. Kalbag, A. D. Mendelow, R. P. Sengupta & A. D. J. Pearson: Prognostic implications of p53 protein, epidermal growth factor receptor, and Ki-67 labelling in brain tumors. Br J Cancer 66, 373-385 (1992)

57. Rainov N G, K. U. Dobberstein, H. Bahn, H. J. Holzhausen, C. Lautenschläger, V. Heidecke & W. Burkert: Prognostic factors in malignant glioma: influences of the overexpression of oncogene and tumor-suppressor gene products on survival. J Neuro-Oncol 35, 13-28 (1997)

58. Newcomb E W, H. Cohen, S. R. Lee, S. K. Bhalla, J. Bloom, R. L. Hayes & D. C. Miller: Survival of patients with glioblastoma multiforme is not influenced by altered expression of p16, p53, EGFR, MDM2 or Bcl-2 genes. Brain Pathol 8, 655-667 (1998)

59. Bouvier-Labit C, O. Chinot, C. Ochi, D. Gambarelli, H. Dufour & D. Figarella-Branger: Prognostic significance of Ki-67, p53 and epidermal growth factor receptor immunostaining in human glioblastomas. Neuropathol Appl Neurobiol 24, 381-388 (1998)

60. Al-Sarraj S & L. R. Bridges: p53 immunoreactivity in astrocytomas and its relationship to survival. Br J Neurosurg 9, 143-149 (1995)

61. Rolhion C, F. Penault-Llorca, J. L. Kemeny, F. Kwiatkowski, J. J. Lemaire, P. Chollet, F. Finat-Duclos & P. Verrelle: O6-methylguanine-DNA methyltransferase gene (MGMT) expression in human glioblastomas in relation to patient characteristics and p53 accumulation. Int J Cancer 416, 416-420 (1999)

62. Ellison D W, P. V. Steart, A. C. Bateman, R. M. Pickering, J. D. Palmer & R. O. Weller: Prognostic indicators in a range of astrocytic tumours: an immunohistochemical study with Ki-67 and p53 antibodies. J Neurol Neurosurg Psychiatry 59, 413-419 (1995)

63. Montine T J, J. M. Bruner, J. J. Vandersteenhoven, R. K. Dodge & P. C. Burger: Prognostic significance of p53 immunoreactivity in adult patients with supratentorial fibriallary astrocytic neoplasms. Diagn Mol Pathol 3, 240-245 (1994)

64. Korkolopoulou P, K. Kouzelis, P. Christodoulou, A. Papanikolaou & E. Thomas-Tsagli: Expression of retinoblastoma gene product and p21 (WAF1/Cip 1) protein in gliomas: correlations with proliferation markers, p53 expression and survival. Acta Neuropathol 95, 617-624 (1998)

65. van Meyel D J, D. A. Ramsay, A. G. Casson, M. Keeney, A. F. Chambers & J. G. Cairncross: p53 mutation, expression, and DNA ploidy in evolving gliomas: evidence for two pathways of progression. J Natl Cancer Inst 86, 1011-1017 (1994)

66. Watanabe K, K, Sato, W. Biernat, O. Tachibana, K. von Ammon, N. Ogata, Y. Yonekawa, P. Kleihues & H. Ohgaki: Incidence and timing of p53 mutations during astrocytoma progression in patients with multiple biopsies. Clin Cancer Res 3, 523-530 (1997)

67. Korkolopoulou P, P. Christodoulou, K. Kouzelis, M. Hadiyannakis, A. Priftis, G. Stamoulis, A. Seretis & E. Thomas-Tsagli: MDM2 and p53 expression in gliomas: a multivariate survival analysis including proliferation markers and epidermal growth factor receptor. Br J Cacner 75, 1269-1278 (1997)

68. Reifenberger G, K. Ichimura, J. Reifenberger, A. G. Elkahloun, P. S. Meltzer & V. P. Collins: Refined mapping of 12q13-q15 amplicons in malignant gliomas suggests CDK4/SAS and MDM2 as independent amplification targets. Cancer Res 56, 5141-5145 (1996)

69. Reifenberger G, L. Liu, K. Ichimura, E. E. Schmidt & V. P. Collins: Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. Cancer Res 53, 2736-2739 (1993)

70. Freeman D A, L. Wu & A. J. Levine: Functions of the MDM2 oncoprotein. Cell Mol Life Sci 55, 96-107 (1999)

71. Stott F J, S. Bates, M. C. James, B. B. McConnel, M. Starborg, S. Brookes, I. Palmero, K. Ryan, E. Hara, K. H. Vousden & G. Peters: The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. EMBO J 17, 5001-5014 (1998)

72. Olson J J, D. Barnett, J. Yang, R. Assietti, G. Cotsonis & C. D. James: Gene amplification as a prognostic factor in primary brain tumors. Clin Cancer Res 4, 215-222 (1998)

73. Galanis E, J. Buckner, D. Kimmel, R. Jenkins, B. Alderete, J. O'fallon, C. H. Wang, B. W. Scheithauer & C. D. James: Gene amplification as a prognostic factor in primary and secondary high-grade malignant gliomas. Int J Oncol 13, 717-724 (1998)

74. Richardson W D, N. Pringle, M. J. Mosley, B. Westermark & M. Dubois-Dalcq: A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 53, 309-319 (1988)

75. Abramovitch R, M. Marikovsky, G. Meir & M. Neeman: Stimulation of tumor growth by wound-derived growth factors. Br J Cancer 79, 1392-1398 (1999)

76. Nistér M, T. A. Libermann, C. Betsholtz, M. Pettersson, L. Claesson-Welsh, C. H. Heldin, J. Schlessinger & B. Westermark: Expression of messenger RNAs for platelet-derived growth factor and transforming growth factor alpha and their receptors in human malignant glioma cell lines. Cancer Res 48, 3910-3918 (1988)

77. Nistér M, L. Claesson-Welsh, A. Eriksson, C. H. Heldin & B. Westermark: Differential expression of platelet-derived growth factor receptors in human malignant glioma cell lines. J Cell Biol 266, 16755-16763 (1991)

78. Hart IK, W. D. Richardson, C. H. Heldin, B. Westermark & Raff M: PDGF receptors on cells of the oligodendrocyte-type-2 astrocyte (O-2A) cell lineage. Development 105, 595-603 (1989)

79. Hammacher A, U. Hellman, A. Johnson, A. Ostman, K. Gunnarson, B. Westmark, A. Wasteson & C. H. Heldin: A major part of platelet-derived growth factor purified from human platelets is a heterodimer of one A and one B chain. J Biol Chem 263, 16493-16498 (1988)

80. Hart C E, J. W. Forstrom, J. D. Kelly, R. A. Seifert, R. A. Smith, R. Ross, M. J. Murray & D. F. Bowen-Pope: Two classes of PDGF-receptors recognize different isoforms of PDGF. Science 240, 1529-1531 (1988)

81. Hermanson M, K. Funa, M. Hartman, L. Claesson-Welsh, C. H. Heldin, B. Westermark & M. Nistér: Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. Cancer Res 52, 3213-3219 (1992)

82. Hermanson M, K. Funa, J. Koopman, D. Maintz, A. Waha, B. Westermark, C. H. Heldin, O. D. Wiestler, D. N. Louis, A. von Deimling & M. Nistér: Association of loss of heterozygosity on chromosome 17p with high platelet-derived growth factor-alpha receptor expression in human malignant gliomas. Cancer Res 56, 164-171 (1996)

83. Maxwell M, S. P. Naber, H. J. Wolfe, T. Galanopoulos, E. T. Hedley-Whyte, P. Black & H. N. Antoniades: Coexpression of platelet-derived growth factor (PDGF) and PDGF-receptor genes by primary human astrocytomas may contribute to their development and maintenance. J Clin Invest 85, 131-40 (1990)

84. Westermark B, C. H. Heldin and M. Nistér: Platelet-derived growth factor in human glioma. Glia 15, 257-263 (1995)

85. Fleming T P, A. Saxena, W. C. Clark, J. T. Robertson, E. H. Oldfield, S. A. Aaronson and I. U. Ali: Amplification and/or overespression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumors. Cancer Res 52, 4550-4553 (1992)

86. Yates A J, H. E. Saqr & J. Van Brocklyn: Ganglioside modeluation of the PDGF receptor. J Neuro-Oncol 24, 65-73 (1995)

87. Shawver L K, D. P. Scwartz, E. Mann, H. Chen, J. Tsai, L. Chu, L. Taylorson, M. Longhi, S. Meredith, L. Germain, J. S. Jacobs, C. Tang, A. Ullrich, M. E. Berens, E. Hersh, G. McMahon, K. P. Hirth & T. J. Powell: Inhibition of platelet-derived growth factor-mediated signal transduction and tumor growth by N-[4-(trifluoromethyl)-phenyl] 5-methylisoxazole-4-carboxamide. Clin Cancer Res 3, 1167-1177 (1997)

88. Chin L S, S. F. Murray, K. M. Zitnay & B. Rami: K252a inhibits proliferation of glioma cells by blocking platelet-derived growth factor signal transduction. Clin Cancer Res 3, 771-776 (1997)

89. Ino Y, J. S. Silver, L. Blazejewski, R. Nishikawa, M. Matsutani, A. von Deimling & D. N. Louis: Common regions of deletion on chromosome 22q12.3-q13.1 and 22q13.2 in human astrocytomas appear related to malignancy grade. J Neuropathol Exp Neurol 58, 881-885 (1999)

90. Fults D, C. A. Pedone, G. A. Thomas, & R. White: Allelotype of human malignant astrocytoma. Cancer Res 50, 5784-5789 (1990)

91. James C D, E. Carlblom, J. P. Dumanski, M. Hansen, M. Nordenskjold, V. P. Collins & W. K. Cavenee: Clonal genomic alterations in glioma malignancy stages. Cancer Res 48, 5546-5551 (1988)

92. Rey J A, M. J. Bello, A. M. Jimenez-Lara, J. Vaquero, M. E. Kusak, J. M. Campos, J. L. Sarasa & A. Pestana: Loss of heterozgosity for distal markers on 22 q in human gliomas. Int J Cancer 51, 703-706 (1992)

93. Hoang-Xuan K, P. Merel, F. Verga, J. P. Hugot, P. Cornu, J. Y. Delattre, M. Poisson, G. Thomas & O. Delattre: Analysis of the NF2 tumor-suppressor gene and of chromosome 22 deletions in gliomas. Int J Cancer 60, 478-481 (1995)

94. Sherr CJ: Cancer cell cycles. Science 274, 1672-1677 (1996)

95. He J, J. J. Olson & C. D. James: Lack of p16INK4 or retinoblastoma protein (pRb), or amplification-associated overexpression of CDK4 is observed in distinct subsets of malignant glial cells. Cancer Res 55, 4833-4836 (1995)

96. Ueki K, Y. Ono, J. W. Henson, A. von Deimling & D. N. Louis: CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. Cancer Res 56, 150-153 (1996)

97. Giani C & G. Finocchiaro: Mutation rate of the CDKN2 gene in malignant gliomas. Cancer Res 54, 6338-6339 (1994)

98. Jen J, W. Harper, S. H. Bigner, D. D. Bigner, N. Papadopoulos, S. Markowitz, J. K. V. Wilson, K. W. Kinzler & B. Vogelstein: Deletion of p16 and p15 genes in brain tumors. Cancer Res 54, 6353-6358 (1994)

99. Schmidt E E, K. Ichimura, G. Reifenberger & V. P. Collins VP: CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. Cancer Res 54, 6321-6324 (1994)

100. Dreyling M H, S. K. Bohlander, M. O. Adeyanju & Olopade OI: Detection of CDKN2 deletions in tumor cell lines and primary glioma by interphase fluorescence in situ hybridization. Cancer Res 55, 984-988 (1995)

101. Moulton T, G. Samara, W. Chung, L. Yuan, R. Desai, M. Sisti, J. Bruce, B. Tycko: MTS1/p16/CDKN2 lesions in primary glioblastoma multiforme. Am J Pathol 146, 613-619 (1995)

102. Walker D G, W. Duan, E. A. Popovic, A. H. Kaye, F. H. Tomlinson & M. Lavin: Homozygous deletions of the multiple tumor suppressor gene 1 in the progression of human astrocytomas. Cancer Res 55, 20-23 (1995)

103. Nobori T, K. Miura, D. J. Wu, A. Lois, K. Takabayashi K & D. A. Carson: Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature 368, 753-756 (1994)

104. Kamb A, N. A. Gruis, J. Weaver-Feldhaus, Q. Liu, K. Harshman, S. V. Tavtigian, E. Stockert, R. S. Day, B. E. Johnson & M. H. Skolnick: A cell cycle regulator potentially involved in genesis of many tumor types. Science 264, 436-440 (1994)

105. Merlo A, J. G. Herman, L. Mao, D. J. Lee, E. Gabrielson, P. C. Burger, S. B. Baylin & D. Sidransky: 5'CpG island methylation is associated with transcriptional silencing of the tumor suppressor p16/CDKN2/MTS1. Nature Med 1, 686-692 (1995)

106. Ueki K, M. P. Rubio, V. Ramesh, K. M. Correa, J. L. Rutter, A. von Deimling, A. J. Buckler, J. F. Gusella & D. N. Louis: MTS1/CDKN2 gene mutations are rare in primary human astrocytomas with allelic loss of chromosome 9p. Hum Molec Genet 3, 1841-1845 (1994)

107. Henson J W, B. L. Schnitker, K. M. Correa, A. von Deimling, F. Fassbender, H. J. Xu, W. F. Benedict, D. W. Yandell & Louis DN: The retinoblastoma gene is involved in malignant progression of astrocytomas. Ann Neurol 36, 714-721 (1995)

108. Ichimura K, E. E. Schmidt, H. M. Goike & V. P. Collins: Human glioblastomas with no alterations of the CDKN2 (p16INK4A, MTS1) and CDK4 genes have frequent mutations of the retinoblastoma gene. Oncogene 13, 1065-1072 (1996)

109. Ono Y, T. Tamiya, T. Ichikawa, K. Kunishio, K. Matsumoto, T. Furuta, T. Ohmoto, K. Ueki & D. N. Louis: Malignant astrocytomas with homozygous CDKN2/p16 gene deletions have higher Ki-67 proliferation indeces. J Neuropathol Exp Neurol 55, 1026-1031 (1996)

110. Nakamura M, N. Konishi, S. Tsunoda, Y. Hiasa, T. Tsuzuki, T. Inui & T. Sakaki: Retinoblastoma protein expression and MIB-1 correlate with survival of patients with malignant astrocytoma. Cancer 80, 242-249 (1997)

111. Mizumatsu S, T. Tamiya, Y. Ono, T. Abe, K. Matsumoto, T. Furuta & T. Ohmoto: Expression of cell regulator p27Kip1 is correlated with survival of patients with astrocytoma. Clin Cancer Res 5, 551-557 (1999)

112. Alleyne C H, Jr, J. He, J. Yang, S. B. Hunter, G. Cotsonis, C. D. James & J. J. Olson: Analysis of cyclin dependent kinase inhibitors in malignant astrocytomas. Int J Oncol 14, 1111-1116 (1999)

113. Miettinen H, J. Kononen, P. Sallinen, H. Alho, P. Helen, H. Helin, H. Kalimo, L. Paljärvi, J. Isola & H. Haapasalo: CDKN2/p16 predicts survival in oligodendrogliomas: comparison with astrocytomas. J Neuro-Oncol 41, 205-211 (1999)

114. Ransom D T, S. R. Ritland, C. A. Moertel, R. J. Dahl, J. R. O'Fallon, B. W. Scheithauer, D. W. Kimmel, P. J. Kelly, O. I. Olopade, M. O. Diaz & R. B. Jenkins: Correlation of cytogenetic analysis and loss of heterozygosity studies in human diffuse astrocytomas and mixed oligo-astrocytomas. Genes Chrom Cancer 5, 357-374 (1992)

115. Ransom D T, S. R. Ritland, R. B. Jenkins, B. Scheithauer, P. J. Kelly & D. W. Kimmel: Loss of heterozygosity studies in human gliomas. Proc Am Assoc Cancer Res 32, 302 (1991)

116. Seizinger B R, H. P. Klinger, C. Junien, Y. Nakamura, M. Le Beau, W. Cavenee, B. Emanuel, B. Ponder, S. Naylor, F. Mitelman, D. N. Louis, A. Menon, I. Henry & A. von Deimling: Report of the committee on chromosome and gene loss in human neoplasia. Cytogenet Cell Genet 58, 1080-1096 (1991)

117. Bello M J, E. Leone, J. Vaquer, J. M. De Campos, M. E. Kusak, J. L. Sarasa, A. Pestana & J. A. Rey: Allelic loss at 1p and 19q frequently occurs in association and may represent early oncogeneic events in oligodendroglial tumors. Int J Cancer 64, 207-210 (1995)

118. von Deimling A, D. N. Louis, K. von Ammon, I. Peterson, O. D. Wiestler & B. R. Seizinger: Evidence for a tumor suppressor gene on chromosome 19q associated with human astrocytomas, oligodendrogliomas, and mixed gliomas. Cancer Res 52, 4277-4279 (1992)

119. Maintz D, K. Fiedler, J. Koopman, B. Rollbrocker, S. Nechev, D. Lenartz, A. P. Stangl, D. N. Louis, J. Schramm, O. D. Wiestler & A. von Deimling: Molecular genetic evidence for subtypes of oligoastrocytomas. J Neuropathol Exp Neurol 56, 1098-1104 (1997)

120. Smith J S, B. Alderete, Y. Minn, T. J. Borell, A. Perry, G. Mohapatra, S. M. Hosek, D. Kimmel, J. O'Fallon, A. Yates, B. G. Feuerstein, P. C. Burger, B. W. Scheithauer & R. B. Jenkins: Localization of common deletion regions on 1p and 19q in human gliomas and their association with histological subtype. Oncogene 18, 4144-4152 (1999)

121. von Deimling A., B. Bender, R. Jahnke, A. Waha, J. Kraus, S. Albrecht, R. Wellenreuther, F. Fassbender, A. G. Menon, D. N. Louis, D. Lenartz, J. Schramm & O. D. Weistler: Loci associated with malignant progression in astrocytomas. Cancer Res 54, 1397-1401 (1994)

122. von Deimling A, J. Nagel, B. Bender, D. Lenartz, J. Schramm, D. N. Louis & O. D. Weistler: Deletion mapping of chromosome 19 in human gliomas. Int J Cancer 57, 676-680 (1994)

123. Rosenberg J E, D. K. Lisle, J. A. Burwick, K. Ueki, A. Mohrenweiser & D. N. Louis: Refined deletion mapping of the chromosome 19q glioma tumor suppressor gene to the D19S412-STD interval. Oncogene 13, 2483-2485 (1996)

124. Smith J S, I. Tachibana, H. K. Lee, J. Qian, U. Pohl, H. W. Mohrenweiser, T. J. Borell, S. M. Hosek, C. L. Soderberg, A. von Deimling, A. Perry, B. W. Scheithauer & R. B. Jenkins: Mapping of the chromosome 19 q-arm glioma tumor suppressor gene using fluorescence in situ hybridization (FISH) and novel microsatellite markers. (submitted)

125. Pohl U, J. S. Smith, I. Tachibana, K. Ueki, H. K. Lee, S. Ramaswamy, Q. Wu, H. W. Mohrenweiser, R. B. Jenkins & D. N. Louis: EHD2, EHD3 and EHD4 encode novel members of a highly conserved family of EH domain containing proteins. Genomics (in press)

126. Peters N, J. S. Smith, I. Tachibana, H. K. Lee, U. Pohl, B. P. Portier, D. N. Louis & R. B. Jenkins: The human glia maturation factor-gamma gene: genomic structure and mutation analysis in gliomas with chromosome 19q loss. Neurogenetics 2, 163-166 (1999)

127. Chou D, T. Miyashita, H. W. Mohrenweiser, K. Ueki, K. Kastury, T. Druck, A. von Deimling, J. C. Reed & D. N. Louis: The BAX gene maps to the glioma candidate region at 19q13.3. but is not altered in human gliomas. Cancer Genet Cytogenet 88, 136-140 (1996)

128. Ueki K, S. Ramaswamy, S. J. Billings, H. W. Mohrenweiser & D. N. Louis: Chromosomal localization to

19q13.3, partial genomic structure and 5' cDNA sequence of the human symplekin gene. Somat Cell Molec Genet 23, 229-231 (1997)

129. Yong W H, K. Ueki, D. Chou, S. A. Reeves, A. von Deimling, J. F. Gusella, H. W. Mohrenweiser, A. J. Buckler & D. N. Louis: Cloning of a highly conserved human protein serine-threonine phosphatase gene from the glioma candidate region on chromosome 19q13.3. Genomics 29, 533-536 (1995)

130. Ueki K, S. Ramaswamy, S. J. Billings, H. W. Mohrenweiser & D. N. Louis: ANOVA, a putative astrocytic RNA-binding protein gene that maps to chromosome 19q13.3. Neurogenetics 1, 31-36 (1997)

131. Smith J S, A. Perry, T. J. Borell, H. K. Lee, J. O'Fallon, S. M. Hosek, D. Kimmel, A. Yates, P. C. Burger, B. W. Scheithauer & R. B. Jenkins: Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol (in press).

132. Weber R G, M. Sabel, J. Reifenberger, C. Sommer, J. Oberstras, G. Reifenberger, M. Kiessling & T. Cremer: Characterization of genomic alterations associated with glioma progression by comparative genomic hybridization. Oncogene 13, 983-994 (1996)

133. Fults D, J. Petronio, B. D. Noblett & C. A. Pedone: Chromosome 11p deletions in human malignant astrocytomas and primitive neuroectodermal tumors. Genomics 14, 799-801 (1992)

134. Sonada Y, M. Iizuka, J. Yasuda, R. Makino, T. Ono, T. Kayama, T. Yoshimoto & T. Sekiya: Loss of heterozygosity at 11p15 in malignant glioma. Cancer Res 55, 2166-2168 (1995)

135. Jenkins R B, D. W. Kimmel, C. A. Moertel, C. G. Schultz, B. W. Scheithauer, P. J. Kelly & G. W. Dewald: A cytogenetic study of 53 human gliomas. Cancer Genet Cytogenet 39, 253-279 (1989)

136. Yarden Y & A. Ullrich: Growth factor receptor tyrosine kinases. Annu Rev Biochem 57, 443-478 (1988)

137. Wong A J, S. H. Bigner, D. D. Bigner, K. W. Kinzler, S. R. Hamilton & B. Vogelstein: Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. Proc Natl Acad Sci USA 84, 6899-6903 (1987)

138. Ekstrand A J, C. D. James, W. K. Cavenee, B. Seliger, R. F. Petterson & V. P. Collins: Genes for epidermal growth factor receptor, transforming growth factor alpha, and epidermal growth factor and their expression in human gliomas. Cancer Res 51, 2164-2172 (1991)

139. von Deimling A, D. N. Louis, K. von Ammon, I. Peterson, T. Hoell, R. Y. Chung, R. Martuza, D. Schoenfeld, M. G. Yasargil, O. D. Wiestler & B. R. Seizinger: Association of epidermal growth factor receptor gene amplification with loss of chromosome 10 in human glioblastoma multiforme. J Neurosurg 77, 295-301 (1992)

140. Ekstrand A J, N. Sugawa, C. D. James & V. P. Collins: Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletion of sequences encoding proteins of the N- and/or C-terminal tails. Proc Natl Acad Sci USA 89, 4309-4313 (1992)

141. Wikstrand C J, R. E. McLendon, A. H. Friedman & D. D. Bigner: Cell surface localization and density of the tumor-associated variant of the epidermal growth factor receptor. Cancer Res 57, 4130-4140 (1997)

142. Dorward N L, R. A. Hawkins & I. R. Whittle: Epidermal growth factor receptor activity and clinical outcome in glioblastoma and meningioma. Br J Neurosurg. 7, 197-200 (1993)

143. Leenstra S, E. K. Bijlsma, D. Troost, J. Oosting, A. Westerveld, D. A. Bosch & T. J. M. Hulsebos: Allele loss on chromosomes 10 and 17p and epidermal growth factor receptor gene amplification in human malignant astrocytoma related to prognosis. Br J Cancer 70, 684-689 (1994)

144. Kordek R, W. Biernat, J. Alwasiak, R. Maculewicz, R. Yanagihara & P. P. Liberski: p53 protein and epidermal growth factor receptor expression in human astrocytomas. J Neuro-Oncol 26, 11-16 (1995)

145. Schober R, T. Bilzer, A. Waha, G. Reifenberger, W. Wechsler, A. von Deimling, O. D. Weistler, M. Westphal, J. T. Kemshead, F. Vega, J. Y. Delattre & P. Stasiecki-Steinfeld: The epidermal growth factor receptor in glioblastoma: genomic amplification, protein expression, and patient survival data in a therapeutic trial. Clin Neuropathol 3, 169-174 (1995)

146. Waha A, A. Baumann, H. K. Wolf, R. Fimmers, J. Neumann, D. Kindermann, K. Astrahantseff, I. Blümcke, A. von Deimling & U. Schlegel: Lack of prognostic relevance of alterations in the epidermal growth factor receptor-transforming growth factor-alpha pathway in human astrocytic gliomas. J Neurosurg 85, 634-641 (1996)

147. Leenstra S, N. T. Oskam, E. H. Bijleveld, D. A. Bosch, D. Troost & T. J. M. Hulsebos: Genetic sub-types of human malignant astrocytoma correlate with survival. Int J Cancer 79, 159-165 (1998)

148. Schlegel J, A. Merdes, G. Stumm, F. K. Albert, M. Forsting, N. Hynes & M. Kiessling: Amplification of the epidermal-growth-factor-receptor gene correlates with different growth behaviour in human glioblastoma. Int J Cancer 56, 72-77 (1994)

149. Hurtt M R, J. Moossy, M. Donovan-Peluso & J. Locker: Amplification of epidermal growth factor receptor gene in gliomas: histopathology and prognosis. J Neuropathol Exp Neurol 61, 84-90 (1992)

150. Torp S H, E. Helseth, A. Dalen & G. Unsgaard: Relationships between Ki-67 labelling index, amplification of

the epidermal growth factor receptor gene, and prognosis in human glioblastomas. Acta Neurochir 117, 182-186 (1992)

151. Hiesiger E M, R. L. Hayes, D. M. Pierz & G. N. Budzilovich: Prognostic relevance of epidermal growth factor receptor (EGF-R) and c-neu/erbB2 expression in glioblastomas (GBMs). J Neuro-Oncol 16, 93-104 (1993)

152. Zhu A, J. Shaeffer, S. Leslie, P. Kolm & A. M. El-Mahdi: Epidermal growth factor receptor: an independent predictor of survival in astrocytic tumors given definitive irradiation. Int J Radiation Oncology Biol Phys 34, 809-815 (1996)

153. Rasheed B K A, G. N. Fuller, A. H. Friedman, D. D. Bigner & S. H. Bigner: Loss of heterozygosity for 10q loci in human gliomas. Genes Chrom Cancer 5, 75-82 (1992)

154. Karlbom A E, C. D. James, J. Boethius, W. K. Cavenee, V. P. Collins, M. Nordenskjold & C. Larsson: Loss of heterozygosity in malignant gliomas involves at least three distinct regions on chromosome 10. Hum Genet 92, 169-174 (1993)

155. Albarosa R, B. M. Colombo, L. Roz, I. Magnani, B. Pollo, N. Cirenei, C. Giani, A. M. Conti, S. DiDonato & G. Finocchiaro: Deletion mapping of gliomas suggests the presence of two small regions for candidate tumor-suppressor genes in a 17-cM interval on chromsome 10q in human glioma. Am J Hum Genet 58, 1260-1267 (1996)

156. Kimmelman A C, D. A. Ross & B. C. Liang: Loss of heterozygosity on chromosome 10p in human gliomas. Genomics 34, 250-254 (1996)

157. Ichimura K, E. E. Schmidt, A. Miyakawa, H. M. Goike & V. P. Collins: Distinct patterns of deletion on 10p and 10q suggest involvement of multiple tumor suppressor genes in the development of astrocytic gliomas of different malignancy grades. Genes Chrom Cancer 22, 9-15 (1998)

158. Li J, C. Yen, D. Liaw, K. Podsypanina, S. Bose, S. I. Wang , J. Puc, C. Miliaresis, L. Rodgers, R. McCombie, S. H. Bigner, B. C. Giovanella, M. Ittmann, B. Tycko, H. Hibshoosh, M. H. Wigler & R. Parsons: PTEN, a putative tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275, 1943-1947 (1997)

159. Steck P A, M. A. Perhouse, S. A. Jasser, W. K. A. Yung, H. Lin, A. H. Ligon, L. A. Langford, M. L. Baumgard, T. Hattier, T. Davis, C. Frye, R. Hu, B. Swedlund, D. H. F. Teng & S. V. Tavtigian: Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nature Genet 17, 32-39 (1997)

160. Teng D H F, R. Hu, H. Lin, T. Davis, D. Iliev, C. Frye, B. Swedlund, K. L. Hansen, V. L. Vinson, K. L. Gumpper, L. Ellis, A. El-Naggar, M. Frazier, S. Jasser, L. A. Langford, J. Lee, G. B. Mills, M. A. Perhouse, R. E. Pollack, C. Tornos, P. Troncoso, W. K. A. Yung, G. Fujii, A. Berson, R. Bookstein, J. B. Bolen, S. V. Tavtigian & P. A. Steck: MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. Cancer Res 57, 5221-5225 (1997)

161. Liu W, C. D. James, L. Frederick, B. E. Alderete & R. B. Jenkins: PTEN/MMAC1 mutations and EGFR amplification in glioblastoma. Cancer Res 57, 5254-5257 (1997)

162. Cantley L C & B. G. Neel: New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci USA 96, 4240-4245 (1999)

163. Mollenhauer J, S. Wiemann, W. Scheurlen, B. Korn, Y. Hayashi, K. K. Wilgenbus, A. von Deimling & A. Poutska: DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3-26.1 is deleted in malignant brain tumours. Nature Genet 17, 32-39 (1997)

164. Law S K, K. J. Micklem, J. M. Shaw, X. P. Zhang, Y. Dong, A. C. Willis & D. Y. Mason: A new macrophage differentiation antigen which is a member of the scavenger receptor suerfamily. Eur J Immunol 23, 2320-2325 (1993)

165. al Awqati Q: Plasticity in epithlial polarity of renal intercalated cells: targeting of the H(+)-ATPase and band 3. Am J Physiol 270, 1571-1580 (1996)

166. Sano T, H. Lin, X. Chen, L. A. Langford, D. Koul, M. L. Bondy, K. R. Hess, J. N. Myers, Y. K. Hong, W. K. A. Yung & P. A. Steck: Differential expression of MMAC/PTEN in glioblastoma multiforme: relationship to localization and prognosis. Cancer Res 59, 1820-1824 (1999)

167. Lin H, M. L. Bondy, L. A. Langford, K. R. Hess, G. L. Delclos, X. Wu, W. Chan, M. A. Perhouse, W. K. A. Yung & P. A. Steck: Allelic deletion analyses of MMAC/PTEN and DMBT1 loci in gliomas: relationship to prognostic significance. Clin Cancer Res 4, 2447-2454 (1998)

168. Mørk S J, K. F. Lindegaad, T. B. Halvorsen, E. H. Lehmann, T. Solgaard, R. Hatlevoll, S. Harvei & J. Ganz: Oligodendroglioma. Incidence and biological behavior in a defined population. J Neurosurg 63, 881-889 (1985)

169. Tola M R, E. Casetta, E. Granieri, L. Pinna, V. Veronesi, R. Tamarozzi, G. Trapella, V. C. Monetti, E. Paolino, V. Govoni & M. Carreras: Intracranial gliomas in Ferrara, Italy, 1976 to 1991. Acta Neurol Scand 90, 312-317 (1994)

170. Kernohan J W, R. F. Mabon, H. J. Svien & A. W. Adson: A simplified classification of the gliomas. Proc Staff Meeting Mayo Clin 24, 71-75 (1949)

171. Ludwig C L, M. T. Smith, A. D. Godfrey & V. W. Armbrustmacher: A clinicopathological study of 323 patients with oligodendroglioma. Ann Neurol 19, 15-21 (1986)

172. Muller W, D. Afra & R. Schroder: Supratentorial recurrences of gliomas: morphological studies in relation to time intervals with oligodendrogliomas. Acta Neurochir 39, 15-25 (1977)

173. Ransom D T, S. R. Ritland, D. W. Kimmel, C. A. Moertel, R. J. Dahl, B. W. Scheithauer, P. J. Kelly & R. B.

Jenkins: Cytogenetic and loss of heterozygosity studies in ependymoma, pilocytic astrocytomas, and oligodendrogliomas. Genes Chrom Cancer 5, 348-356 (1992)

174. Zhu J J, T. Santarius, X. Wu, J. Tsong, A. Guha, J. K. Wu, T. J. Hudson & P. McL Black: Screening for loss of heterozygosity and microsatellite instability in oligodendrogliomas. Genes Chrom Cancer 21, 207-216 (1998)

175. Luider T M, J. M. Kros, P. A. Sillevis Smitt, M. J. van den Bent & C. J. Vecht: Glial fibrillary acidic protein and its fragments discriminate astrocytoma from oligodendroglioma. Electrophoresis 20, 1087-1091 (1999)

176. Morris H H, M. L. Estes, R. Gilmore, P. C. Van Ness, G. H. Barnett & J. Turnball: Chronic intractable epilepsy as the only symptom of primary brain tumor. Epilepsia 34, 1038-1043 (1993)

177. Sjors K, G. Blennow & G. Lantz: Seizures as the presenting symptom of brain tumors in children. Acta Paediatr 82, 66-70 (1993)

178. Mason W P, G. S. Krol & L. M. DeAngelis: Low-grade oligodendroglioma responds to chemotherapy. Neurology 46, 2093-2097 (1996)

179. S M Sagar & M Israel: Tumors of the nervous system. In: Harrison's Principles of Internal Medicine. Fourteenth Edition. Eds: Fauci A S, Braunwald E, Isselbacher K J, Wilson J D, Martin J B, Kasper D L, Hauser S L, Longo D L, McGraw-Hill, NY 2398-2409 (1998)

180. Shaw E G, B. W. Scheithauer, J. R. O'Fallon, H. D. Tazalaar & D. H. Davis: Oligodendogliomas. The Mayo Clinic experience. J Neurosurg 76, 428-434 (1992)

181. Kros J M, H. Pieterman, C. G. van Eden & C. J. Avezaat: Oligodendroglioma, the Rotterdam-Dijkzigt experience. Neurosurgery 34, 959-966 (1994)

182. Wallner K E, M. Gonzales & G. E. Sheline: Treatment of oligodendrogliomas with or without postoperative irradiation. J Neurosurg 68, 684-688 (1988)

183. Mahaley M S, C. Mettlin, N. Natarajan & E. B. Laws, Jr.: National survey of patterns of care for brain-tumor patients. J Neurosurg 71, 826-836 (1989)

184. Sun Z M, S. Genka, N. Shitara, A. Akanuma & K. Takakura: Factors possibly influencing the prognosis of oligodendroglioma. Neurosurgery 22, 886-891 (1988)

185. Burger P C, C. E. Rawlings, E. B. Cox, R. E. McLendon, S. C. Schold, Jr & D. E. Bullard: Clinicopathologic correlations in oligodendroglioma. Cancer 59, 1345-1352 (1987)

186. Smith M T, C. L. Ludwig, A. D. Godfrey & V. W. Armbrustmacher: Grading of oligodendrogliomas. Cancer 52, 2107-2114 (1983)

187. Macdonald D: Low grade gliomas, mixed gliomas and oligodendrogliomas. Semin Oncol 21, 236-248 (1994)

188. Westergaard L, F. Gjerris & L. Klinken: Prognostic factors in oligodendroglioma. Acta Neurochir (Wien) 139, 600-605 (1997)

189. Schiffer D, A. Dutto, P. Cavalla, I. Bosone, A. Chiò, R. Villani & C. Bellotti: Prognostic factors in oligodendroglioma. Can J Neurol Sci 24, 313-319 (1997)

190. Cairncoss J G & D. R. Macdonald: Successful chemotherapy for recurrent malignant oligodendroglioma. Ann Neurol 23, 350-364 (1988)

191. Glass J, F. H. Hochberg, M. L. Gruber, D. N. Louis, D. Smith & B. R. Rattner: The treatment of oligodendrogliomas and mixed oligodendroglioma-astrocytomas with PCV chemotherapy. J Neurosurg 76, 741-745 (1992)

192. Cairncross J G, D. Macdonald, S. Ludwin, D. Lee, T. Cascino, J. Buckner, D. Fulton, E. Dropcho, D. Stewart, J. Schold, N. Wainman & E. Eisenhauer: Chemotherapy for anaplastic oligodendroglioma: National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 12, 2013-2021 (1994)

193. P L Lantos, S R Vandenberg & P Kleihues: Tumours of the nervous system. In: Greenfield's Neuropathology. Sixth Edition. Eds: Graham D I, Lantos P L, Oxford University Press, NY 583-795 (1997)

194. Cairncross J G, K. Ueki, M. C. Zlatescu, D. K. Lisle, D. K. Lisle, D. M. Finkelstein, R. R. Hammond, J. S. Silver, P. C. Stark, D. R. Macdonald, Y. Ino, D. A. Ramsay & D. N. Louis: Specific genetic predictors of chemotherapeutic response and survival in patients with anplastic oligodendroglioma. J Natl Cancer Inst 90, 1473-1479 (1998)

195. Reifenberger J, G. Reifenberger, L. Liu, C. D. James, W. Wechsler & V. P. Collins: Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol 145, 1175-1190 (1994)

196. Kraus J A, J. Koopman, P. Kaskel, D. Maintz, S. Brandner, D. N. Louis, O. D. Weistler & A. von Deimling: Shared allelic losses on chromosomes 1p and 19q suggest a common origin of oligodendrogliomas and oligoastrocytomas. J Neuropathol Exp Neurol 54, 91-95 (1995)

197. Bello M J, J. M. De Campos, J. Vaquero, M. E. Kusak, J. L. Sarasa, J. Saez-Castresana, A. Pestana & J. A. Rey: Molecular analysis of chromosome 1 abnormalities in human gliomas reveals frequent loss of 1p in oligodendroglial tumors. Int J Cancer 57, 172-175 (1994)

198. Husemann K, M. Wolter, R. Büschges, J. Boström, M. Sabel & G. Reifenberger: Identification of two distinct deleted regions on the short arm of chromosome 1 and rare mutation of the CDKN2C gene from 1p32 in oligodendroglial tumors. J Neuropathol Exp Neurol 58, 1041-1050 (1999)

199. Sato K, B. Schauble, P. Kleihues & H. Ohgaki: Infrequent alterations of the p15, p16, CDK4, and cyclin D1 genes in non-astrocytic human brain tumors. Int J Cancer 66, 305-308 (1996)

200. Jeuken J W M, S. H. E. Sprenger, P. Wesseling, M. V. E. Macville, A. von Deimling, H. L. J. M. Teepen, J. J. van Overbeeke & R. H. Boerman: Identification of subgroups of high-grade oligodendroglial tumors by comparative genomic hybridization. J Neuropathol Exp Neurol 58, 606-612 (1999)

201. Reifenberger J, G. Reifenberger, K. Ichimura, E. E. Schmidt, W. Wechsler & V. P. Collins: Epidermal growth factor receptor expression in oligodendroglial tumors. Am J Pathol 149, 29-35 (1996)

202. Reifenberger G, R. Prior, M. Deckert & W. Weschsler: Epidermal growth factor receptor expression and growth fraction in human tumors of the central nervous system. Virchows Ach A Pathol Anat 414, 147-155 (1989)

203. Hawkins R A, E. R. Killen, W. J. Jack & U. Chetty: Epidermal growth factor receptors in intracranial and breast tumors: their clinical significance. Br J Cancer 63, 553-560 (1991)

204. Diedrich U, S. Soja, J. Behnke & B. Zoll: Amplification of the c-erb-B oncogene is associated with malignancy in primary tumors of neuroepithelial tissue. J Neurol 238, 221-224 (1991)

205. Cooper E R A: The relation of oligocytes and astrocytes in cerebral tumors. J Pathol 41, 259-266 (1935)

206. P C Burger & B W Scheithaer: Tumors of the Central Nervous System. Atlas of Tumor Pathology. Armed Forces Institute of Pathology, Washington DC (1994)

207. Burger P C, Y. Minn, J. S. Smith, T. J. Borell, A. E. Jedlicka, B. K. Huntley & R. B. Jenkins: Comparative genomic hybridization, fluorescence in situ hybridization, PCR, and p53 gene sequencing in the diagnosis of oligodendrogliomas. (submitted)

208. Jakolsky D, M. Zawirski, W. Papierz & Z. Kotwica: Mixed gliomas. Their clinical course and results of surgery. Zentralbl Neurochir 48, 120-123 (1987)

209. Shaw E G, B. W. Scheithauer, J. R. O'Fallon & D. H. Davis: Mixed oligoastrocytomas: a survival and prognostic factor analysis. Neurosurgery 34, 577-582 (1994)

210. Helseth A & S. J. Mørk: Neoplasms of the central nervous system in Norway. III. Epidemiological chracteristics of intracranial gliomas according to histology. Acta Pathol Microbiol Immunol Scand 97, 547-555 (1989)

211. Hart M N, C. K. Petito & K. M. Earle: Mixed gliomas. Cancer 33, 134-140 (1974)

212. Kim L, F. H. Hochberg, A. F. Thornton, G. R. Harsh, H. Patel, D. Finkelstein & D. N. Louis: Procarbazine, lomustine, and vincristine (PCV) chemotherapy for grade III and grade IV oligoastrocytomas. J Neurosurg 85, 602-607 (1996)

213. Donahue B, C. B. Scott, J S. Nelson, M. Rotman, K. J. Murray, D. F. Nelson, F. L. Banker, J. D. Earle, J. A. Fischbach, S. O. Asbell, L. E. Gaspar, A. M. Markoe & W. Curran: Influence of an oligodendroglial component on the survival of patients with anaplastic astrocytomas: a report of radiation therapy oncology group 83-02. Int J Radiation Oncology Biol Phys 38, 911-914 (1997)

Key Words: Diffuse Glioma, Astrocytoma, Oligodendroglioma, Mixed Oligoastrocytomas, Prognosis, Therapy, Stratification, Molecular Genetics, Review

Send correspondence to: R. B. Jenkins, M.D., Ph.D., Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905, Tel: 507-284-9617, Fax: 507- 284-0043, E-mail: rjenkins@mayo.edu

This manuscript is available on line at:

http://www.bioscience.org/2000/v5/d/smith/fulltext.htm