In more than 80% of cases, the first manifestation of low-grade glioma (LGG) is a partial or (secondarily) generalized seizure in a “young” adult (median age, 35–39 years) who is otherwise in good general health with a normal neurological examination. Much more rarely, progressive deficit, cognitive dysfunction, or intracranial hypertension will reveal the disease. In this setting, MRI of the brain with and without gadolinium infusion is mandatory. The MRI aspect is not specific, but the diagnosis is strongly suspected when a nonenhancing mass (hypointense on T1-weighted images and hyperintense on T2-weighted images or FLAIR sequences) is discovered in the “frontal-temporal-insular” region or within the “parasagittal” frontal-parietal regions, the most frequent and characteristic locations of LGG (Duffau and Capelle, 2004). Occasionally, contrast enhancement is seen, or the tumor widely infiltrates the brain at the onset (gliomatous cerebri). MR spectroscopy and PET scan (fluorodeoxyglucose and methionin) are useful to guide a biopsy and for differential diagnosis. The most frequent tumors, such as astrocytic or glioblastomas encephalitis in young patients and stroke in older patients (Calli et al., 2002). Definite diagnosis eventually relies on microscopic and genetic examination of a tumor sample. Microscopic examination is crucial to exclude tumors that can be age, performance status, histological subtype, or tumor size at diagnosis. Indeed, the WHO classification of LGGs (Kleihues, 2000) relies on morphological criteria to define astrocytomas, oligodendrogliomas, or mixed gliomas. This classification remains imperfect because of incomplete reproducibility, even in a single observer, and lack of specific marker of tumor subtype (Mokhtari et al., 2005). This fact is illustrated by a dramatic rise in the incidence of oligodendrogliomas in many centers over the last decade associated with a concomitant reduction of astrocytomas, even if the diagnostic criteria did not change significantly during this period (Burger, 2002, and data not shown). As a consequence, the respective frequency of tumor subtypes varies considerably among institutions, a feature with practical implications because evidence suggests that the management of these tumors should be tailored according to the main tumor cell type (Hoang-Xuan et al., 2004).

Molecular classification is an important adjunct to the classification of LGGs. Up to 80% of LGGs have one of the two key “early” genetic alterations reported in gliomas, including chromosome 1p loss or 19p (which is linked to P53 mutation), and 20% to 25% have both alterations or neither. Overall, these two molecular alterations are strongly mutually exclusive. Tumors with both loss almost always have a morphological pattern of oligodendrogliomas (“honeycomb” appearance) or rarely a mixed glialoma pattern (Reifenberger et al., 1994). On the other hand, tumors with P53 expression are more heterogeneous: 50% of them are fibrillary astrocytomas, but the other half have either an oligodendroglial or a mixed glialoma pattern. Thus, an astrocytoma pattern is practically never associated with 1p loss alone, but an oligodendroglioma or mixed glioma pattern may be associated with either 1p loss or P53 expression. There is a correlation between the profile of molecular alterations and tumor location (Laigle-Donadey et al., 2004; Zlatescu et al., 2001). Chromosome 1p-deleted tumors are preferentially located in the frontal-parietal-temporal occipital regions and are more circumscribed on MRI. Interestingly, a correlation between P53 mutation and preferential topography of the tumor has also been noted in some gliomas and colon cancers.

Knowledge of the genetic status of LGGs is important consequences because chromosome 1p loss seems to be one of the most important favorable prognostic factors in LGG, possibly surpassing classic factors such as age, performance status, histological subtype, or tumor size at diagnosis. Furthermore, 1p-deleted LGGs are chemosensitive in half of the cases, but 1p-intact tumors much more rarely respond to chemotherapy (Hoang-Xuan et al., 2004). Thus, a biopsy does not necessarily encompass the most aggressive part of a tumor, microscopic examination of a small specimen taken at the periphery of the lesion may be falsely reassuring when it detects only features of LGG. In these circumstances, detection of the so-called late genetic alterations of gliomas, particularly the ominous combination of EGFR amplification with chromosome 10q loss, may alert the clinician that the underlying tumor is much more malignant than expected by histology.

The natural history of LGG is changing, with a striking “increase of survival” over the last two decades. This feature reflects the fact that the diagnosis is made much more earlier than before and underlines the need for a careful evaluation of the risk/benefit ratio before administration of potentially toxic treatment. An important observation is that LGGs grow inexorably over time during the period, which often lasts many years, preceding the malignant transformation and terminal phase of an LGG course (Mandonnet et al., 2003), a feature pleading for early surgery when it is possible.

References


2. EXTENDED ABSTRACT: PROSPECTIVE CLINICAL TRIALS OF ADULT-SUPRATENTORIAL LOW-GRADE GLIOMA

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There are three current controversies in the radiotherapeutic management of adults with supratentorial low-grade glioma (LGG) based on numerous retrospective studies that have been published in the medical literature. The first is the optimum timing of radiation therapy (RT). Following maximum surgical resection, should RT be given immediately postoperatively, or is surgery alone adequate treatment, with RT added only if imaging progression occurs? Second, if RT is to be given, is there an advantage in terms of local control and survival for higher rather than moderate to lower doses of RT? Third, given the modest cure rates of surgery with or...
without RT, will disease-free and overall survival be improved by adding chemotherapy to RT? Four prospective randomized clinical trials in adults with supratentorial LGG were conducted in the United States, Canada, and Europe during the 1980s and 1990s that addressed these questions (Eyre et al., 1993; Karim et al., 1996, 2002; Shaw et al., 2002; van den Bent et al., 2004). The data from the nearly 1000 patients treated in these studies are summarized in this abstract. In addition, the schema from recently completed and planned phase 2 and phase 3 clinical trials in the United States and Europe will be reviewed.

Summary of Published Clinical Trials: The five-year overall survival and progression-free survival rates in these four published studies ranged from 58% to 72% and from 37% to 55%, respectively. European Organization for Research and Treatment of Cancer (EORTC) Study 22845 randomized 311 adults to postoperative observation or RT. There was no difference in the five-year survival rate between the two arms. Irradiated patients had a significantly higher 5-year progression-free survival rate. EORTC Study 22844 randomized 379 adults to low- versus high-dose RT. Similarly, an intergroup study conducted by the North Central Cancer Treatment Group, Radiation Therapy Oncology Group (RTOG), and the Eastern Cooperative Group randomized 211 adults to low- versus high-dose RT. There was no difference in the five-year overall or progression-free survival rates between the two dose groups in either study. A Southwest Oncology Group study randomized 60 adults with incompletely resected LGG to RT alone or RT plus lomustine (CCNU) chemotherapy. There was no difference in outcome between the two treatment arms. Important prognostic factors for overall survival in these trials include extent of surgical resection, histologic tumor grade, and age.

Summary of Recently Completed and Planned Clinical Trials: The RTOG recently completed Study 9802, in which adults with supratentorial LGG were placed into risk groups and treated accordingly. Low-risk patients were defined as those younger than 45 years and who underwent gross total resection postoperatively. The preliminary data from 111 low-risk patients will be presented at this meeting (see Shaw et al., 2002). High-risk patients in RTOG 9802, defined as those aged 40 years or older who underwent subtotal or biopsy, were randomized to RT alone (54 Gy to tumor with a 2 cm margin) or RT followed by six cycles of PCV chemotherapy (procarbazine, CCNU, and vincristine). It will be several years before data from the high-risk group will be available. The RTOG has just opened a randomized phase 2 study of RT (54 Gy) followed by temozolomide chemotherapy, or temozolomide alone. A phase 3 trial in adults with supratentorial LGG, randomizing them to RT (50.4 Gy to tumor with a 2 cm margin) or RT followed by temozolomide chemotherapy, or temozolomide both during and following RT. The Eastern Cooperative Oncology Group has just submitted a protocol to the National Cancer Institute for a phase 3 clinical trial in “high-risk” adults with supratentorial LGG, randomizing them to RT (50.4 Gy to tumor with a 2 cm margin) or RT followed by six cycles of temozolomide chemotherapy. The EORTC is planning a phase 3 clinical trial in adults with supratentorial LGG, randomizing them to RT (50.4 Gy) or temozolomide (no RT), stratifying patients by chromosome 1p deletion status (present versus absent) (Dr. Martin van den Bent, personal communication, November 30, 2004).

Conclusions: Based on the information presented, the following conclusions can be made: In adults with LGG, there is no difference in overall survival whether RT is given postoperatively or delayed to the time of recurrence. However, about two-thirds of adults with LGG will develop tumor progression by 15 years following surgery alone. When RT is administered, lower doses produce a similar survival outcome as higher doses with less neurotoxicity. Data on whether chemotherapy (PCV or temozolomide) should be added to RT in “high-risk” adults with supratentorial LGG, randomizing them to RT alone (54 Gy to tumor with a 2 cm margin) or RT followed by six cycles of PCV chemotherapy (procarbazine, CCNU, and vincristine) will be several years before data from the high-risk group will be available. The RTOG has just opened a randomized phase 2 study of RT (54 Gy) followed by temozolomide chemotherapy, or temozolomide alone. A phase 3 trial in adults with supratentorial LGG, randomizing them to RT (50.4 Gy to tumor with a 2 cm margin) or RT followed by temozolomide chemotherapy, or temozolomide both during and following RT. The Eastern Cooperative Oncology Group has just submitted a protocol to the National Cancer Institute for a phase 3 clinical trial in “high-risk” adults with supratentorial LGG, randomizing them to RT (50.4 Gy to tumor with a 2 cm margin) or RT followed by six cycles of temozolomide chemotherapy. The EORTC is planning a phase 3 clinical trial in adults with supratentorial LGG, randomizing them to RT (50.4 Gy) or temozolomide (no RT), stratifying patients by chromosome 1p deletion status (present versus absent) (Dr. Martin van den Bent, personal communication, November 30, 2004).

5. EXTENDED ABSTRACT: OVERVIEW AND CURRENT STATUS OF BRAIN TUMOR EPIGENOLOGY

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Epidemiology aims to describe the occurrence of a disease in time and place, understand and explain the distribution and causes of the disease, and address factors that influence survival after diagnosis. Major challenges in conducting epidemiologic studies of brain tumors include (1) substantial histologic and molecular heterogeneity of tumors between and within histologic groups, (2) the relative rarity of any given subtype, (3) geographic and temporal heterogeneity in reporting requirements and classification systems, (4) a paucity of well-established risk factors, and (5) the rapid fatality associated with the most common and lethal form of primary malignant brain tumors, glioblastoma multiforme, necessitating studies that often involve the use of proxy respondents for gathering potentially relevant life-history information. To meet these challenges, recent descriptive epidemiologic studies of primary brain tumors highlight strengths and weaknesses in population data to encourage consistent and relevant brain tumor surveillance systems. While efforts to facilitate etiologic studies, establishing awareness of the disease, and ultimately, for the prevention of all brain tumors. Etiologic and prognostic studies of brain tumors increasingly require multidisciplinary collaboration between surgeons, neuro-oncologists, geneticists, toxicologists, epidemiologists, and molecular and environmental scientists. Molecular tumor markers are identified that predict survival and treatment response with the goal of even greater gains in this area with emerging array technologies. Regarding risk factors, studies of inherited susceptibility and constitutive polymorphisms in genes pertinent to carcinogenesis (e.g., DNA repair, detoxification, and immune function genes and mutation sensitivity) have revealed provocative findings. Consistent inverse associations of history of...
allergies with glioma risk observed in several large studies suggest a possible role of immune factors in gliomagenesis or progression. Studies also continue to suggest that brain tumors might result from workplace, dietary, and other personal and residential exposures. Recent studies of cell phone use have suggested that acoustic neuromas, but not other primary brain tumors, might be influenced by cell phone use. Only a small proportion of primary brain tumors are attributable to proven and widely accepted causes of brain injuries (i.e., rare hereditary radia-
tion, and immune suppression giving rise to brain lymphomas), suggesting the need for further discovery. Progress in understanding primary brain tumors will require large studies of well-defined histologic and molecular traits incorporating assessment of potential relevant information on subject susceptibility and environmental and noninherited endogenous factors (viruses, radiation, and carcinogenic or protective chemical exposures through diet, workplace, oxidative metabolism, or other sources). To follow these trends, in the low-dose group (Kiebert et al., 1998).

In two randomized trials the impact of temozolomide on HRQOL in glioblastoma multiforme patients was investigated. No negative impact of temozolomide on the patients’ HRQOL was observed, in contrast to the toxic effect of procarbazine (Osoba et al., 2000a; Taphoorn et al., 2004).

Supportive Treatment: The brain tumor and its treatment may have significant physical, cognitive, emotional, and social effects on the patient. The patient’s partner and family may also experience a negative emotional and social impact. Apart from treatment of the tumor itself, supportive treatment of the patient and the patient’s family may include medication (anti-epileptic drugs, steroids, antidepressants) and/or psychological and/or cognitive support. As there is a steady increase in effective treatments for brain tumor patients, the number of long-term survivors will grow. Long-term negative effects of the tumor and its treatment demand an increasing effort of doctors, nurses, and psychologists for supportive care (Remer and Murphy, 2004).

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Abstracts from the World Federation of Neuro-Oncology Meeting

Compliance with serial assessments of HRQOL is one of the major problems in randomized trials. A low compliance with HRQOL assessment is related to performance status and outcome and may cause a serious bias in results (Osoba et al., 2004).

Health-related quality of life in newly diagnosed high-grade glioma patients appeared to be comparable to that in lung cancer patients and was significantly worse than that of healthy controls (Klein et al., 2001). In contrast, cognitive deficit was far more pronounced in glioma patients than in lung cancer patients, reflecting the specific neurological deficit. From studies in both newly diagnosed and recurrent high-grade glioma it is known that HRQOL is related to disease burden and neurological deficit (Osoba et al., 1997).

In a comparison between low-dose and high-dose radiation schedules in low-grade glioma patients, there appeared to be no difference in survival, but HRQOL was more seriously impaired in the high-dose group than in the low-dose group (Kiebert et al., 1998).

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The objective of any anticancer therapy extends well beyond prolonga-
tion of survival. Palliation of symptoms and maintenance or improvement of quality of life are important goals of therapy. Thus, the benefits of exist-
ing or new cancer treatments that maintain or extend survival time need to be weighed against side effects and a possible decrease in the patient’s health-related quality of life (HRQOL). The health-related quality of life become an increasingly important endpoint in cancer studies, next to outcome measures such as overall survival and disease-free survival, and is most relevant in patients who cannot be cured of their disease (Efficace and Bot-

tomley, 2003).

In brain tumor patients, palliating symptoms and maintaining or improving HRQOL is particularly relevant for several reasons. First, patients with primary brain tumors (mainly gliomas) or metastatic tumors in the central nervous system have a dismal prognosis and cannot be cured of their disease. Second, brain tumor patients not only have to cope with clinical symptoms, such as motor deficit and epilepsy, but they are usually also confronted with a decline in cognitive and emotional functioning as a result of cerebral disease. Third, side effects of treatment for brain tumors may have an even further negative impact on cerebral functioning (Mey-
er, 1997).

Measurement of HRQOL: Despite the specific relevance for measuring HRQOL in brain tumor patients, the interest in HRQOL emerged relatively late in this patient group compared with more common cancers such as breast or lung cancer. This has to do with the low incidence of primary brain tumors, a former therapeutic nihilism toward brain cancer, and the fact that the subjective nature of HRQOL assessment may be problematic in brain tumor patients with mental impairments.

Health-related quality of life is defined as a multidimensional concept consisting of at least physical, psychological, and social phenomena. Measuring outcome in terms of tumor size, time to tumor progression, and overall survival is relatively simple compared with outcome measures such as impairment, disability, or handicap, which require (symptom) scales (Heimans and Taphoorn, 2002). These objective scales, however, do not adequately measure the patient’s HRQOL. Health-related quality of life is an even more complex outcome measure, demanding a multidimensional instrument that should be filled out by the patient (self-report questionnaire). Both generic and disease-specific questionnaires have been developed and validated to assess HRQOL in cancer patients. To measure HRQOL in brain tumor patients, the generic European Organization for Treatment and Research of Cancer Quality of Life Questionnaire (EORTC QLQ-C 30) is used in combination with the H&N20 (EORTC QLQ-H&N 20) or the Functional Assessment of Cancer Therapy (FACT) generic question-
naire together with the FACT brain module (Osaba et al., 1996; Weitzner et al., 1995).

Health-Related Quality of Life in Brain Tumor Patients: HRQOL is increasingly being measured as a secondary outcome in brain tumor trials in which the efficacy of a (new) therapy is being evaluated. This holds true for both low-grade and high-grade glioma patients.


7. PRELIMINARY RESULTS OF RTOG PROTOCOL 9802: A PHASE II STUDY OF OBSERVATION IN COMPLETELY RESECTED ADULT LOW-GRADE GLIOMA

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In 1998, the RTOG initiated Protocol 9802 for adults with supratentorial low-grade glioma. Patients were divided into two groups based on risk. Low risk was defined as age. These patients were observed postoperatively with serial magnetic resonance imaging (MRI) scans and form the basis of this report. Eligibility criteria included histologically proven WHO grade II A, O, or OA based on central pathology review, age <18 and >60, Neuropsychologic Function Score (NFS) <3, supratentorial tumor location, GTR, and pre- and post-operative MRI scans available, and signed consent form. MRI scans were obtained every 6 months. Prognostic factors analyzed for their effect on overall survival (OS), progression-free survival (PFS), and tumor recurrence included age (>30 years), gender (male vs. female), KPS (<90 vs. 100), NFS (0 vs. 1–3), histology (A = astrocytoma or astrocytoma-dominant OA vs. O = oligodendroglioma or oligodendroglioma-dominant OA), contrast enhancement on pre-operative MRI scan (present vs. absent), pre-operative tumor diameter (>4cm), and baseline mini-mental status exam score. Between 1998 and 2002, 116 patients were entered, 111 of whom were analyzable. OS and PFS at 3 years for all patients was 97% and 73%, respectively. The only two prognostic factors predicting for significantly poorer PFS in univariate analysis were histology (univariate P = 0.02, multivariate P = 0.03, hazard ratio = 2.33) and pre-operative tumor diameter (univariate P = 0.002, multivariate P = 0.006, hazard ratio = 2.90). The crude incidence of tumor recurrence was 38% for vs. 20% for O and 43% for tumors >4 cm vs. 18% for tumors <4 cm. The 3-year PFS was 89% for O tumors >4 cm. An assessment of extent of surgical resection based on the pre- vs. postoperative MRI scans is ongoing and will also be analyzed. Other investigations pointed out that in bilingual patients common areas with different languages. It has been reported that in bilingual patients multiple and separate areas of the cortex mediate the different languages. Other investigations pointed out that in bilingual patients common areas of the brain are responsible for those functions. We report here five cases of patients harboring a left fronto temporal glioma who were proficient with three to five different languages in which a multiple language brain mapping was undertaken during awake craniotomies for tumor removal. They were 3 males and 2 females, age ranging from 34 to 61 years. Language proficiency was tested by submitting patients to confrontation tests for each language during the pre-operative examination. Each language was tested serially starting from the first acquired language. Language mapping was undertaken during asleep awake craniotomy, by the use of an Ojemann cortical stimulator and by using the largest current that did not produce afterdischarge (from 3.5 to 6 mA) during counting and confrontation naming tests. Subcortical stimulation by using the same current threshold was also applied during tumor resection, in a back and forth fashion. Our data showed that each language has separate and distinct cortical cortices. Cortical areas for first acquired language had a larger cortical representation, whereas those for the secondly acquired languages were localized in more distinct cortical sites. Subcortical stimulations found tracks for the first acquired language in 4 patients, whereas those for the other languages in 3 patients. Subcortical tracts for the first language had a larger representation. Three patients experienced immediate post-operative surgery, mainly affecting the first acquired language, which fully recovered in two patients in two months and partially in one. These findings support the existence of language-specific cortical sites and the concept that intraoperative mapping should be performed for all the languages the patient is fluent in, to maximally preserve functional language integrity. In addition, confrontation naming test is more accurate than counting for localization of functional areas.

8. STUDY OF THE INDIVIDUAL CORTICAL ORGANIZATION, CONNECTIVITY, AND PLASTICITY APPLIED TO THE SURGERY OF LOW-GRADE GLIOMAS: FUNCTIONAL AND ONCOLOGICAL RESULTS

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While considered by more and more authors, surgery of low-grade gliomas (LGG) remains matter of debate, because of (1) the risk for generating neuro-oncology.oxfordjournals.org Downloaded from

10. 5-AMINOLEVULINIC ACID-BASED PHOTODYNAMIC DETECTION OF VARIOUS BRAIN TUMORS

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It has been established that 5-aminolevulinic acid (ALA) induces the accumulation of fluorescent porphyrins in malignant gliomas, a phenomenon potentially exploitable in surgical resection of tumors with no or slight deficit. ALA was subsequently used for the diagnosis of various brain tumors, including low-grade tumors, with or without surgical resection. However, the usefulness of fluorescence-guided resections not been studied. Here, we examined the value of ALA-induced fluorescence for detecting various brain tumors including low-grade tumors. Forty-seven patients underwent ALA-induced protoporphyrin fluorescence detection. Three hours before the induction of anesthesia, 1 g 5-ALA/Body was administered orally. Intraoperatively, red porphyrin fluorescence was observed with a 453-nm long-pass filter after excitation with violet-blue (405 nm) light. Fluorescing and nonfluorescing organization of the functional networks was possible in all cases. Despite a frequent immediate post-operative worsening, 94% of patients recovered their preoperative status or (even improved) and returned to a normal socio-professional function within 3 months after surgery. The resection was total or subtotal in 88% of cases. With a median follow-up of 50 months, a significant statistical relationship was observed between the survival and the quality of resection (P = 0.02). Moreover, by comparing these results with 30% LGG operated on in the same institution without intraoperative functional mapping, we showed that electrical stimulation allowed a significant decrease of postoperative sequelae (5% vs. 17%, P = 0.002) and a significant increase of the quality of resection (53% vs. 35% of subtotal and 12% vs. 3% of total), (P < 0.001).

These findings suggest a dynamic spatio-temporal functional organization, with (a) before surgery, the recruitment of compensatory areas, explaining the lack of deficit despite the tumor growth in eloquent regions, (b) immediate after surgery, the occurrence of a deficit, likely due to the resection of invaded areas participating (but not essential) to the function, and (c) 3 months after surgery, a recovery due to long-term functional reshaping. The application of this brain dynamic potential can be considered to significantly extend the limits of surgery in eloquent areas while minimizing the risk of sequelae.

9. INTRAOPERATIVE LANGUAGE MAPPING IN MULTILINGUAL PATIENTS WITH GLIOMAS

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Surgical removal of lesions located close to or in areas of the brains mediating speech requires the use of intraoperative techniques to localize which cortical areas and subcortical tracts have those functions. Localization of speech is particularly problematic in patients that are usually fluent with different languages. It has been reported that in bilingual patients multiple and separate areas of the cortex mediate the different languages. Other investigations pointed out that in bilingual patients common areas of the brain are responsible for those functions. We report here five cases of patients harboring a left fronto temporal glioma who were proficient with three to five different languages in which a multiple language brain mapping was undertaken during awake craniotomies for tumor removal. They were 3 males and 2 females, age ranging from 34 to 61 years. Language proficiency was tested by submitting patients to confrontation tests for each language during the pre-operative examination. Each language was tested serially starting from the first acquired language. Language mapping was undertaken during asleep awake craniotomy, by the use of an Ojemann cortical stimulator and by using the largest current that did not produce afterdischarge (from 3.5 to 6 mA) during counting and confrontation naming tests. Subcortical stimulation by using the same current threshold was also applied during tumor resection, in a back and forth fashion. Our data showed that each language has separate and distinct cortical cortices. Cortical areas for first acquired language had a larger cortical representation, whereas those for the secondly acquired languages were localized in more distinct cortical sites. Subcortical stimulations found tracks for the first acquired language in 4 patients, whereas those for the other languages in 3 patients. Subcortical tracts for the first language had a larger representation. Three patients experienced immediate post-operative surgery, mainly affecting the first acquired language, which fully recovered in two patients in two months and partially in one. These findings support the existence of language-specific cortical sites and the concept that intraoperative mapping should be performed for all the languages the patient is fluent in, to maximally preserve functional language integrity. In addition, confrontation naming test is more accurate than counting for localization of functional areas.

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samples taken from the tumor tissues were examined histologically. Bright red fluorescent tumor tissues were observed in 100% (9 of 9) of glioblastomas, 66% (2 of 3) of anaplastic astrocytomas, 100% (3 of 3) of anaplastic oligodendrogliomas, and 83% (9 of 11) of pilocytic astrocytomas, 72% (8 of 11) of meningiomas (WHO grade I), 33% (1 of 3) of atypical meningiomas, 62% (5 of 8) of germ cell tumors, 66% (2 of 3) of malignant lymphoma, and 50% (1 of 2) of hemangioblastomas. The observation of this study indicates that the presence of 5-ALA-induced tumor fluorescence not only for guiding malignant glioma resection but also for guiding resection of benign tumors such as pilocytic astrocytoma or meningioma. ALA-mediated fluorescence detection may enhance completeness of benign tumor removal and increase the diagnostic accuracy of intraoperative biopsies.

11. VOLUMETRIC EXTENT OF RESECTION AND RESIDUAL CONTRAST ENHANCEMENT AT INITIAL SURGERY AS PREDICTORS OF OUTCOME IN ADULT PATIENTS WITH HEMISPHERIC ANAPLASTIC ASTROCYTOMA

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The available literature evaluating the role of extent of resection for anaplastic astrocytomas, as a distinct histological group, is limited and assessment of residual disease has not been quantitative. To investigate the prognostic significance of volumetrically assessed extent of resection on time to tumor progression (TTP), overall survival (OS), and tumor recurrence patterns, we retrospectively analyzed preoperative and postoperative tumor volumes on 102 adult patients from the time of the initial resection for a hemispheric anaplastic astrocytoma. Histological diagnosis of anaplastic astrocytoma was confirmed based on pathology re-review for all patients using current World Health Organization criteria. Patients with recurrent anaplastic astrocytoma were not included in this study. Quantiﬁcation of tumor volumes was based on a previously described method involving computerized image analysis of magnetic resonance imaging scans. Volumetric analysis was conducted on contrast-enhancing tumor volumes on T1-weighted MR images for 67 patients who had contrast-enhancing tumors, in addition to measurements of T2 hyperintensity for all patients. The variables analyzed included age, Karnofsky Performance Status (KPS), preoperative tumor volume (T1 enhancement and T2 hyperintensity), percent of resection (POR), and volume of residual disease (VRD) (T1 enhancement and T2 hyperintensity). All patients had postoperative radiotherapy, and 94% (96/102) of the patients received chemotherapy. Presence or absence of preresection enhancement, actual volume of this enhancement, and the percentage of preoperative enhancement as it relates to the total T2 tumor volume did not have a statistically signiﬁcant impact on TTP or OS. In addition to age, VRD measured on T2-weighted MR scans was the most signiﬁcant predictor of TTP. Unlike low-grade gliomas, there was no statistically signiﬁcant relationship between extent of resection, i.e., POR and VRD, and histology at the time of recurrence, i.e., grade 3 vs. grade 4. This retrospective analysis of anaplastic astrocytomas treated in the MR era suggests that residual tumor volumes, as documented on postoperative imaging studies, may be a prognostic factor for time to progression and survival for this patient population.

12. POST-OPERATIVE OUTCOME OF ANTERIOR SKULL BASE MENINGIOMAS

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The purpose of this retrospective study was to analyze the outcome and recurrence of anterior skull base meningiomas. A total of 571 meningiomas that were operated upon at the Neurosurgical Department, Technical University of Dresden, between January 1994 and December 2002. Of these 571 meningiomas, 151 were located within the anterior skull base, namely, the frontal base, sphenoid wing, tuberculum sellae, cavernous sinus, and olfactory groove. One hundred forty-one patients were amenable for follow-up including regular ambulatory visits and a questionnaire. The median follow-up including MR imaging was 3.5 years (0.7–6.6 years). The median age was 62.5 years (range 14–91 years). The female to male ratio was 1:2:5. The patients most commonly presented with visual deﬁcits (37%), headaches (25%), dizziness (16%), seizures (12%), or symptoms of organic psychosis (11%). Median tumor volume was 9.7 cm3, mean volume was 26.1 ± 33.6 cm3. Most frequent histological subtypes were meningothelial (67%) and transitional (18%) meningiomas, whereas WHO grade II meningiomas appeared only twice (1.4%). Seizures were most frequently associated with lateral sphenoid wing and frontal base tumors, whereas visual acuity changes, dizziness, and double vision were associated with medial sphenoid wing, olfactory groove, and tuberculoma sellae tumors. Tumor size correlated with appearance of symptoms. Intraoperative radicality was in 16% Simpson grade 1, 59% grade 2, 13% grade 3, and 13% grade 4. Biopsies (grade 5) were not done. Operative radicality in 87% (9 of 10) of differentiated astrocytoma and oligodendroglioma, 100% (3 of 3) of pilocytic astrocytomas, 72% (8 of 11) of meningiomas (WHO grade I), 33% (1 of 3) of atypical meningiomas, 62% (5 of 8) of germ cell tumors, 66% (2 of 3) of malignant lymphoma, and 50% (1 of 2) of hemangioblastomas. The observation of this study indicates that the presence of 5-ALA-induced tumor fluorescence not only for guiding malignant glioma resection but also for guiding resection of benign tumors such as pilocytic astrocytoma or meningioma. ALA-mediated fluorescence detection may enhance completeness of benign tumor removal and increase the diagnostic accuracy of intraoperative biopsies.

13. TAILORING OF ANTI ANGIOGENIC THERAPIES TO TUMOR STAGE IMPROVES THERAPEUTIC EFFICACY IN MOUSE MODELS OF HUMAN GLIOMAS

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Systemic administration of inhibitors of angiogenesis, migration, and proliferation successfully inhibits the growth of experimental malignant gliomas. Nevertheless, after some time, tumor escapes treatment. In this work we investigated the role of changes in tumor vasculature during glioma development on the significance of the therapeutic effect. We initially studied the changes in tumor vasculature occurring in the well-established and surgical resection glioma models in nude mice. Brains from animals sacrificed at various time points from tumor cell injection or tumor removal were studied by immunofluorescence, immunohistochemical, and vessel casting techniques. Early tumors were composed of irregular highly angiogenic vessels, uncovered from pericytes. Tumor vasculature in late tumors showed a complex regional heterogeneity, with highly angiogenic areas and areas composed of more regular vessels covered by pericytes. We then investigated the effect of different inhibitors on various stage of vasculature development. Inhibitors that exhibit various activities on tumor or endothelial cells and that act by different mechanisms were adminis- tered sequentially and simultaneously. The drugs that inhibit FGF and VEGF and act on both tumor and endothelial cells, mainly by inhibiting FGF, was more effective on early tumors; PF-4/DLR, which inhibits FGF and VEGF and acts on both tumor and endothelial cells, was effective on both early and late tumors. A low-dose chemotherapy regimen based on carboplatin and etoposide was more active on late tumors that were more dependent on their location and involvement of other structures, such as vessels and cranial nerves. The most important predictors of postoperative outcome were Simpson grade, age, and location. Repeated MR is required for reliable assessment of recurrent tumor growth.
14. VEGF COOPERATES WITH SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE (SPARC) TO ACTIVATE INTRACELLULAR GLIOMA SURVIVAL PATHWAYS
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Secreted protein acidic rich in cysteine (SPARC) is an extracellular matrix protein expressed in many advanced cancers, including malignant gliomas. We and others have previously shown that human glioma cell lines engineered to overexpress SPARC adopt an invasive phenotype. We now show that SPARC expression increases cell survival under stress-induced by serum withdrawal through a decrease in apoptosis. Phosphatidylinositol 3- OH kinase (PI3K)/AKT is a potent pro-survival pathway that contributes to the malignancy of gliomas. Cells expressing SPARC display increased AKT activation with decreased caspase 3/7 activity. Exogenous SPARC rapidly induces AKT phosphorylation, an effect that is blocked by a neutralizing SPARC antibody. Further, AKT activation is essential for the anti-apoptotic effects of SPARC, as the decreased apoptosis associated with SPARC expression can be blocked with dominant-negative AKT or a specific AKT inhibitor. As tumor cells face stressful microenvironments, particularly during the process of invasion, these results suggest that SPARC functions, in part, to promote tumor progression by enabling tumor cells to survive under stressful conditions. We have now profiled the signaling events upstream from AKT activation by SPARC. SPARC binds and regulates growth factor presentation to cells. We examined the impact of SPARC and VEGF on growth factors on glioma invasion, including AKT while IGF1 induced AKT phosphorylation, the addition of SPARC did not alter this activity. In contrast, VEGF and SPARC each modestly increased AKT phosphorylation the combination more significantly increased AKT expression. Additionally, focal adhesion kinase (FAK) appears upstream of AKT as both SPARC alone and in combination with VEGF induced FAK phosphorylation before AKT phosphorylation. Dominant negative FAK (FRNK) induced cell death suggesting – like AKT, FAK is essential to cell survival. In summary, we have now directly linked SPARC to essential tumor processes – including invasion, survival, and angiogenesis. SPARC warrants further investigation as a contributor to malignancy and as a potential therapeutic target. (J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar. This work was also supported by NIH grant NS047409 to J.N.R.).

15. INHIBITION OF INTRACEREBRAL GliOMA GLIOBLASTOMA GROWTH BY TREATMENT WITH A NOVEL ONE-ARMED ANTI-MET ANTIBODY
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The proto-oncogene encoded tyrosine kinase receptor MET and its ligand scatter factor/hepatocyte growth factor (SF/HGF) are strongly upregulated in malignant gliomas. The SF/HGF/MET system is important for glioma cell migration, invasion, proliferation, and angiogenesis. We used a novel single-chain anti-MET antibody to inhibit glioma growth in an orthotopic model. U87 glioblastoma cells were xenografted into the brains of nude mice. On day 1 or day 7 after tumor cell injection, osmotic minipumps with intracranial catheters were implanted. The one-armed anti-MET antibody (40 mg/day) was infused intratumorally until day 3 weeks after tumor cell injection. Tumor size, proliferation, apoptosis, microvessel density, and expression of extracellular matrix (ECM) molecules were determined by immunohistochemistry and cDNA arrays. We performed to determine the effect of the anti-MET antibody on the expression of invasion-related genes in vitro. Functional effects of the anti-MET antibody were analyzed in vitro. The effects of the anti-MET treatment on tumor size and morphology were very similar, regardless whether treatment was initiated on day 1 or day 7. Tumor volumes were reduced by >95% (P < 0.001) in animals treated with the anti-MET antibody compared with controls. Tumor cell proliferation was reduced by >75% (P < 0.001) in treated tumors; microvessel density was reduced by >90% (P < 0.001) in apoptosis was increased by >60% (P < 0.05). Interestingly, the tumor cell density was >2-fold higher in controls than in treated tumors, in which a striking increase in ECM deposition between tumor cells was apparent. Immunohistochemically, strongly increased intensities for laminin, fibronectin, and tenascin were found in tumors treated with the anti-MET antibody. cDNA arrays revealed downregulation of uPA, tPA, MMP7, MMP15, and MMP16 and upregulation of PAI-1 in U87 cells treated with the anti-MET antibody, which may explain the change in ECM proteins in vivo. Proliferation and migration of U87 cells in vitro were inhibited by the anti-MET antibody. Local treatment with the one-armed anti-MET antibody can inhibit intracranial glioblastoma growth almost completely. The responsible mechanisms appear to include anti-proliferative, anti-angiogenic, anti-migratory, and pro-apoptotic effects as well as enhancement of ECM deposition, presumably caused by decreased matrix degradation.

16. ANGIOPOIETIN-2 INDUCES GLIOMA CELL INVASION BY STIMULATING MMP-2, MT1-MMP AND LN 5 GAMMA 2 EXPRESSION THROUGH TIE2-INDEPENDENT PATHWAYS
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A hallmark of malignant human gliomas is their ability to diffusely invade into surrounding brain tissues. We have previously reported that angiopoietin-2 (Ang2), a known angiogenic factor, induces glioma cell invasion through the activation of matrix metalloproteinase-2 (MMP-2). In this study, we analyzed 57 human glioma biopsies (WHO grade I to IV) displaying a distinct invasive edge and 39 glioma specimens that only contain the central region of the tumors and found that Ang2, MMP-2, MT1-MMP, and LN 5 gamma 2 were co-overexpressed in the invasive areas, but not in the central regions of the glioma tissues. Statistical analyses revealed a significant link between the preferential expression of these molecules and invasiveness. Western blot analyses of total protein extracted from the microdissected primary glioma specimens showed an upregulation and increased expression of MT1-MMP and LN 5 gamma 2 at the invasive edge of the tumors versus the tumor center, supporting this observation. Analysis of engineered U87MG glioma xenografts with expression Ang2, revealed an increased expression of MMP-2, MT1-MMP and LN 5 gamma 2 immediately invad ing glioma cells, along with MMP-2 upregulation. Stimulation of glioma cells by overexpressing Ang or exposure to exogenous Ang2 promoted the expression and activation of MMP-2, MT1-MMP, and LN 5 gamma 2. Furthermore, Ang2 directly binds to beta 1 integrin in Tie2-deficient U87MG cells inducing the activation of FAK, p30Cas, ERK/1,2, and JNK. Ang2-stimulated MMP-2 expression and secretion was attenuated by a functional neutralizing anti-beta 1 antibody, by an ERK1/2 inhibitor, and by a JNK inhibitor. Stable expression of a specific negative regulator of FAK, FAK related non-kinase (FRNK) but mutant FRNK-S1034, inhibited Ang2-stimulated phosphorylation of FAK and p30Cas, blocked the activation of ERK and JNK, and decreased Ang2-stimulated MMP-2 expression and secretion. Inhibition of beta 1 integrin, FAK, p30Cas, ERK1/2, and JNK also attenuated Ang2-stimulated glioma cell invasion. Lastly, expression of FRNK, but not FRNK-S1034, by glioma xenografts in the brain derived from US7MG/Ang2 cells suppressed Ang2-induced glioma cell infiltration and MMP-2 expression. These data suggest that upregulation of Ang2, MMP-2, MT1-MMP, and LN 5 gamma 2 is associated with glioma invasiveness and demonstrate a mechanism whereby binding of Ang2 to glioma cells regulates MMP-2 expression and secretion through the integrin and FAK signaling pathways.

17. PROMOTION OF MALIGNANT GLIAL CELL POLARITY AND INVASION BY DRR-1
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Invasion is a major factor responsible for the failure of brain tumor treatment. There are currently no chemotherapeutics directed at controlling the infiltration of malignant glial cells into normal brain. In order to identify invasion-associated genes we designed an unbiased, genome-wide functional screen based on tumor cell invasion. A normal adult human brain cDNA library was stably infected into a glioma cell line using retroviral transduction. Tumor spheroids generated from these cells were implanted into a 3D collagen matrix. Hypervascular cells were isolated from the matrix and cDNA inserts identified. Dose-regulated in renal cell carcinoma (Dr1) was identified as a potent mediator of glial cell hyperinvasion in this screen. Time-lapse video microscopy reveals that Dr1 overexpression promotes invasion, whereas RNAi-mediated expression of Dr1 in actively invading a 3D tumor model. Importantly, human glioma sampling reveals that Dr1 is highly expressed in invasive gliomas, whereas expression is not detectable in noninvasive gliomas. Further, we have identified Dr1 as a novel activator of microtubule polylisterin that promotes invasion by enhancing cell polarity using a pericentriolocalization assay. These findings uncover a novel glioma invasion gene and its mechanism of action, which provides an explanation for the highly invasive nature of Dr1-expressing human gliomas.
The mammalian target of rapamycin mTOR controls a spectrum of cellular events such as initiation of translation, ribosome biogenesis, and cell growth and proliferation. Pharmacologic inhibition of mTOR activity by rapamycin has been shown to elicit antitumor activity possibly through G1 cell cycle arrest and inhibition of VEGF expression. Currently, an analogue of rapamycin, RAD001, is being used in clinical trial for different cancers including gliomas. Glioblastoma multiforme is a malignant tumor that is extremely refractory to therapy because of rapid growth and local invasive potential of these tumors. In this study, we sought to examine the effect of antagonizing mTOR activity by RAD001 on glioma tumor invasion in vitro. Four different glioma cell lines were treated with RAD001 for 72 h before harvesting for Western blotting, RT-PCR, microarray, and in vitro Matrigel gel invasion analyses. Glioma cells were stably transfected with NRP-1 expression and control vector followed by G418 selection. The resulting G418-resistant cells were examined for their NRP-1 expression and in vitro invasion propensity. Glioma cell lines treated with RAD001 resulted in a reduction of mTOR downstream target genes expression such as S6K1 and the eukaryotic initiation factor 4E-binding protein 1. Inhibition of mTOR activity with RAD001 in tumor cells also leads to G1 cell cycle arrest and reduction in VEGF secretion consistent with previous documented studies. Importantly, invasion propensity of tumor cells is greatly inhibited by RAD001 as assessed in an in vitro Matrigel invasion assay. Interestingly, RAD001 treatment does not affect either the expression or the activity of MTA1. Using Affymetrix gene expression arrays we have observed the expression of neuropilin-1 (NRP-1) is decreased by RAD001. NRP-1, initially found to be involved in axon growth during neuronal development, is expressed in both tumor cells and endothelial cells. It has been shown that overexpression of NRP-1 is associated with angiogenesis and tumor growth. In this study, we demonstrated that a polymorphism in NRP-1 affects NRP-1 mRNA expression. Exogenous NRP-1 expression significantly increases glioma cell invasion and promotes anchorage-independent growth. Our results demonstrate that antiangiogenic activity of RAD001 may be a combination of growth arrest, antiangiogenesist, and anti-invasion effects. Furthermore, the potential anti-invasion activity of RAD001 is likely through controlling cell motility by inhibiting NRP-1 expression.

19. POLYMORPHISMS IN DNA REPAIR GENES AND SUSCEPTIBILITY TO PRIMARY INTRACRANIAL BRAIN GLIOMAS
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Enzymes in base excision (BER), nucleotide excision (NER), double strand break/recombination (DSB/RR), mismatch (MMR), and direct-damage repair pathways are important in the repair of diverse types of DNA damage. Polymorphisms in many of the genes encoding these enzymes have been identified as risk factors for environmentally and occupationally caused cancers. We evaluated the associations of polymorphisms in genes in BER (ADPRT V762A, APEX D148E, MUTYH Ex1+1>A, OGG1 S326C, POLB IVS11-233>A>G, XRCC1 R399Q, R280H, R194W, LIG1 Ex2-24C>T, PCNA IVS1-242C>T), NER (ERCC2 D312N, K751Q, ERCC4 R415Q, ERCC5 H1104D, RAD52 A249V, LIG1 Ex2-24C>T, PCNA IVS1-242C>T), DSB/RR (NBS1 Q185E, RAD54 Y210stop, XRCC2 R186H, XRCC3 T241M, XRCC4 N298S), MMR (MLH1 I219V, MSH2 G232D), and direct-damage repair (MGM1 H43V, R178K, L484F) as risk factors for primary intracranial gliomas in the Upper Midwest Health Study, a population-based case-control study including rural residents of four states with high glioma incidence. Glioma cases (N = 798) were identified from hospitals, private physicians, and registries. Control participants (N = 1175) were stratified samples of licensed drivers and HCAF enrollees. Questionnaires elicited occupational and environmental exposures. DNA was obtained from 451 controls with no self-reported cancer and from 316 cases. TaqMan and MGB Eclipse methodology were used to characterize genotypes. In unadjusted analyses, a polymorphism in ADPRT V762A (VN 67% of controls, 75% of cases, odds ratio [OR] 1.48, 95% confidence interval [CI] 1.07–2.04) had a statistically significant association with glioma, and polymorphisms in three other genes showed associations with gliomas of borderline statistical significance: (1) R178K controls, 38% of cases, OR 1.32, CI 0.97–1.78; (2) ERCC3 H1104, 61% of controls, 68% of cases, OR 1.33, CI 0.98–1.78; and (3) XRCC4 N4074% of controls, 80% of cases, OR 1.37, CI 0.57–1.94. For each DNA repair pathway, multivariate logistic analyses included all polymorphisms in the pathway plus ever/never living on a farm and ever/never smoking, as surrogates for occupational and environmental exposure. Adjusting for these factors did not change odds ratios substantially. Our results offer additional, if preliminary, evidence for the role of specific DNA repair genotype in glioma risk. Further analyses of our data will include assessing the risk of DNA repair polymorphisms under specific exposure conditions, such as exposures to pesticides, solvents, and UV light.

20. A GLTSCR1 HAPLOTYPE IS ASSOCIATED WITH OLIGODENDROGLIOMA DEVELOPMENT
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Deletions of 19q have been associated with gliomas, especially oligodendrogliomas. In addition, oligodendrogliomas with 19q deletion have a better survival. We have previously described a 150-kb minimal deletion region in gliomas that maps to 19q13.33 that contains three novel candidate genes (GLTSCR1, EHHD2, and GLTSCR2). Polymorphisms in loci near this deletion region (ERCC1, ERCC2, RAI, ASE, and D19S246) have been associated with basal cell, breast and lung carcinoma, and mixed oligodendrogliomas. A polymorphism in GLTSCR1 has been associated with prostate cancer aggressiveness. We have recently shown that a polymorphism in GLTSCR1 (SNP rs1035938) is associated with oligodendroglioma development. We have now evaluated five additional SNPs within GLTSCR1 utilizing a larger and more comprehensive set of controls. The polymorphisms of (Novel 4 – A to G at position 304 from the putative transcription start site) is a novel SNP discovered during GLTSCR1 mutation screening studies of sporadic glioma specimens. Of these five SNP, four (Novel 1, 2, 4 and controls cell membrane) were found to have allelic association with oligodendroglioma development by allele-based analysis (P for both = 0.032). However, the prevalence of the at-risk allele was significantly decreased in patients whose oligodendroglioma have 19q deletion (P for Novel 4 = 0.025; P for rs1005911 = 0.014). By genotyping 10 CEPI families we were able to determine the most prevalent haplotypes for the six total GLTSCR1 SNPs we tested. We then assessed the prevalence of these haplotypes in the cases with glioma and the normal controls. One haplotype (haplotype 1, ACTCGG) was more prevalent in gliomas than in controls (27.4% vs. 20.4%, P = 0.067). The prevalence increased to 36.7% in gliomas with 19q deletion (P vs. controls = 0.009; P vs. gliomas without 19q deletion = 0.02). The increased prevalence of this haplotype in gliomas was primarily due to its prevalence in oligodendrogliomas (34%, P vs. controls = 0.010) and among the oligodendrogliomas, those with 19q deletion (45%, P vs. controls = 0.001; P vs. oligodendrogliomas without 19q deletion = 0.002). Interestingly, this haplotype is retained in 8 of 10 oligodendrogliomas with 19q deletion. Even though it had a low overall prevalence, another haplotype (ACCCCGG) was significantly more prevalent in the controls than the cases (2% versus 0%; P = 0.033). The high-risk and low-risk haplotypes on the presence of glioma showed that the presence of GLTSCR1 haplotype 1 allele (rs1035938). These preliminary data strongly suggest that a polymorphism (mutation) is in linkage disequilibrium with the GLTSCR1 ACTCGG haplotype and is associated with oligodendroglioma development, especially those with 19q deletion.
22. PROSPECTIVE QUALITY OF LIFE ASSESSMENT USING EORTC QLQ 30 AND BRAIN CANCER MODULE (BN 20) IN 257 ADULT PATIENTS WITH PRIMARY BRAIN TUMORS SEEN CONSECUTIVELY IN A TYPICAL NEUROONCOLOGY CLINIC

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A majority of quality of life (QOL) data for patients with brain tumors is available as a part of some other study, and there is a relative lack of information in patients seen routinely in clinical practice. The purpose of this study was to evaluate QOL in patients with primary brain tumors as seen in a typical neurooncology clinic, in our setup using validated local language questionnaires. Two hundred and fifty-seven adult patients presenting consecutively in our neurooncology clinic from January 2003 to 31 December 2003 were prospectively accrued in the study. A majority of the patients had some sort of surgical intervention before the accrual. All patients completed a detailed neurological examination, using EORTC questionnaire (QLQ-30), specific Brain Cancer module (BN 20), and daily activities by modified Barthel's index. Assessments were done before starting treatment (typically radiotherapy [RT] or chemotherapy [CT]) in some patients), at completion of RT/CT, and at each follow-up (at least two). The questionnaires were administered in English, Hindi, and Marathi according to the patients' needs using EORTC-rattled versions of Hindi and Marathi questionnaires. MSK post-treatment QOL was compared with the pre RT values. Initial evaluation was done for patients accrued in the first 6 months (N = 137), including 48% below the age of 40 years, 45% between 41 and 60 years, and 6% above 60 years old in a male-to-female ratio of 2.5:1. Eighty-five percent of the patients had at least primary education, and KPS was above 90 in 68% of the patients.

Ninety-seven patients received RT up front and form the analyzed patient population. Sixty-one percent of the patients completed the questionnaire by themselves, 16% required assistance because of poor neurological condition, 11.5% because of illiteracy, and 11.5% because of other causes. At the end of RT, there was a statistically significant improvement in the overall global QOL score, the benefit of which continued at 3 months. Significant improvement was seen in functional scores of physical (P = 0.001) and role domain (P = 0.007), while the difference in the emotional, cognitive, and social scores were not significant. There was deterioration in the symptom scale with respect to nausea and vomiting (P = 0.04). In the BN 20 module there was a significant deterioration in scores of hair loss (P = 0.001) and local itching (P = 0.01). QOL assessment using EORTC QOL 30 and BN20 in validated local language is simple to administer, is generally well understood, and can be used even in routine patients in a busy neurooncology clinic. Patients receiving RT as an up-front modality showed a statistically significant improvement in global, physical, and role functioning and transient worsening in scores of hair loss and itching of skin. An update of all 257 patients at an extended follow up will be presented in the meeting.

23. HUMAN TELOMERASE GENETIC VARIATION PREDICTS SURVIVAL OF PATIENTS WITH GIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most common glioma with the poorest survival. Although extent of surgery or a combination of radiotherapy or chemotherapy may improve the outcome, it is important to identify and evaluate biomarkers that might be useful for screening patients and possibly modifying treatment. Level of human telomerase (hTERT) mRNA or protein in some patients receiving RT as an upfront therapy showed a statistically significant improvement in global, physical, and role functioning and transient worsening in scores of hair loss and itching of skin. An update of all 257 patients at an extended follow up will be presented in the meeting.

24. VALIDATION OF BLINDED EVENTS REVIEW COMMITTEE (ERC)–DETERMINED TIME TO NEUROLOGIC PROGRESSION (TTNP) DEMONSTRATES CORRELATION WITH SURVIVAL, RADIOLOGIC PROGRESSION, AND FUNCTIONAL INDEPENDENCE END POINTS

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Survival is a standard end point for brain metastasis trials, but it inadequately measures treatment benefit because of competing risks for death from systemic disease and central nervous system progression, and it does not account for neurologic function, an important quality of life consideration. There is no standardized and validated tool to measure TTNP. The purpose of this study was to test the validity of ERC-determined TTNP. In a prospective, randomized phase 3 trial of whole brain radiation versus placebo (10%), with brain metastases, 401 pts underwent standardized neurologic exam, neurocognitive tests (NCT), evaluation of symptoms, functional independence (Barthel Index), and MRI. Using a prespecified algorithm, the ERC reviewed blinded clinical data, excluding MRI, to score progression if 2 or 3 functional domains (NCT, neuro exam, neuro symptoms) showed progression on consecutive visits (Mehta, J. Clin. Oncol. 21, 2529, 2003). TTNP was compared with survival, MRI progression, and time to loss of functional independence by using log-rank tests and Kaplan–Meier plots. Patients with TTNP = 1, 2, 3, or 4 months had a shorter median survival (by 4.4, 3.9, 3, and 2.4 months, respectively), compared with pts who did not have neurologic progression at those times. The validity of a blinded ERC-determined TTNP end point was demonstrated by high correlations with survival, radiologic progression, and loss of functional independence end points. We conclude that ERC-determined TTNP measures clinical benefit and is sensitive to change.

25. ADVANCES IN DRUG DELIVERY

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Drug therapy for gliomas has been consistently hampered by lack of specificity, systemic toxicity, lack of penetration, limitations to size of the molecules, and poor diffusion into normal brain. Therefore, methods have been developed for local delivery. Intracavitary delivery has been used mostly in the context of slow-release biodegradable polymers, which has resulted in high local drug concentration but variable depth of diffusion in the tissue. Releasing BCNU, this technique has gone through successful phase 3 trials. Under investigation for the same technique are different compounds, modified release kinetics, higher doses, and drug combinations. Periventricular injection of slow-release polymer with 5-FU after resection of the tumor is used as a radiation sensitization, and a phase 2 study is under published and phase 3 in preparation. A major advance is the direct, slow infusion of therapeutic compounds into tissue, either the tumor or the surrounding “normal” brain. This convection-enhanced delivery (CED) allows for any substance to be delivered on the “other side” of the blood-brain barrier. Large and complex molecules can be used, with their distribution properties depending on their physicochemical characteristics (charge, solubility, size). Presently, large fusion molecules of ligands for cell surface receptors with toxins are under evaluation in phase 2 and 3. Being directed at cell surface molecules expressed only on tumor cells and not on normal brain cells, this therapy is aimed to be in the crosshairs of compartmental delivery and a compartmental specificity. Many more molecules can be designed, depending only on compartmental specificity, especially with larger, either naked or coupled to effector molecules. Distribution will be different for every new compound and will also depend on heterogeneous tissue factors like scars, cysts, and hemorrhages in a tumor but also favorable white matter tracts and possibly less permeative areas like basal ganglia or areas of...
prior injury in “normal” brain. Further development requires cooperation with neuroradiologists to study fluid movements with DT, which does not really prove where the compounds go. Also, computer modeling is currently being explored for its predictive value to create “isodistributed” curves. In addition to the large protein-based therapeutics, classical compounds like taxol are under investigation for CED. Prodrug therapy is another way to reduce systemic toxicity but requires the specific transduction of glioma cells with a prodrug to an active enzyme. This is a known vector, and a new phase 3 trial based on a TK transducing adenovirus will start in 2005. Research into the biology of the specialized endothelial cells of the blood-brain barrier has revealed transporter molecules which when expressed, will afford specific delivery of conjugated therapeutics across the blood-brain barrier.

26. ADVANCES IN MOLECULAR DIAGNOSIS

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Diffuse gliomas are clinically, pathologically, and genetically heterogeneous malignancies. Patient age and histology are the two most powerful prognostic parameters, though there remains significant clinical variability, both in terms of overall survival and response to therapies. Our understanding of glioma tumorigenesis and progression has advanced greatly over the last 15 years, though few observations have successfully translated into molecular diagnostic assays applied daily in neuropathology and neuro-oncology. The most common is chromosome 1p and 19q testing with combined 1p/19q deletions identifying “genetically favorable” oligodendrogial tumors with enhanced survival and responsiveness to alkylating chemotherapeutic agents and radiation. It is predominately, but not exclusively, associated with histologically classic grade II and III oligodendrogliomas (ODG), where codeletions are found in up to 80% to 90%, suggesting that this represents an early event. It is rare in pediatric ODGs, which suggests an alternate tumorigenic pathway in children. Mixed oligoastrocytomas are diagnostically challenging and are genetically heterogeneous. We recently found that survival was enhanced in those with 1p/19q codeletion, 19q deletion alone, or no detectable alterations, whereas it was decreased in those harboring 9p deletion, 10q deletion, and/or EGFR amplification. Small interstitial 1p and/or 19q deletions are also seen in some astrocytomas, though the whole arm 1p/19q codeletions are fairly specific to ODGs. We have not seen them in morphologic mimics, such as DNT, clear cell ependymoma, central neurocytoma, and small cell glioblastoma. The latter enters the differential diagnosis most often. However, survival is short and it is genetically characterized by 1qq deletion (>98%), EGFR amplification (70%), and EGFR-III expression (50%). We have also seen rare “extraventricular neurocytomas” with 1p/19q codeletion, though there is evidence that some oligodendrogliomas undergo neuronal differentiation and these 2 entities are likely related. Lastly, high-throughput technologies, such as expression profiling and array CGH, provide new opportunities to identify candidate genes and groups of genes that may lead to molecular diagnostics applications in the future. The ultimate goal will be to provide accurate and cost-effective glioma phenotyping that will enable targeted therapies with the highest likelihood of success for each individual patient.

27. MALIGNANT GLIOMA: ADVANCES IN RADIATION THERAPY

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Randomized clinical trials have established a survival benefit with adjuvant radiotherapy for malignant glioma, and indirect evidence supports a dose-survival relationship, between 0 and 60 Gy. Escalation beyond 60 Gy has been limited primarily by toxicities, but innovative approaches to achieve this aim have been developed, piloted, and after promising initial results, tested in phase 3 trials, only to result in negative findings. The best examples of these technologies include brachytherapy and radiosurgery, for both of which, promising phase 2 results were not corroborated in phase 3 trials. New technologies continue to be developed with the ultimate objective of achieving dose-escalation. Examples include 3-dimensional radiotherapy delivery techniques, with which doses up to 90 Gy have been explored (without improved local control), the balloon brachytherapy device known as a “novel modulator” of radiation, intensity modulated radiotherapy (IMRT), etc. The purpose of IMRT is to produce exquisite shaping of the radiation dose distribution to mimic the exact shape of the tumor, with a dramatic avoidance of nearby critical structures. The value and success of this modality is contingent on the hypothesis that prior dose-escalation efforts have failed as a consequence of inadequate target definition due to inherent limitations of current MRI methods. Perhaps with the advent of functional imaging with MR and PET, a more precise definition of the target might be achieved, and the irregularly shaped tumor could be accurately and conformally targeted by using IMRT for dose-escalation, ultimately resulting in improved local control. Other strategies have been used to achieve dose-escalation focus on molecular targeting. In the simplest of these approaches, a tumor-specific antigen is targeted with a “radiolabeled antibody” to achieve a high target dose, and clinical trials evaluating this approach are under way. Other targeted approaches have attempted to identify molecularly targeted pathways that support proliferation and angiogenesis and co-delivered with this strategy are radioactive iodine or Yttrium-90. Two of these pathways are being explored as a way of increasing tumor dose-intensity, by exploiting the preferential dose-localization properties of neutron interaction with elements such as boron, which have a high cross-sectional area of intersection for neutrons. In order for such a strategy to be successful, tumor-specific preferential localization of boron needs to occur at an adequate concentration level. First- and second-generation boronated compounds have not met these criteria, and newer efforts are focused on two fronts: (1) developing tumor-targeted boron-containing compounds by tagging EGF R ligands and (2) evaluating tumor-specific gadolinium-containing agents, since gadolinium has a superior cross-sectional area of intersection with neutrons.

28. DEVELOPMENTAL ANOMALIES AND ONCOGENESIS IN THE BRAIN OF A TRANSGENIC E2F1 MOUSE MODEL


The E2F family of transcription factors are involved in tumor suppression and tumor generation. Although the Rb/E2F pathway is deregulated in most brain tumors, there is no direct evidence of the role of E2F1 in the generation or maintenance of brain tumors. To address this question, we generated a transgenic animal model driving expression of E2F1 through the GFP promoter to glial cells and neuronal/glial precursors. Histological analysis of brain tissue revealed expression of the transgene in astrocytes, ependyma, and the Bergman glial cells of the cerebellum. Importantly, cells positive for E2F1 were also positive for proliferation markers such as PCNA and BRDU as well as apoptosis, detected through TUNEL assay. Overexpression of E2F1 led to the generation of a phenotype characterized by numerous neurological defects without a clear pathological frame. The early onset of these phenotypes suggests a developmental abnormality. When overexpression of E2F1 was introduced into an E2F4 null background, a new phenotype, not seen in either background alone, was induced and characterized by the development of a domed head, hyps-arachy, and seizures. MR analysis of these mice revealed a dramatic decrease in brain surface area. Pathologic examination of the brains uncovered congenital triventricular hydrocephalus due to E2F1-induced hyperproliferation of the ependyma in the aqueduct, resulting in the death of the animal by 5 weeks of age. Parallel studies involving the overexpression of E2F1 and the concomitant nevus of p53 resulted in neurological signs including tremors, ataxia, seizures, head tilt and paresias of the posterior limbs. Histological analyses of the brains from 3-month-old transgenic animals unveiled the production of neoplasms including a highly undifferentiated choroid plexus carcinoma with papillary and glandular features and an aggressive embryonal tumor of the cerebellum, expressing the pathologic features of medulloblastoma. Furthermore, immunohistochemical studies confirmed proliferation of cells expressing the hE2F1 transgene within the tumor tissue. This study is the first to provide direct evidence that E2F1 functions as an oncogene through the induction of brain cancer.

29. FOS-RELATED ANTIGEN 1 (FRA-1) MODULATES MALIGNANT FEATURES OF GLIOMA CELLS

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We uncovered that a c-fos inducible vascular endothelial growth factor D (VEGF-D) is ubiquitously upregulated in high-grade gliomas (HGG). However, HGG overexpress a Fos-related antigen 1 (Fra-1) rather than c-Fos among the activating protein 1 (AP-1) transcription factors. Therefore, we have examined an effect of ectopic Fra-1 or a knockdown of this transcription factor in H4 (low Fra-1, non-tumorigenic), U-87 MG (high Fra-1, highly tumorigenic), and A-172 MG (moderate Fra-1, non-tumorigenic) malignant glioma cells. We have transfigured glioma cells with fra-1...
in both sense (+) and anti-sense (−) orientation. The ectopic Fra-1 evoked prominent phenotypic changes in all cell lines studied: The cells became more polar with larger number of elongated processes. This was seen by standard microscopy and by changes in actin architecture using phalloidin staining. We noticed that fra-1 siRNA, but not nonsense nucleotides, produced reversal of the morphological features associated with the ectopic Fra-1 (H4). The characteristic change in the phenotype seen in cell in vitro was carried over to tumors grown in vivo (U-87 MG). Of interest, completely non-tumorigenic H4 cells started to form tumors when transfected with fra-1 transgene, at an 80% of tumor take. Moreover, the genotype of H4(fra-1−/−) cells changed significantly, since 18 different genes became overexpressed, at least fourfold vs. controls, with a targeted cDNA microarray analysis (1056 genes). Conversely, fra-1(−/−) altered profoundly the morphology (U-87 and A-172), anchorage-independent growth (U-87 and A-172), tumorigenic potential (U-87), and the expression of Fra-1 effectors, such as VEG-F (U-87 and A-172). For example, fra-1(−/−) made cells more rounded, with fewer and shorter cellular processes (U-87 and A-172). Also, the U-87(fra-1−/−) cells lost an ability to grow in agar, while U-87(fra-1+/+) cells started to form multiple colonies; similar results were seen in A-172 cells. Furthermore, we found that by day 22, there were 80% U-87(fra-1−/−) tumors formed (8 out of 10) of an average size 20 mm^3 while the size of U-87(fra-1+/+) tumors (10 out of 10) was 135 mm^3. In addition, the U-87(fra-1−/−) tumors were poorly vascularized, and the levels of VEG-F in tumor cells were low compared to parental U-87 tumors. Thus, Fra-1 induces profound phenotypic changes in malignant glioma cells with associated significant changes in their genotype. Fra-1 engages mechanisms that promote tumorigenesis and anchorage-independent growth. Being that Fra-1 is frequently upregulated and also accumulates at high levels in response to AP-1 activation in malignant glioma cells, this transcriptional factor may likely play an important role in the maintenance or progression of malignant gliomas and potentially represents a new target for therapeutic interventions.

31. HUMAN BRAIN SLICES AS CONTROL ASSAY FOR TUMOR-CIBER, IDEMPERI, K. P. Weenink, 1, C. M. F. Dirven, 2, W. van Houdt, 2, D. P. Noske, 5, S. Idema, 2, M. L. M. Lambers, 2, V. W. van Beusechem, 1, and R. W. H. Verwer 2

To develop novel tumor-targeted anticancer drugs, such as oncolytic adenoviruses, early in vitro testing of efficacy is a prerequisite. True selectivity of the targeted drug toward tumor cells can be reliably assessed only when results with tumor tissue can be compared to those obtained with “normal tissue”. Here we describe the use of cultured slices of normal human brain, obtained by epilepsy surgery, as a control assay for testing selective cytopathic activity of oncolytic adenoviruses. Methods to evaluate the cytopathic effect in relation to the viability of the brain slices are studied. Fresh surgical specimens of healthy cerebral cortex acquired during epilepsy surgery are cryopreserved whole or cut into 200-μm-thick slices, and kept in 24-well plates in culture medium (Verwer et al., FASEB J. 16 (2002) 54–60). Slices were infected with several different adenoviruses, i.e., wild-type Ad5 and replication-defective adenovirus vectors, Ad.CMV.Luc, and Ad.survivin.Luc (the latter only expressing the luciferase transgene in cells with active survivin transcription, i.e., tumor cells). Viability of cells was assessed before and after treatment by using the MTT-treated WST-1 assay (Roche), Live/Dead kit (L/D, Molecular Probes), and cytochrome oxidase activity histochemistry. Our results indicate that wild-type adenovirus can efficiently infect the brain slices. This resulted in a significant reduction of viability and energy metabolism, as measured by the L/D kit and cytochrome oxidase activity, respectively. The WST-1 assay appeared to be insufficiently sensitive. Experiments with Ad.CMV.Luc and Ad.survivin.Luc show that luciferase expression is significantly lower when using the virus with the tumor-specific promoter. The brain slice model is a valuable tool for assessment of the selectivity of tumor-targeted agents in neuro-oncology, such as modified adenoviruses. The brain slice model is particularly useful when the targeting strategy of the oncolytic drug is based on species-specific (human) proteins, causing xenografted animal tumor models to be of limited use for addressing the question of tumor selectivity. In the context of oncolytic agents, cytochrome oxidase activity appears to be the most favorable method to assess viability of brain slices.

32. NOVEL APPROACHES TO METASTATIC ANIMAL MODELS: A PILOT STUDY FOR BRAIN METASTASIS IN MICE, 3D TUMOR GROWTH IN CELLOUSE MATRIX, AND IN VIVO REAL-TIME IMAGING WITH BIOULUMINESCENCE

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Animal models for brain tumors have provided a significant insight in understanding molecular mechanisms of disease progression and development of new treatments. Despite the presence of many models available currently for glial tumors, there is no reproducible model to mimic human cancers that metastasize to brain in animals. This is a pilot study of a “brain melanoma metastasis model in mice” that may re-create the metastatic cascade of cancer. We have chosen melanoma, which is a tumor with high predisposition for metastasizing to brain. Human melanoma cell lines derived from patient’s specimens with metastatic melanoma to the brain are harvested and cultured (VMM1 and B6). Cells are transfected with luciferase marker gene and after assessing its expression; cells are xenografted into two different groups of immunocompromised mice in this pilot study. In the first group made up of four animals, tumors embedded into cellulose matrix (Gelfoam) are injected stereotactically into brain to allow “activation” of melanoma cells in brain tissue milieu. In the second group of four animals, tumors are injected intradurally to re-create the metastatic cascade resulting in brain metastasis. Multiple transfers of cells from xenografts of melanoma, whose tumor burden in the brain, in turn, will ensure continuity of the cycle. Both intradermal and intracranial injection groups are imaged for luciferase activity as reflecting tumor growth and patterns of metastasis without sacrificing the animals. All four animals in both groups reflected tumor growth in their origin, with luciferase activity detected within four weeks. Luciferase bioluminescence may be imaged with a simple, cooled, charged couple device (CCD) camera and is an inexpensive, non-invasive screening and sensitive way of assessing tumor growth even after intra-cranial injections. Application of the bioluminescence techniques provides in vivo, real-time imaging that is used for the first time in a brain metastasis model. This is a pilot study that utilizes novel approaches to metastatic animal models; namely, this is the first study to mimic the metastatic cascade to the central nervous system using cancer cell lines that are “preadapted” to brain metastasis and to evaluate in vivo, real-time imaging enabled by luciferase gene transfection into tumor cells.

33. LOCAL TREATMENT WITH AN IMMUNOSTIMULATORY CPG-OLIGONUCLEOTIDE IN PATIENTS WITH RECURRENT GLOBLASTOMA: RESULTS OF A PHASE I TRIAL

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Oligonucleotides containing one or several CpG motifs (CpG-ODN) display a strong immunostimulating activity, driving the immune response toward the Th1 phenotype. In preclinical studies, they have shown promising efficacy when injected locally in several cancer models, including gliomas. Limursen, a new phosphorothioate CpG-ODN, was administered intratumorally by convection-enhanced delivery in patients (pts) with recurrent glioblastomas (GBMs). Increasing doses were injected, starting at 0.5 mg and escalating to 1, 2, 5, 10 or 20 mg in cohorts of 3 to 6 patients. The primary objective was to determine the safety profile of intratumoral Limursen in patients with recurrent GBM. Twenty-four pts were enrolled in the study. All patients were previously treated with radiotherapy and, in most cases, with one or several lines of chemotherapy (1 chemotherapy in 10 pts, 2+ in 11 pts). At the time of inclusion, the median age was 36 years (range, 24–72 years), and median KPS was 80% (range, 60%–100%). Two adverse events were considered related to the procedure, a local hemorrhage along the catheter track and a pulmonary embolism secondary to the interruption of a long-term anticoagulant therapy. Adverse effects possibly probably related to the studied drug were moderate. Spontaneously regressive grade 3 lymphopenia was reported in 6 patients. Grade 3 nonhematological toxicity consisted of reversible ALT elevation (2 pts at the highest dose). Six patients experienced fever above 38°C, mainly at higher doses. The fever peaked on day 3 and disappeared within a few days. Transient worsening of neurological conditions was observed at the highest dose in 3 patients. Preliminary evidence of antitumor activity was suggested with a local response at the site of injections in 2 patients. Three other patients had a stable disease for more than 3 months. Updated data for survival will be presented at the meeting. In conclusion, Limursen was well tolerated in patients with recurrent GBM, with sides effects mainly limited to transient worsening of neurological conditions and fever in a few patients. Limursen is now applied in a multicentric phase 2 study in recurrent glioblastomas.
34. EARLY PHYSIOLOGICAL AND METABOLIC EFFECTS OF INTRATUMORAL BCNU ON UNTREATED HUMAN GLIOMAS
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DTI-015 (BCNU dissolved in ethanol) utilizes solvent-facilitated perfusion for the intratumoral treatment of gliomas. We have investigated the early biological effects of intratumoral DTI-015 on newly diagnosed, circumscribed malignant gliomas. Magnetic resonance imaging (MRI) and spectroscopy (MRS), single photon emission computed tomography (SPECT), and computed tomography (CT) perfusion studies were used to assess the effect of DTI-015 on in vivo tumor physiology and metabolism. Nine patients (2 female, 7 male) with a median age of 58 years (range: 47–70 years) were enrolled into the study. Histological diagnosis was anaplastic astrocytoma (n = 3) and glioblastoma multiforme (n = 6). The median Karnofsky performance score was 90. Tumor volume was 13.6 ± 7.6 cm³ (mean ± SD). The volume of DTI-015 injected was 4.3 ± 1.4 ml (mean ± SD) with a BCNU dose of 256 ± 82 mg (mean ± SD). Mean tumoral cerebral blood flow significantly reduced within 72 h of DTI-015 injection (paired t-test; mean reduction 17.3, P = 0.001, 95% CI, 10.0–24.6). Relative cerebral blood flow also reduced significantly (paired t-test; mean reduction 12.7, P = 0.017, 95% CI, 2.9–22.3). There was a significant reduction in FDG utilization (paired t-test; mean reduction 28.0, P = 0.001, 95% CI, 0.16–0.41) and thallium uptake (paired t-test; mean reduction 3.26, P = 0.001, 95% CI, 1.78–7.47), with an increase in the Lip1/Cr ratio (Wilcoxon signed ranks; P = 0.034) after DTI-015 injection. The data forms a biological basis for understanding the effects of high-dose BCNU on malignant gliomas. Early effects can be seen on the tumor vasculature and metabolism, resulting in a pattern of ischemic tumor necrosis.

35. IMATINIB MESYLATE (GLEEVEC) PLUS HYDROXYUREA: AN EFFECTIVE REGIMEN IN THE TREATMENT OF RECURRENT MALIGNANT GLIOMA: PHASE 2 STUDY RESULTS
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In this phase 2 study, we evaluated the activity of imatinib mesylate (Gleevec), an inhibitor of the PDGF receptor tyrosine kinase with antiangiogenic activity and the ability to decrease tumor interstitial pressure, combined with hydroxyurea in the treatment of patients with recurrent malignant glioma. Eligibility criteria include the following: recurrent malignant glioma; age ≥18 years; KPS 60% or greater, less than grade 3 intratumoral hemorrhage; adequate hepatic, renal, and bone marrow function. Hydroxyurea is administered at 1000 mg orally BID while Imatinib is administered at 500 mg BID for patients on enzyme-inducing anticonvulsants (EIAC; phenytoin, carbamazepine, and phenobarbital) and at 400 mg QD for those not on EIAC. Each treatment cycle is 28 days, and patients are evaluated for response every other cycle. Sixty-four patients have been enrolled to date, including 32 with recurrent GBM and 32 with recurrent AA/30. The median age is 46 (range 21 to 68); 55% are male and 45% are on EIAC. All patients had prior XRT. The median number of prior chemotherapy agents was 3 (range, 1–5), and the median number of prior progressions was 2 (range, 1–7). Toxicity has been limited to grade 3 or 4 hematologic events in 20% and 5%, respectively, grade 3 edema in 8%, and grade 3 LFT abnormalities in 3%. Among GBM patients, radiographic responses have been observed in 9%, while 35% have achieved stable disease. Median progression-free survival (PFS) for patients with recurrent AA/30 and GBM are 10.9 and 14.4 weeks, respectively. At 6 months, 26.3% of GBM patients remain progression free. The rate of radiographic response, median PFS, and 6-month PFS rate observed in this study among heavily pretreated patients with recurrent GBM compare favorably to results achieved with temozolomide in first relapse, indicating that a randomized trial of imatinib mesylate plus hydroxyurea versus temozolomide is warranted.

36. IMPROVED SURVIVAL OF HIGH-GRADE GLIOMA PATIENTS AFTER GENE THERAPY WITH AN ADENOVIRAL VECTOR CONTAINING THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE: A PHASE 2 STUDY
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A phase 2 study was conducted to evaluate the efficacy and safety of using Herpes Simplex virus thymidine kinase in an adenoviral vector AdvHSV-tk (Cerepreo, Ark Therapeutics Ltd) with intravenous ganciclovir in malignant glioma patients. This was a single center, randomized, controlled study involving 36 patients with operable primary or recurrent high-grade glioma. Seventeen patients were randomized to receive AdvHSV-tk gene therapy (3 × 10^10 pfu) by local injection into the wound bed at the time of tumor resection, followed by intravenous ganciclovir, 5 mg/kg twice daily for 14 days. The control group of 19 patients received standard care consisting of radical excision. Patients in both groups with primary tumors received postoperative radiotherapy. AdvHSV-tk gene therapy increased mean survival from 39.0 ± 19.7 (SD) in control patients to 70.6 ± 52.9 weeks in patients in the active group (log-rank regression P = 0.0095). Median survival increased from 57.7 to 62.4 weeks. The percentage increase in mean survival was 81% and median survival was 65%. The therapy was well tolerated as assessed by adverse events, clinical chemistry, hematology, and immunology. There was no evidence of any deterioration in quality of life or increase used of concomitant medications. AdvHSV-tk gene therapy with ganciclovir is a new, well-tolerated, potentially effective therapy for operable high-grade glioma.

37. ROLES OF AURORA A MITOTIC KINASE IN THE DEVELOPMENT OF MALIGNANT GLIOMAS
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Chromosomal instability and aneuploidy are remarkable hallmarks of human cancers. In most cancers, high rates of chromosome gains/losses leading to aneuploidy have been observed. The causes of aneuploidy involve various critical mitotic events, including centrosome separation, chromosome alignment, chromosome segregation, and completion of cytokinesis. The error-free mitosis that is important to genomic integrity is regulated by phosphorylation reactions driven by several evolutionarily conserved serine/threonine kinases, known as mitotic kinases. Mitotic kinases include cyclin-dependent kinase 1 (Cdk1) and Polo-related, NimA-related, Aurora-related, and Warts-related kinases. In mammals, three members of this kinase family, Aurora-A, -B and –C, were identified. Recently, observations have revealed that Aurora-A kinase activity is required for various events during mitosis, such as transition from G2-M to M transition at M phase, centrosome separation, chromosome alignment and cytokinesis (Hirota et al., Cell 114, 585, 2003; Kunitoku et al., Dev. Cell 5, 853, 2003; Marumoto et al., J. Biol. Chem. 278, 11786, 2003). Given that only elevated expression of Aurora-A but also depletion of Aurora-A leads to mitotic failure and multinucleation, it is speculated that the proper timing and amplitude of Aurora-A expression is important for accurate chromosome segregation and fidelity of chromosome transmission (Marumoto et al., Nat. Rev. Cancer, in press, 2005). Furthermore, we generated a transgenic mouse model to investigate the involvement of Aurora-A overexpression in the tumorigenesis and found that elevated Aurora-A expression induces malignant transformation in the mouse in the presence of p53 mutation/loss (Zhang et al., Oncogene 23, 8720, 2004). These findings indicate that mitotic aberrations induced by Aurora-A overexpression with p53-dependent checkpoint abnormality are critical factors for the cancer formation. We have analyzed Aurora-A expression in malignant glioma and found that it is frequently overexpressed in anaplastic astrocytomas and glioblastomas. Especially, the Aurora-A overexpression is well correlated with giant cell formation in those tumors, which is consistent with data obtained in our transgenic mouse model. Our findings strongly suggest that aberrant expression of Aurora-A is a high risk factor for malignant progression of astrocytic tumors.
38. REGULATION OF PI3K SIGNALING AND TRANSFORMATION BY PTEN C-TERMINAL-INTERACTING PROTEINS
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The PTEN tumor suppressor gene is frequently mutated in diverse tumor types, including those of endometrium, breast, prostate, lung, and high-grade glioma. The protein possesses an amino-terminal catalytic domain with lipid phosphatase activity which regulates the AKT pathway through modulation of the second messenger product of PI3K, PI(3,4,5)P3. While this is catalytically active protein, which controls G0, cell cycle progression and hence suppresses tumor formation, has been fundamentally defined, the function(s) of the carboxy-terminal region of the protein remain largely unknown. To address the issue we searched for PTEN-interacting proteins by yeast two-hybrid screening using the PTEN carboxy-terminal domain, which has been reported to contribute membrane localization and protein stability as governed by casein kinase II-directed phosphorylation. Here we report the identification and characterization of two PTEN-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-

39. RAS/RAL-PATHWAY ACTIVATION SUPPRESSES CDC42 FLIPS EXPRESSION AND SENSITIZES GLIOMA CELLS TO TRAIL-INDUCED APOPTOSIS
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The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein is an attractive therapeutic molecule because it induces apoptosis in glioma cells, but not in normal astrocytes. The present study was initiated to better understand the basis for this tumor selectivity. Normal human astrocytes express TRAIL receptors but not TRAIL, whereas glioblastoma multiforme cells express both TRAIL and TRAIL receptors. The TRAIL-induced apoptosis was determined by flow cytometry. Immortalized astrocytes were also retrovirally infected with constructs encoding mutant forms of Ras that selectively activated the Ras-Ral pathway (C40 Ras, Ras-Ral (S33 Ras), or Ras-Ral (G37 Ras) pathways, after which the effects of PI3K, Raf, or Ral activation on cellular transformation; the levels of the Ras/Ral target cdc42; FLIPS levels; and TRAIL sensitivity were monitored. While both normal and immortalized human astrocytes were resistant to TRAIL-induced apoptosis (up to 1000 ng/ml TRAIL) and expressed high levels of FLIPS, V12 H-Ras-transformed astrocytes exhibited low levels of FLIPS and underwent apoptosis following exposure to as little as 200 ng/ml TRAIL. Only expression of a retroviral construct encoding the Ras/Ral activation domain resulted in an increase in FLIPS levels and enhanced TRAIL sensitivity. The mechanism underlying the effect of HDAC inhibitors remain unclear. In this study, we investigated the functional antiproliferative effect of HDAC inhibitors, N-butyric acid and trichostatin A, on human malignant glioma cell lines, U251-MG and D54. MTT assay showed dose-dependent inhibition of cell proliferation in both cell lines. Cell cycle analysis revealed increased sub-G1 population in both lines, and G0 arrest only in U251-MG cells. Induction of apoptosis was also supported by the occurrence of DNA fragmentation in tumor cells treated with HDAC inhibitors. Furthermore, histone deacetylase inhibitor assay indicated that HDAC inhibitors-induced apoptosis was caspase dependent. Interestingly, neither mitochondrial membrane potential nor the expression of caspase-3 was changed by treatment with HDAC inhibitors. These results show that activation of the Ras/Ral pathway not only leads to cellular transformation, but also to inhibition of cdc42 activation/FLIPS expression and sensitization of glioma cells to TRAIL-induced apoptosis. The extent of Ras pathway activation (which has been shown to be proportional to glioma grade) may therefore be helpful in predicting the sensitivity of gliomas to TRAIL-induced apoptosis.

40. HISTONE DEACYLASE INHIBITORS, N-BUTYRIC ACID AND TRICHOSTATIN A, INDUCE CASPASE-8-DEPENDENT BUT NOT CASPASE-9-DEPENDENT APOPTOSIS IN HUMAN MALIGNANT GLIOMA CELLS
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Histone deacetylase (HDAC) inhibitors have both apoptotic and differentiating effects on various tumor cells. However, the mechanisms underlying the effect of HDAC inhibitors remain unclear. In this study, we investigated the function of antiproliferative effect of HDAC inhibitors, N-butyric acid and trichostatin A, on human malignant glioma cell lines, U251-MG and D54. MTT assay showed dose-dependent inhibition of cell cycle arrest. The retention of PI3K/AKT signaling and cell cycle regulatory activities of acetylation-resistant PTEN K125R and K128R mutants in the presence of enforced PCAF expression supports a causal relationship. The association of PTEN with MSP58 was mediated to the MSP58 FHA domain and required PTEN threonine 366, a site reported to be phosphorylated in vivo. Additionally, we showed that while MSP58 transformed per-cellular MEF cells, concurrent introduction of wild-type PTEN caused a dramatic reduction in the number of MSP58-induced colonies. This inhibition of cellular transformation required interaction with PTEN since a point mutant of its interaction domain (Thr366Ala) was without effect. Importantly, transformation inhibition did not, however, require PTEN to be catalytically active, as its G129R mutant could inhibit MSP58-driven transformation. Thus, the C-terminal region of PTEN provides novel biological functions to this tumor suppressor gene in its ability to regulate its lipid phosphatase activity through interaction with PCAF and in its ability to regulate cellular transformation through interaction with MSP58.

41. LOW-MOLECULAR-WEIGHT CALDESIOMON (L-CAD) AS A NEW SERUM MARKER FOR GLIOMA
42. HIGH POSITIVE PREDICTIVE VALUE OF HEMIZYGOUS DELETIONS AT THE NOTCH2 LOCUS FOR SURVIVAL OF BRAIN TUMOR PATIENTS R. Scupin,1, 2A. Parada,1, 3 J. Mihai-Co,1, 4 T. Elisabeth,1, 5D. Beatrice,1 G. Anthony6, Z. Christian,7 M. Adrian,8, 9 M. André,10, 11 and R. Guido12; 1Neuro Oncology and 2Neurosurgical Clinic, University Hospital, Basel, Switzerland; 3University Hospital, Department of Internal Medicine, Bruderholz, Switzerland; 4Heinrich-Heine University, Department of Neuropathology, Düsseldorf, Germany

Loss of heterozygosity (LOH) on chromosome 1p predicts responsiveness to chemotherapy in 70% of malignant oligodendrogliomas (OGs), pointing to a genetic factor for the response that distinguishes OGs from resistant glioblastomas (GBMs). We defined eight distinct haplotypes on a somatic deletion map on chromosome 1 of 26 OGs and 50 GBMs. In search for correlation between survival and particular haplotypes, factor analysis, multivariate analysis, and non-parametric Kaplan-Meier curves were used. Test accuracy was determined by receiver operating characteristic (ROC) analyses. A consistent centromeric recombination breakpoint, clustered within 1 centimorgan between markers D1S2696 and D1S344, was prevalent in OGs, but not in GBMs (P < 0.0001). Hemizygous deletions at D1S2696, located within intron 12 of the Notch2 gene, correlated with better outcome (P < 0.0001). Interestingly, primary OGs and a subgroup of GBMs harbored overlapping single-copy microdeletions, defining a minimally lost region of 45 kb within the coding sequence of the Notch2 gene. LOH at the marker D1S2696 defines a new molecular classification that is independent of age and gender and equally well predicts survival time as histological and immunohistochemical classification (P < 0.0001). By ROC analysis, a cut-off of 24 months of survival time was defined resulting in a sensitivity and specificity for molecular classification of 80.8% and 90.2%. Simple, rapid, and highly reproducible molecular classification better outcome (P < 0.0001). Hemizygous deletions in OGs, but not in GBMs (P < 0.0001). Hemizygous deletions at D1S2696, located within intron 12 of the Notch2 gene, correlated with better outcome (P < 0.0001). Interestingly, primary OGs and a subgroup of GBMs harbored overlapping single-copy microdeletions, defining a minimally lost region of 45 kb within the coding sequence of the Notch2 gene. LOH at the marker D1S2696 defines a new molecular classification that is independent of age and gender and equally well predicts survival time as histological and immunohistochemical classification (P < 0.0001). By ROC analysis, a cut-off of 24 months of survival time was defined resulting in a sensitivity and specificity for molecular classification of 80.8% and 90.2%). Simple, rapid, and highly reproducible molecular classification using marker D1S2696 at the Notch2 locus adds independent prognostic information to histological classification and identifies a subgroup of OG-like GBMs with long-term survival.

43. GENE EXPRESSION PROFILING LINKS INVASION-RELATED GENES TO POOR SURVIVAL IN OLDER GLIOBLASTOMA PATIENTS J. Rich,1 C. Hans,2 B. Jones,3 R. McLendon,1 B. Rasheed,1 A. Dobra,1 H. Dressman,4 D. Bigner,3 J. Nevins,4 and M. West; 1Department of Medicine, 2Institutes of Statistics and Decision Sciences, and Departments of 3Pathology and 4Molecular Genetics & Microbiology, Duke University Medical Center, Durham, North Carolina, USA

Despite the strikingly grave prognosis for older patients with glioblastomas, significant variability in patient outcome is experienced. To explore the potential for developing improved prognostic capabilities based on the elucidation of potential biological relationships, we performed analyses of genes commonly mutated, amplified, or deleted in glioblastomas and Affymetrix DNA microarray gene expression data from tumors of 43 glioblastoma patients of age greater than 50 for whom survival is known. No prognostic significance was associated with genetic changes in EGFR, TP53, p16(INK4A), or PTEN. Statistical analysis of the gene expression data in connection with survival involved exploration of regression models of genes, based on computational search over multiple regression models with cross-validation to assess predictive validity. The analysis generated a set of regression models that, when weighted and combined according to posterior probabilities implied by the statistical analysis, identify patterns in expression of a small subset of genes that are associated with survival and have value in assessing survival risks. The dominant genes across multiple such regression models involve three key genes, secreted protein acidic and rich in cysteine (SPARC, osteonectin), Doublecortin and Semaphorin3B, which play roles in cellular migration processes. Additional analysis, based on statistical graphical association models constructed by using similar computational analysis methods, reveals others genes that support the view that multiple mediators of tumor invasion may be important prognostic factors in glioblastomas in older patients. No previous studies of which we are aware have elucidated conclusive links between expression of specific gene and survival of older glioblastoma patients. Our regression analyses using gene expression as explanatory of survival outcomes revealed that genes whose primary cellular effects may be the regulation of cellular migration appear as candidate markers of poor survival. Together these results suggest that tumor migration may represent an important effector of glioblastoma malignancy and may warrant accelerated development of specific therapies. Future studies will prospectively determine the link between the expression of SPARC, doublecortin, and SEMA3B in gliomas of all ages and patient outcome. This work was supported by a grant from the W.M. Keck Foundation, J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar. This work was also supported by NIH grants NS047409 (J.N.R.).

44. CRITICAL ANALYSIS OF THE WHO HISTOPATHOLOGICAL GRADING FOR MENINGIOMAS: IMPACT ON POSTOPERATIVE RADIOTHERAPY AND FOLLOW UP IN GERMANY M. Simon, J. Boström, P. Koch, and J. Schramm; University Hospital Bonn, Department of Neurosurgery, Bonn, Germany

We critically analyzed the clinical impact of the WHO histological grading for meningiomas including its role for postoperative radiotherapy/radiosurgery indications and MR follow-up protocols. The current (2000) and the 1993 WHO classifications were used to review the histological grade of 57 meningiomas operated at our institution. All German Neurosurgical Departments performing intracranial microsurgery were asked to detail their guidelines for radiation therapy and follow-up for meningiomas of different WHO grades. Comparing both WHO classifications, the current criteria downgraded 7/13 (54%) atypical (WHO grade II, MI) meningiomas to grade I (MI) and 4/6 (67%) anaplastic (WHO grade III, MIH) tumors to grade II. The use of specific criteria to diagnose atypia (MIBI index >5%) and malignancy (brain invasion) only during the first review accounted for 3 grade II to I and all grade III to II reclassifications, respectively. Indications for radiation therapy and MR follow-up protocols varied substantially with the histological grade and between institutions. After an incomplete resection, radiotherapy recommendations differed between MI and MIH in 30/58 (52%), and between MI and MIH in 34/56 (61%) units. Our data document a considerable impact of the histological grading for meningiomas in clinical practice. However, the use of changing grading paradigms and gender renders clinical decision making based on local and published experience difficult. The categories atypical and anaplastic meningioma, WHO grade II and III, respectively, have probably described quite different tumors in recent years. The clinical relevance of meningioma grading will only be properly recognized if diagnostic neuropathological labels are used consistently.

45. NOVEL STRATEGIES OF IMMUNOTHERAPY FOR MALIGNANT GLIOMA M. Weller; University of Tubingen, Neurologische Klinik, Tubingen, Germany

The limited efficacy of surgery, radiotherapy, and chemotherapy in the treatment of malignant glioma calls for innovative treatment approaches targeting specific biological features of these tumors. Malignant glioma cells have long been known for the release of multiple established or putative immunosuppressive molecules, including transforming growth factor-(TGF)-beta, prostaglandins, interleukin 10, CD95 ligand, or HLA-E/G. The very low frequency of systemic metastases in tumors which otherwise exhibit all features of malignancy has been attributed to an efficient glioma immune surveillance outside, but not inside, the central nervous system. Accordingly, promising strategies of immunotherapy based on these observations include (i) targeting the synthesis, release, or activity of glioma-derived immunosuppressive molecules and (ii) taking the presumably effective immune environment of gliomas from the outside into the central nervous system. Paradigmatically, TGF-beta, the prime suspect for glioma-associated immunosuppression, may be antagonized by RNA interference, inhibition of proprotepin production or TGF-beta receptor antagonists, conferring resistance to TGF-beta-induced immune paralysis. In fact, while the biological neutralization of glioma-associated immunosuppressive molecules alone may not induce the immune rejection of these tumors, it may still be a precondition for active strategies of immunotherapy to be successful. Until specific tumor antigens for malignant glioma may be identified, the most promising strategies of immunotherapy include those based on the vaccination with autologous, genetically modified tumor cells in the context of a stimulatory immune environment, possibly employing dendritic cell therapy. Future experimental trials in glioma-bearing rodents and phase 1/2 clinical trials will have to demonstrate whether the initiation of an efficient effenter immune response against malignant glioma cells may be more easily triggered by peripheral vaccination or by efforts to create a strong immune stimulatory environment within a Postsurgical tumor cavity.
46. PSEUDOTYPED, ONCOLYTIC ADENOVIRUSES USED TO TARGET GLIOMA STEM CELLS

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We have developed a number of oncolytic viruses based on a controlled gene expression by the human telomerase reverse transcriptase promoter. We describe five different oncolytic viruses: (1) an adenovirus using the CMV promoter to express the herpes simplex virus thymidine kinase gene (HSV-TK) in combination with Gancyclovir, (2) an adenovirus using the hTERT promoter to express the HSV-TK promoter to express the human telomerase reverse transcriptase (hTERT) promoter, (3) an adenovirus using the hTERT promoter to express the cytodeaminase gene fused to a uracil kinase gene in combination with 5-F-deoxycytidine, (4) an adenovirus using the hTERT promoter to express the adenoviral E1 proteins for replication in and lysis of hTERT positive cells, and (5) an adenovirus using the hTERT promoter to express a modified drosophila cytidine kinase, developed by ZGENE (Denmark) in combination with Gancyclovir. All of the hTERT promoter-containing viruses have upstream chicken insulator element to prevent activation by cis-acting enhancer/promoters. This insulator element increases the specificity and integrity of the hTERT promoter. The proof of concept about this promoter construct is available (Edqvist et al. ibid.). The adenovirus we use is a pseudotyped adenovirus that uses the CD46 molecule as the receptor instead of the coxsackie adenovirus receptor (CAR). The knob and the shaft of the fiber protein come from adenovirus type 35. We will test these viruses on at least 20 different primary glioma cell cultures and xenografted subcutaneously growing gliomas. The frequency of hTERT positive cells before and after the treatment will be evaluated. Preliminary data will be given and discussed.

47. ANTISENSE-MEDIATED SUPPRESSION OF HEPARANASE GENE INHIBITS BRAIN METASTASIS OF MELANOMA CELLS

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Brain metastasis occurs in most of all human cancers and is a frequent manifestation of malignant melanoma progression. Successful invasion into brain by tumor cells must include attachment to microvessel brain endothelial cells, and, of relevance, degradation of surrounding extracellular matrix (ECM). Heparan sulfate proteoglycans (HSPG) are essential and ubiquitous macromolecules associated with the cell surface and ECM of a wide range of cells and tissues. Heparanase (HPSE-1) is an ECM degrada
tive enzyme acting as an endo-beta-glucuronidase which degrades the heparan sulfate (HS) chains of HSPG at specific intrachain sites, resulting in bioactiv
ive HS fragments of discrete molecular weight size. To investigate effects of changes in heparanase gene expression in brain-metastatic melanoma (BMM) cells, we constructed adenoviral vectors containing the full-length human HPSE-1 cDNA in both sense (Ad-S/hep) and antisense orientation (Ad-A/shep). We found increased HPSE-1 expression and activity in BMM following Ad-S/hep infection by Western blot analyses and specific HPSE-1 activity assays. Conversely, HPSE-1 content was significantly inhibited following infection with Ad-A/shep. Importantly, HPSE-1 modulation by these adenoviral constructs correlated with brain invasive cellular properties in vitro. Moreover, extensive brain metastasis formation was observed in athymic nu/nu mice injected with Ad-S/hep-infected BMM cells, while none of the mice injected with Ad-A/shep showed any evidence of macroscopic malignancy. Our results suggest that HPSE-1 not only contributes to the brain-metastatic phenotype of melanoma cells, but also that the Ad-A/shep-mediated inhibition of its enzymatic activity can be efficacious in the prevention and treatment of melanoma brain metastasis.

48. ADENOVIRAL VECTOR MEDIATED DETECTION OF TELOMERASE ACTIVITY AT SINGLE LIVING CELLS

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Telomerase activity is normally observed in highly proliferative tissues, stem cells, and most malignant cells and therefore offers an attractive target for therapeutic intervention and for diagnostic and prognostic purposes. The telomerase activity is predominantly controlled by the regulated expression of the catalytic subunit telomerase reverse transcriptase (hTERT) at the transcriptional level. Here, we developed adenoviral vectors for detecting telomerase activity in single living cells. In these vectors, the expression of destabilized enhanced green fluorescence protein with a half-life of 2 h (d2EGFP) is under the control of the hTERT promoter. Insulator DNA sequences were introduced to shield the hTERT promoter from cis-activating elements in the adenoviral vector backbone. Moreover, the vectors were retargeted to ubiquitously expressed CD46 as a cellular receptor. Following infection of telomerase positive (HeLa and A549 cells) or negative cells (fibroblast and WI-38 cells) with such vectors, the d2EGFP expression was detected in a telomerase activity-dependent manner, which correlated with the hTERT expression as assessed with real-time PCR. Furthermore, about 50% of the promyelocytic leukemic HL-60 cells were expressing d2EGFP following infection with telomerase reporting adenoviral vector, and the d2EGFP expression was significantly diminished in retinoic acid–induced differentiating HL-60 cells compared with nontreated control cells. In all cell types tested, the expression level of d2EGFP in the presence of SP600125, a highly specific chemical inhibitor of JNK, was reduced. The results indicate that (i) Ras activates JNK during cell death induced by transient knockdown of JNK and (ii) telomerase activity at single living cells can be identified using adenoviral reporting vectors.
50. THE MOST CONSTITUTIVELY ACTIVE JNK ISOFORM, JNK2a2, IS PREFERENTIALLY EXPRESSED IN GliOBlastomas: IDENTIFICATION OF SPECIFIC SEQUENCES LEADING TO ITS ACTIVATION

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C-jun N-terminal kinases (JNKs) are critical to regulating cell growth, proliferation, and apoptosis. Activation of the JNK pathway has been implicated in the formation of several human tumors, particularly gliomas. There are 10 different JNK isoforms. We previously showed that a 55KD JNK isoform is constitutively activated in 86% of human gliomas, which makes it the most frequent signaling alteration found in these tumors. We found that this isoform was specifically a JNK2 isoform and likely to be either JNK2a2 or JNK2b2. Notably, we showed that JNK2a2 possesses the strongest autophosphorylation activity among all isoforms. We now report our efforts to identify specific sequences that contribute to JNK2a2 activation and how this isoform contributes to gliomogenicity. We generated a series of chimeric cDNAs that join portions of JNK1a2, which lacks detectable autophosphorylation activity, with portions of JNK2a2, which has the strongest activity. Through in vivo and in vitro kinase assays, we defined a domain within JNK2a2 from amino acid 218 to 226 that is required for its autophosphorylation activity. Mutation of JNK2a2 to its counterpart of JNK1a2 in this region abrogated the autophosphorylation activity and c-jun substrate kinase activity in vivo and in vitro. The switching of JNK1a2 to JNK2a2 at this region enables JNK1a2 to gain autophosphorylation activity. Next, we performed sequence analysis and expression of JNK2a2 and JNK2b2 in normal brain specimens and glioblastomas by RT-PCR. All four isoforms were expressed in normal brains (3/3), JNK1a2, JNK2b2, and JNK2b2 were found in 18% of glioblastomas (10/11). We then transfected U87-MG cells with GFC1-JNK1a2, GFC1-JNK2a2, and GFC1-JNK2a2/APF (a dominant negative mutant form of JNK2a2) and obtained stable clones expressing similar levels of protein. We assessed the effects on parameters relevant to tumor genesis including cell proliferation and tumor formation, and tumor formation in athymic mice. JNK2a2 was consistently the most effective in promoting proliferation and tumor growth where the relative order was JNK2a2 > JNK1a1 > JNK2a2/APF. Since JNK2a2 activates transcription factors, we profiled gene expression using cDNA microarrays. There were 16 genes whose expression was upregulated by JNK2a2 but suppressed by JNK2a2/APF, including EIF-4E and TGF-a, which indicates that the mechanism for JNK2a2 catalysis transformation is different from that of JNK1a2. Our data suggest that glioblastomas specifically upregulate the most active JNK isoform which promotes tumorigenesis through upregulation of specific genes. The identification of specific sequences that lead to JNK2a2 activation will allow us to design specific inhibitors.

51. ESTABLISHMENT OF A CELL LINE DERIVED FROM HUMAN CENTRAL NERVOUS SYSTEM LYMPHOMA

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Primary central nervous system lymphoma (PCNSL) exhibits several characters different from systemic lymphoma cells. PCNSL are usually confined to the central nervous system, vanish completely when treated with steroids, anticancer drugs or radiation, but relapse very rapidly. Little has been reported, however, on the genetic and biological nature of PCNSL, because of the scare tissue specimens derived from stereotactic biopsy. We established a cell line from human PCNSL and aimed to inquire its character of PCNSL. The cell line was established from the surgical specimen of PCNSL in the right putamen of a 68-year-old female, using primary explant technique. We investigated the character of lymphoma cells by immunocytochemistry, electron microscopic observation, and immunoblot analysis and at the same time, studied the response of cells against dexamethasone and methotrexate (MTX) in vitro. Apoptosis was analyzed by the Tunel method. To check the genetic changes, spectral karyotyping was performed. The population-doubling time of the cultured cell was 20 h. The cultured cell in RPMI1640 supplemented with 10% fetal bovine serum reacted with anti CD20, BCL2, and BCL6 antibodies. Electron microscopic observation revealed nuclear bleb, which was specific for lymphoma cell. Immunoblot analysis revealed BCL-2 and BCL-6 are expressed in the cultured cell. Representative karyotype was interpreted as 50.XX, +3(3); t(4;15)(q31;q15), del(6)(q21q25), +18(10). Inhibition dose (ID50) of MTX was 6.6 M, and ID50 of dexamethasone was 2 nM. Apoptosis was detected after either MTX or dexamethasone treatment. Our results demonstrated that the established cell line (designated MCL2) could be the useful in vitro model of PCNSL.

52. THE MITOCHONDRIAL PATHWAY IS CENTRAL TO ERUCYLPHOSPHOCHOLINE-MEDIATED APOPTOSIS IN HUMAN GLIOMA CELL LINES

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Glioblastomas are highly chemoresistant and radioresistant tumours of the CNS. Therefore, new therapeutic approaches are urgently needed. Alkylphosphocholines (APCs) are membranophilic agents not directly targeting cellular DNA. Erucylphosphocholine (ErPC) represents the prototype of a promising class of APC for parenteral administration. It has potent antineoplastic activity on various malignant tumours of different origin and accumulates within the brain. Of particular interest, ErPC induces apoptosis in glioma cells resistant to treatment with standard chemotherapeutics. Recently, we have shown that ErPC mediates apoptosis independent of p53 signaling and death receptor/linker systems, whereas activation of caspases via mitochon- dria plays a major role. To analyze the contribution of the mitochondrial pathway in more detail, we investigated the cytotoxic effects of ErPC in tumour cells with defects in apoptosis. To this end, we silenced the expression of Apaf-1 and caspases-3 and -9 in human glioma cell lines by specific small interfering RNAs (siRNAs). The different siRNAs showed a variable degree of knockdown efficiency with respect to protein expression and induction of apoptosis arguing in favor of an essential role of the intrinsic pathway. As a further proof that the poor response to ErPC is due to the lack of an essential apoptosis gene, we used HeLa cells stably transfected with a dominant-negative caspase-9 construct, Apaf-1-negative melanoma cells, and MCF-7 cells harboring a deletion in the Casp-3 gene. In these cell lines, cytotoxicity and apoptosis induction was drastically decreased compared to vector controls, to cells harboring a functional Apaf-1 gene, and to cells stably transfected with the Casp-3 cDNA, which thus corroborated our results in glioma cells. In particular, we provide evidence that caspase-3 is required for the activation of caspases-2, -6, -8 and -9 also participates in a feedback amplification loop. Together, our data suggest that components of the mitochondrial apoptosis pathway are essential for ErPC to effectively induce apoptosis, whereas elements of the death receptor pathway are dispensable. This work was supported by B. Braun-Stiftung.

53. TRAIL INDUCES PROLIFERATION OF MALIGNANT GLIOMA CELLS THROUGH C-FLIP-MEDIATED ERK1/2 PATHWAY

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in TRAIL-sensitive human malignant glioma cells. Here we show that TRAIL stimulated cell growth in TRAIL-resistant glioma cells. TRAIL-induced cell growth in the resistant cells occurred through increased cell cycle progression as determined by flow cytometry analysis of propidium iodide–stained cells. Western blot analysis of TRAIL-treated cells revealed phosphorylation of pRb protein (pRb) phosphorylation. Western blot analysis of TRAIL-treated resistant cells revealed phosphorylation of ERK1/2 proteins, and in vitro kinase analysis confirmed the activation of the ERK1/2 kinases. ERK1/2 kinases were activated through a mechanism dependent on MEK phosphorylation and MAPK/ERK kinase (MEK). Treatment of the resistant cells with MEK1 inhibitor PD98059 eliminated TRAIL-induced ERK1/2 activation and cell proliferation. These results suggested that TRAIL-induced cell proliferation occurs through activation of the ERK1/2 pathway in TRAIL-resistant glioma cells. Inhibition of cell death domain–like interleukin-1β-converting enzyme–inhibitory protein (c-FLIP) with small interfering RNA (siRNA) eliminated TRAIL-induced ERK1/2 activation, and proliferation as determined by cell viability assay, propidium iodide staining, and flow cytometry, and pRb phosphorylation. The results indicate that TRAIL-induced ERK1/2 activation and proliferation in TRAIL-resistant glioma cell lines is dependent upon the expression of the caspase-8 inhibitor c-FLIP. Furthermore, inhibition of c-FLIP expression sensitized the resistant cells to TRAIL-induced apoptosis as demonstrated by the cleavage of caspases. In conclusion, TRAIL triggers growth in TRAIL-resistant malignant glioma cells through c-FLIP-mediated ERK1/2 pathway, and thus targeting c-FLIP/ERK1/2 pathway may overcome the resistance of malignant glioma cells to TRAIL treatment.
54. ROCK INHIBITION INDUCES ASTROCYTOMA MOTILITY IN A RAC-DEPENDENT MANNER
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The intracellular mechanisms governing astrocytoma motility while poorly understood require modifications of the cytoskeleton. The Rho-GTPases (Rac, Cdc42 and Rho) are pivotal regulators of cytoskeletal organization and cell motility. We have shown that inhibition of ROCK, a serine/threonine effector kinase of Rho, with Y27632 or the dominantly-neg-ative mutant inhibits stress fibers and focal adhesions induced by LPA. In contrast to several studies demonstrating that inhibition of ROCK can decrease tumor cell invasiveness and motility, we found using a 2-dimen-sional radial migration assay that astrocytoma migration was significantly increased following treatment with Y27632. LPA also significantly stimu-lated the motility of astrocytoma cells. The observation that ROCK inhibit-ion also led to increased membrane ruffling suggested that Rac activation was a possible mechanism in Y27632-induced motility. Rac-GTPases are thought to regulate membrane ruffling formation and cell migration in large part by stimulating actin polymerization. We demonstrated for the first time both directly and indirectly that Rac activation is an outcome of ROCK inhibition in astrocytoma cells. First, using a Rac-GTP pull-down assay, we show that U251 cells treated with Y27632 increased Rac activity com-pared to untreated controls. In addition, LPA alone or in combination with Y27632 also increased the levels of Rac-GTP. Next we show that Y27632 induces membrane ruffling in a Rac1-dependent manner since depletion of Rac1 strongly inhibits Y27632-induced membrane ruffles. These cells also re-cycle the stellate phenotype of Y27632 treatment without regaining actin stress fibers. In addition, Rac1-directed siRNA effectively overcame Y27632-induced motility by nearly 2-fold. Furthermore, Rac1 depletion also inhibited the migration of U251 cells by about 50%, whereas, in all malignancies, our data show that inhibition of ROCK plays a major role in regulating astrocytoma cell morphology, actin cytoskeleton, and migration through the activation of Rac1. In addition, astrocytoma migration seems to be occurring by two separate, Rho-independent, Rac-dependent mechanisms; ROCK inhibition and LPA stimulation. Our future studies will be directed toward determining the mechanisms that lead to Rac activation following ROCK inhibition or LPA stimulation in our cells. Increasing our un-derstanding of the significant cross-talk that appears to be occurring between Rho-GTPase family members and their effector proteins will be important to identifying key elements within these pathways that can be exploited to inhibit the growth and invasiveness of human astrocytoma cells.

55. MEMBERS OF THE ETS FAMILY OF TRANSCRIPTION FACTORS BIND TO THE SITE INTRODUCED BY A SINGLE NUCLEOTIDE POLYMORPHISM IN THE MMP-1 PROMOTER
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Matrix metalloproteinase-1 has been implicated in the metastasis and invasion of many types of tumors. A polymorphism exists in the proximal promoter region of MMP-1 that regulates MMP-1 transcription. This polymorphism consists of either the presence (2G allele) or absence (1G allele) of a guanine nucleotide adjacent to a pre-existing guanine nucleotide. This additional guanine nucleotide creates a binding site for the ETS family of transcription factors and leads to increases in MMP-1 transcription. In several aggressive and metastatic tumors studied, the incidence of the 2G allele is significantly higher. We found a significant difference in the distribution of the genotypes between healthy individuals and glioblastoma patients (P = 0.031) with an increase in the percentage of the 2G/2G genotype in the tumor population (P = 0.018). The aim of this study was to identify if the additional ETS binding site regulates the MMP-1 promoter in glioma cells. Transfection of three glioma cell lines with a 2G MMP-1 promoter reporter construct resulted in increased transcription when compared to transfection with the 1G reporter construct (P = 0.02). Identification of the proteins binding to the 2G promoter is the first step in understanding the regulatory effects this polymorphism has on MMP-1 transcription. ETS transcription factors are divided into subfamilies according to structural similarities. Results from RT-PCR and Western blot indicate that all members of the Ets and PEA3 subfamilies are present in seven glioma cell lines. To determine which members of these subfamilies bind to the 2G promoter, we performed DNA-protein pull-down assays. Our data indicates that Ets-1 binds the 2G promoter, and we are currently evaluating the binding capa-bility of the other members of these subfamilies. We are also conducting chromatin immunoprecipitation assays to determine which proteins bind to the 2G allele in the context of the cellular environment. Both hepatocyte growth factor and phorbol myristate acetate have been shown to increase binding of ETS members to the MMP-1 promoter leading to increases in MMP-1 promoter activity. We are assessing the ability of HGF and PMA to influence the binding of ETS proteins to the 2G promoter and subsequent MMP-1 transcriptional activity. Results from these studies will increase our knowledge of how MMP-1 is regulated in glioma cells. This information may lead to advances in therapies that artificially lower the levels of MMP-1 and indirectly control glioma invasion.

56. REGULATION OF UNCOUPLING PROTEIN-2 (UCP-2) EXPRESSION IN HUMAN GLIOMA CELLS BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) AGONISTS
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Recent studies have suggested that the glitazones, a group of PPAR ago-nists commonly prescribed as therapy in type 2 diabetes, could have a role in regulation of cell viability in astrocytomas and glioma cell lines, possibly due to a modulation of reactive oxygen species (ROS) production. PPAR agonists are also known to regulate expression of the mitochondrial protein UCP-2, and UCP-2 has a purported role in ROS regulation amongst others. This study investigated the expression of UCP-2 in U251MG glioma cells and its regulation by PPAR agonists. U251MG glioma cells were cultured according to standard methods and treated with PPAR alpha, delta, and gamma agonists (10 μM WY14643, 10 nM PGJ2, 10 μM rosiglitazone and 10 nM PGJ2, respectively) for 24 h. Total RNA was subsequently extracted and semiquantitative RT-PCR used to evaluate UCP-2 mRNA expression. Results showed that UCP-2 was upregulated in all control cells com-pared to untreated controls. In addition, LPA alone or in combination with Y27632-induced membrane ruffles. These cells also re-cycle the stellate phenotype of Y27632 treatment without regaining actin stress fibers. In addition, Rac1-directed siRNA effectively overcame Y27632-induced motility by nearly 2-fold. Furthermore, Rac1 depletion also inhibited the migration of U251 cells by about 50%, whereas, in all malignancies, our data show that inhibition of ROCK plays a major role in regulating astrocytoma cell morphology, actin cytoskeleton, and migration through the activation of Rac1. In addition, astrocytoma migration seems to be occurring by two separate, Rho-independent, Rac-dependent mechanisms; ROCK inhibition and LPA stimulation. Our future studies will be directed toward determining the mechanisms that lead to Rac activation following ROCK inhibition or LPA stimulation in our cells. Increasing our un-derstanding of the significant cross-talk that appears to be occurring between Rho-GTPase family members and their effector proteins will be important to identifying key elements within these pathways that can be exploited to inhibit the growth and invasiveness of human astrocytoma cells. UCP-2 expression was regulated differentially by the various PPAR subtype agonists tested. This novel finding that UCP-2 is expressed in glioma cells, and that its expression is regulated by PPAR agonists, sug-gests potential mechanisms of the cytotoxic effects of glitazones that have been previously reported, and describes a mechanism which could possibly be manipulated as a potential therapeutic avenue in the future.
58. NOTCH2 INTRODUCES CBF1-INDEPENDENT PATHWAY IN GliOBlASTOMA: EVIDENCE OF NOVEL NOTCH SIGNAL ELEMENT

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Notch signaling is well known to play a crucial role in cell fate decisions during development. Furthermore, in humans, four different subtypes of Notch have already been recognized. In the past decade, many reports have implicated the notch protein in tumorigenesis, specifically because of overexpression of the Notch intracellular domains as the constitutive active form of Notch. However, the function of this truncated Notch is still not clear, and a few reports have described the correlation between each subtype of Notch and tumorigenesis. We previously reported that Notch signaling is associated with the evasion of apoptosis in glioblastoma. In this report, we focus on Notch1 and Notch2 expression in glioblastoma, and we discuss the function of Notch signaling in this tumor. Glioblastoma cell lines U87MG, U251MG, A172, and T98G were investigated for expression of Notch1 and Notch2 protein and mRNA by using Western blot and RT-PCR, respectively. The intracellular distribution of these two Notch proteins was then revealed individually by immunocytochemistry using specific antibody to Notch1 and Notch2. Downstream of Notch signaling, truncated Notch protein binds CBF1 and expresses HES1. Such protein-to-protein interaction was also confirmed in glioblastoma cell lines by co-immunoprecipitation. Finally, by using western blot analysis of HES1 expression was silenced, and subsequently, mRNA expression of HES1, as the target gene of Notch signaling, was measured by real-time PCR. All cell lines expressed Notch1 and Notch2 and showed a tendency to express Notch1 dominantly. In addition, intracellular Notch expression was strongly, which indicates that Notch signaling was well activated in glioblastoma as well as in carcinoma of other organs. Immunocytochemistry, immunoprecipitation, and gene silencing by siRNA more clearly differentiated Notch1 and Notch2 attributes in glioblastoma. We used mouse astrocytes and NIH3T3 cells to study the molecular mechanisms involved in regulation of PTEN nuclear export. In agreement with other reports, we show that PTEN is preferentially localized in the nucleus of HES1 was not affected, which suggests that Notch2 might introduce a novel CBF1-independent pathway in glioblastoma cell lines. Notch2 signaling might play an unexpected role in glioblastoma, through novel unidentified Notch pathway components.

59. CYTOPLASMIC TRANSLOCATION OF PTEN TUMOR SUPPRESSOR IS MEDIATED BY PI3K/AKT/MTOR/P70S6K SIGNALING CASCADES

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The PTEN tumor suppressor gene is commonly deleted or mutated in a large number of advanced tumors including GBMs. PTEN functions primarily as a phosphoinositide phosphatase that specifically antagonizes PI3K-mediated signaling pathways. PTEN is preferentially expressed in the nucleus of differentiated or resting cells, and increased nuclear PTEN expression is associated with G0/G1 phase. However, the regulation of PTEN’s nuclear translocation is poorly understood. In this study, we used mouse astrocytes and NIH3T3 cells to study the molecular mechanisms involved in regulation of PTEN nuclear export. In agreement with other reports, we show that PTEN is preferentially localized in the nucleus during G0/G1 phase and is exported into the cytoplasm during G2/S transition. We further demonstrate that dominant-negative mutants for Akt (Akt-AAA) and for p70S6K (K113R) as well as inhibitors for PI3K (LY294002), PD1 ( Staurosporine), mTOR (RAD-001), and sodium salicylate (for p70S6K), but not for MEK (PD98059), suppress the nuclear export of PTEN protein. Conversely, constitutively active AKT mutant (Akt-DD) promotes PTEN’s cytosolic translocation. In addition, we also demonstrate that PTEN interacts with p70S6K in vivo and in vitro. Taken together, these findings strongly suggest that PI3K/AKT/MTOR/p70S6K signaling cascades, p70S6K in particular, are pivotal in regulating PTEN’s subcellular localization. This scenario is reminiscent of “yin and yang” reciprocal regulation between PI3K and PTEN during the course of cell cycle progression. Interestingly, our immunohistochemistry results show that PTEN is predominantly expressed in the cytoplasm of GBM tumors that positively correlates with the phosphorylation of S6. Furthermore, we also observe preferentially cytoplasmic localization of PTEN in a GBM cell line, LN229, which is presumably due to the constitutive activation of p70S6K that can be blocked by sodium salicylate but not by RAD-001. Our long-term goal is to establish the relationship between PTEN’s subcellular localization and the status of activation of PI3K downstream effectors, such as AKT, mTOR, or p70S6K in order to improve molecular diagnosis, prognostics, and individualized therapy for GBMs.

60. THE NOTCH SIGNALING PATHWAY AND GROUCHO/TLE CO-REPRESSORS IN MENINGIOMA PATHOGENESIS

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Meningiomas constitute the second most common central nervous system tumor. Atypical and malignant meningiomas are associated with a poor clinical outcome and higher rates of recurrence when compared to benign meningiomas. Relatively little is known about the molecular events important in the pathogenesis and malignant progression of meningiomas. To determine the molecular changes associated with meningiomas, we used serial analysis of gene expression (SAGE). We focused our initial analysis on the 165 genes that are induced in high-grade meningiomas because this population is expected to contain components of activated signal transduction pathways. A novel finding from this screen is the induction of three downstream components of the Notch signaling pathway: the transcription factor, Hairy and Enhancer of Split 1 (HES1), and two members of the Groucho/Transducin like enhancer of split (Gro/TLE) family of co-repressors, TLE2 and TLE3. Gro/TLE co-repressors interact and modulate the activity of a wide range of transcriptional regulatory systems, one of which is HES1. The SAGE results were validated by performing quantitative PCR on a larger, independent set of meningiomas. We confirm that HES1 transcript levels are induced in meningiomas of all three grades while induction of TLE2 and TLE3 is specific to high-grade meningiomas. In particular, overexpression of TLE3 occurs in 50% of malignant meningiomas and is associated with reduced patient survival. Immunohistochemistry revealed that TLE3 is correctly localized to the nucleus, where it is supposed to function as a transcriptional co-repressor. We also find induction of other components of the Notch signaling pathway: Notch2 targeted genes such as HES1. The above results lead us to hypothesize that Gro/TLEs are important for the malignant progression of meningiomas and that one of the mechanisms by which this occurs is by modulation of the Notch signaling pathway. We are currently studying the overexpression of Gro/TLEs and their role in glioblastoma. We are also interested in identifying additional components of the Notch signaling pathway and TLE repressors to delineate their relevance in meningioma growth and tumorigenesis.

61. THE IN VITRO ANTI-CANCER ACTIVITY OF GLIVEC AGAINST GliOBLASTOMA MULTIFORME IS NOT RELATED TO PDGFRAND KIT EXPRESSION

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Overexpression of the tyrosine kinase receptors (RTKs) PDGFRa/b and c-kit is a common event in glioblastomas, therefore representing obvious targets for inhibitor Glivec. Moreover, the PDGFRa and c-kit gene is amplified in a small subgroup of glioblastoma patients. The aim of our study was to compare the expression of PDGFR isoforms a and b as well as c-kit with the therapeutic efficacy of Glivec in glioblastoma primary tumor cultures. Western blot and RT-PCR analysis of the PDGFR levels of PDGFRa/b and c-kit in 43 primary cell cultures from astrocytic brain tumor surgery specimens. Tumor samples were obtained during surgery and histologically verified according to WHO criteria as glioblastoma multiforme. Immunohistochemical expression of PDGFRa and c-kit was determined in selected paraffin sections (N = 5). Sensitivity against Glivec (10–50 μM) was analyzed by MTI tests. Chromosomal aberrations were studied by means of comparative genomic hybridization (CGH) using DNA isolated from cell cultures as well as corresponding tumor sections. Of 43 primary cell cultures, 30 (70%) expressed detectable levels of PDGFRa; 34/43 (79%) displayed PDGFRb protein expression, whereas 25/43 (58%) showed expression of both receptor isoforms. Expression of c-kit was detected in 17/43 (40%) of the analyzed cell cultures. In one highly PDGFRa-overexpressing cell culture, high-level amplification of the respective gene at chromosome 4q12 was detected by CGH. This expression level was well in accordance with response to Glivec. Four of five tumor sections displayed heterogenous PDGFRa immunostaining. Staining intensity differed between and within samples and was high in giant tumor cells and endothelial cells. Widespread but weak staining could be observed in fibrillar tumor cells. In one case, we observed preferentially cytoplasmic localization of PDGFRa in meningiomas, with the amplitude of induction ranging from four- to 65-fold. TLE3 is specifc to high-grade meningiomas. In particular, overexpression of TLE3 occurs in 50% of malignant meningiomas and is associated with reduced patient survival. Immunohistochemistry revealed that TLE3 is correctly localized to the nucleus, where it is supposed to function as a transcriptional co-repressor. We also find induction of other components of the Notch signaling pathway: Notch2 targeted genes such as HES1. The above results lead us to hypothesize that Gro/TLEs are important for the malignant progression of meningiomas and that one of the mechanisms by which this occurs is by modulation of the Notch signaling pathway. We are currently studying the overexpression of Gro/TLEs and their role in glioblastoma. We are also interested in identifying additional components of the Notch signaling pathway and TLE repressors to delineate their relevance in meningioma growth and tumorigenesis.
is not related to expression levels of these targeted RTKs. The relationship of PDGFRA gene amplification in a small subgroup of glioma patients and Gleevec sensitivity should be further investigated, since a glialblastoma cell culture with PDGFRA gene amplification was sensitive against Gleevec in our in vitro experiments.

62. THE PTEN TUMOR SUPPRESSOR GENE SHIFTS CELLULAR RESPONSES TO TRANSFORMING GROWTH FACTOR-β TOWARDS TUMOR SUPPRESSION IN MALIGNANT GLIOMAS
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Transforming growth factor-β (TGFβ) is a multifunctional cytokine commonly expressed by malignant gliomas that regulates a diverse set of biological activities, including proliferation, apoptosis, differentiation, motility, extracellular matrix deposition, and angiogenesis. Although glial cells are growth inhibited by TGFβ, glioma cell lines are resistant to TGFβ-mediated growth inhibition yet retain responsiveness to the effects of TGFβ on the neoplastic phenotype—secretion of angiogenic factors, induction of invasion, and immune escape. The molecular mechanisms through which TGFβ shifts from a tumor suppressor to a tumor enhancer in advanced cancer are poorly understood. Recent work suggests that the phosphodi-3-1,OH inositol kinase (PI3K) pathway interacts with TGFβ signaling at multiple levels. We therefore sought to determine the functional significance of PTEN expression on TGFβ-mediated transcriptions. Restoration of wild-type PTEN in PTEN null glioma cell line inhibited TGFβ transcriptions in a PTEN concentration-dependent fashion, whereas mutant PTEN lacking both protein and lipid phosphatase activity did not have a similar effect. In a cell line with wild-type PTEN expression, stable knockdown of PTEN expression with short hairpin RNA (shRNA) increased TGFβ transcriptional activation or inhibition in multiple TGFβ responsive promoters. Results with these luciferase reporters suggest that PTEN functions through a mechanism distinct from other PI3K pathway components previously shown to interact with SMADs. To elucidate the mechanism by which PTEN inhibits TGFβ transcription, we examined TGFβ-induced phosphorylation of key intracellular mediators (the SMADs) with PTEN expression. C-terminal SMAD phosphorylation was moderately decreased with wild-type PTEN expression but not mutant PTEN, including a mutant that retains protein phosphatase activity. Reconstitution of wild type but not phosphatase dead mutant PTEN into PTEN null glioma cell lines re-established growth inhibition in response to TGFβ. In keeping with the biphasic nature of TGFβ signaling, PTEN reconstitution blocked invasion through an artificial matrix induced by TGFβ. Reciprocally, inhibiting PTEN expression in a PTEN wild type cell line (through expression of shRNA directed against PTEN inhibited TGFβ-induced motility. Thus, loss of PTEN expression may promote cellular responses to TGFβ involved in tumor progression. Reintroduction of PTEN may shift TGFβ cellular responses towards a tumor suppressive phenotype by restoring sensitivity of glioma cell lines to TGFβ-mediated growth inhibition while blocking TGFβ-mediated invasion. This work was also supported by NIH grants NS047409. J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

63. RNAI-MEDIATED SIMULTANEOUS DOWNREGULATION OF uPA AND uPA RESULTS IN THE DOWNREGULATION OF THE REGULATORY ASSOCIATED PROTEIN OF mTOR (RAPTOR), A COLLAPSE IN MITOCOCHONDRIAL DY, AND THE INDUCTION OF PRO-APOPTOTIC GENES IN SBN19 HUMAN GLIOMA CELLS
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Our previous results have demonstrated the ability of a plasmid-based RNAI system to silence uPAR and uPA using a bicistronic construct. Uroki-nase plasminogen activator (uPA) and its receptor (uPAR) are overexpressed during glioma cell invasion and progression. Pro-uPA, when bound to its receptor uPAR, is activated to uPA, which in turn activates plasminogen to plasmin, which is known to be a key mechanism involved in angiogenesis and metastasis. uPA/uPAR signaling involves mediators mediated by MEK and PI-3. SBN19 cells were transfected with plasmid-expressing RNAi targeting uPAR (pUR), uPA (pUP), uPAR–uPA simultaneously (pUP2), empty vector (EV), or scrambled vector (SV). Western blot analysis of RAPTOR, an upstream regulated protein of mTOR showed a marked decrease in expression levels when transfected with plasmids expressing either pUP or pUP2. In contrast, cells transfected with pUR did not exhibit a decrease in RAPTOR levels, which indicates that uPA/uPAR levels are integral in maintaining the activity of mTOR. In addi-

tion, K667 levels decreased in pUP- and pUP2-transfected cells. However, downregulation of uPAR alone did not result in a decrease of K667 levels, further indicating the involvement of uPA with proteins other than its tra-
ditional receptor. Mitochondrial uptake and processing of fluorescent cation dye 5,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidaz-

azolocarbocyanin iodide, where red fluorescence indicates DY collapse. The simultaneous downregulation of uPAR and uPA resulted in a decrease of red fluorescence, whereas the downregulation of either uPAR or uPA did not. These data strongly suggest the involvement of the uPA/uPAR complex in mitochondrial DY maintenance and collapse. The simultaneous downregulation of uPAR and uPA also induced caspase 8 activation accompanied by cytochrome c release, thereby indicating the initiation of apoptosis. Of further interest is that, in pUP2-transfected cells, PARP levels were elevated and cleaved PARP levels were decreased 72 h after transfection, but 120 h after transfection, PARP cleavage, an indicator of apoptotic progression, was observed. In conclusion, the simultaneous downregulation of uPAR and uPA inhibited cap-dependent mRNA translation via downregulation of RAPTOR, initiated apoptosis by mediating the collapse of mitochondrial DY, and induced caspase 8 and PARP cleavage. These results clearly suggest that the simultaneous targeting of uPAR and uPA has potential for cancer gene therapy.

64. OVEREXPRESSION OF MARCKS IN EGFRvIII-EXPRESSING GLIOBLASTOMA MULTIFORME
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The most common gain of function mutation in malignant astrocytomas (GBMs) is amplification or overexpression of the wild-type (wt) and activated receptor tyrosine kinases (RTKs), the most common of which is EGFRvIII. The differential signaling pathways utilized by EGFRvIII vs. wt-EGFR, contributing to its more aggressive behavior, is not known. ICAT, a mass spec–based analytical technique to evaluate differential protein profiles, demonstrated that GBM explant xenografts harboring EGFRvIII expressed much higher levels of MARCKS, as compared to GBMs, which express only wt-EGFR. MARCKS has been previ-
ously implicated in tumor invasion and breast cancers, but not in gliomas or EGFR signaling. MARCKS overexpression in EGFRvIII GBMs was verified by Western immunoblot analysis on a larger panel of GBM cell lines with Tet-off expression of EGFRvIII or wt-EGFR. GBM xenografts with 2× EGFRvIII and GBM operative specimens with 2× EGFRvIII Differences at the level of the proteome, evaluated by proteomics-based techniques such as ICAT, allow us to understand biological and clinical similarities or differences between subtypes of human diseases, such as GBMs. Current work involves assessing the functional role of MARCKS overexpression in GBMs. We will discuss our experiments with siRNA downregulation of MARCKS in GBMs expressing EGFRvIII and MARCKS overexpression in GBMs expressing only wt-EGFR. If MARCKS contributes to the aggressive biology of EGFRvIII expressing GBMs, it may be an additional biological target.

65. OVEREXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND MAINTENANCE OF MALIGNANT PHENOTYPE IN MENINGIOMAS
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Because metastatic activity, host defense mechanisms, and level of dif-
ferentiation seem to be correlated to iNOS expression, a role for iNOS has been proposed in the pathogenesis of meningiomas. Under physiological condition, NO acts as an intracellular secondary messenger and provides an efficient system for cellular regulation, interaction, and defense. The expression of 3 isoforms of NOS in human gliomas and in peritumoral areas has been analyzed by several groups. Although the induction of iNOS has been noted in human meningioma (Bakshi et al., 1998; Broholm et al., 2003; Ellis et al., 1995; Harr et al., 1996), a clear correlation between meningiomas and iNOS expression has not been established. Human meningiomas of benign, atypical, and anaplastic/malignant grade (n = 10) were excised and primary explant cultures obtained. Histopathological confirmation of grade was obtained. By using Western blot, expression of inducible nitric oxide synthase (iNOS) was demonstrated for cultured cells and intact tissue fragments. Integrity of downstream NO signaling pathways was tested by exposing cultured cells at early passage to NO donor compounds. The downstream actions of NO take two forms, (1) cGMP-dependent; and (2) cGMP-independent, which are mediated by reactive nitrogen species produced by the interaction of
66. MOLECULAR GENETIC STUDY FOR HEMANGIOMATOSAS AND FUNCTIONS OF VHL GENE

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Molecular genetic analysis for germline or somatic mutation of von Hippel-Lindau (VHL) gene in central nervous system (CNS) hemangioblastomas (HBs) with and without VHL disease contributes to surgical treatment and follow-up. Forty-nine patients bearing HBs (sporadic 33, VHL 16) underwent surgery and genetic diagnosis for germline and somatic VHL mutations. Locations of the tumors were cerebellum, 39; brain stem, 10; and spinal cord, 10. Eighteen of 16 HB cases had multiple tumors while 3 of 26 sporadic HBs had one tumor. Twenty sporadic HBs showed somatic mutations (missense 6, truncation-type 6) but not germline mutations. In addition, 24 sporadic HBs showed loss of heterozygosity (LOH) on 3p, in which the VHL gene is located. These results suggested that the inactiva-

tion of VHL genes on both alleles was a cause of genesis of the majority of sporadic hemangioblastomas and that the VHL gene functioned as a tumor suppressor gene. Eleven of 15 VHL cases showed VHL germline mutations (missense 8, truncation-type 4). Patients with truncation-type VHL germ-

line mutation were more frequently associated with renal cell cancer (RCC). Causes of death were postoperative complications in 2 sporadic patients and tumor development in 2 VHL patients. Clinically ambiguous cases, whether sporadic or VHL, should be analyzed for VHL germline muta-
tion. It might be recommended that HBs with VHL should be surgically treated if symptomatic, while asymptomatic small ones should be observed or treated with radiosurgery. Functions of the VHL gene include not only tumor suppression in HB as the above but also neuronal differentiation, which we demonstrated. Herein we show neuronal regeneration with donor of VHL-gene or peptide transferred stem cells (neural stem cell, bone marrow stromal cell, skin stem cell, ES cell). Transplantation with VHL-gene transferred stem cells dramatically improved symptoms of neuronal disease model rats (Parkinson, cerebral infarction in vivo and neuronal function in vitro), and they functioned as neurons in the brain. It was suggested that neuronal differ-

entiation by VHL protein was related to ubiquitination and resolution of Notch under normoxia but not under hypoxia. In addition, synthetic VHL oligopeptide (elongin-binding site at a-domain) showed induction potential for neuronal differentiation equal to transduction with viral vector. In the future, neuronal regeneration with VHL gene or peptide would be useful for the clinical level.

67. LIGAND-INDEPENDENT ACTIVATION OF THE EGFRvIII: A NATURALLY OCCURRING MUTATION OF THE EGFR COMMONLY EXPRESSED IN GLIOMA

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Mutations of the epidermal growth factor receptor (EGFR) gene are found at a relatively high frequency in glioma, with the most common being the de2-7 EGFR (or EGFRvIII). This mutation arises from an in-frame dele-
tion of exons 2-7, which removes 267 amino acids from the extracellular domain of the receptor. Despite being unable to bind ligand, the de2-7 EGFR is constitutively active at a low level. Transfection of human glioma cells with the de2-7 EGFR has little effect in vitro, but when grown as tumor xenografts this mutated receptor imparts a dramatic growth advantage. We

have now mapped the phosphorylation pattern of de2-7 EGFR, both in vivo and in vitro, using a panel of antibodies unique to the different phosphory-
lated tyrosine residues. Phosphorylation of de2-7 EGFR was detected con-
stitutively both in vitro and in vivo, including tyrosine 845, a known target in the wild-type EGFR for src kinase. There was a substantial upregulation of phosphorylation at every tyrosine residue of the de2-7 EGFR when cells were grown in vivo compared to the receptor isolated from were very low in vitro. Upregulation of phosphorylation could be mimicked in vitro by the addition of specific components of the ECM such as collagen via an integrin-dependent mechanism. Since this increase in in vivo phosphorylation enhances de2-7 EGFR signaling, this observa-
tion has clinical relevance as it points to the potential for pharmaceutical treatment of glioblastoma. We conclude that de2-7 EGFR is largely restricted to the in vivo environment. In a second set of experiments we analyzed the interaction between EGFRvIII and ErbB2. Co-expression of these proteins in NR6 cells, a mouse fibroblast line devoid of ErbB family members, dramatically enhanced in vivo tumorigenicity of these cells compared to cells expressing either protein alone. Detailed analysis of these xenografts demonstrated that EGFRvIII could heterodimerize and trans-

phosphorylate the ErbB2. Since both EGFRvIII and ErbB2 are commonly expressed at gliomas, this data suggests that the co-expression of these two proteins may enhance glioma tumorigenicity.

68. LONG-TERM SURVIVAL IN PATIENTS WITH GLIOBLASTOMA MULTIFORME TREATED IN PHASE 2 STUDIES WITH ANP

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The purpose of this study was to determine the frequency of long-term survivors in patients with glioblastoma multiforme (GBM) treated in FDA and Institutional Review Board monitored phase 2 studies with ANP (anti-
nexin). Since the beginning of clinical trials in 1991, over 300 patients with GBM who could be evaluated were accrued to FDA-monitored phase 2 trials. Seventy-nine patients were admitted to the study protocols (SP), and an additional group of 94 patients were treated under special exception (SE). There were 15.5% long-term survival in the SP group and 7.1% in the SE group. The maximum survival in the SP group was more than 12 years and in the SE group was more than 10 years. Survival was significantly reduced in the SE group, which consisted of patients with lower KPS. The data indicate that more than 15% of evaluable patients with GBM treated with ANP in phase 2 studies were long-term survivors. The results are significantly worse in a group of patients with lower KPS, but compare favorably with radiation therapy and chemotherapy.

69. RESPONSES OF THE ADULT MAMMALIAN CENTRAL NERVOUS SYSTEM TO EXPERIMENTAL INTRACRANIAL GLIOMA

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In the brain, tumor cells and normal cells are the interacting elements of a two-part system connected by competition for physical space, extracellular matrix components, and secreted soluble factors. Our goal is to study how normal brain cells respond to, and feed back onto, tumor cells. In the present experiments, U251 human glioma cells were injected into cortices of adult nude mice to induce experimental glioma. Brains were examined over time when tumors were 0.5 to 4 mm in diameter and occupying an increasing portion of the frontal area. Markers of cell type and status are examined by immunofluorescence in horizontal sections. Nestin immunoreactivity (ir) was detected in tumor and host cells and separated using species-specific antibodies (Chemicon; MAB3262, MAB3264, MAB3267) for mouse, MAB3262 for rat, and MAB3264 for the ipsilateral side, nestin ir was observed in host cells along both lateral and medial ventricular walls, in cells positioned between the tumor mass and the pia, in cells dispersed around the tumor and just inside its edge, and in the corpus callosum and adjacent tumor. Some nestin-immunoreactive (nestin-ir) cells, especially those in the parenchyma lateral to the ventricle and inside the tumor mass, had the morphology of neural progenitor cells (NPCs). Other nestin-ir cells, particularly those interposed between the
tumor and the pia, had the fibrous morphology of reactive astrocytes, and some of these cells were also GFAP-ir. The presence of proliferating cells was probed using antibody to PCNA (Chemicon MAB424). In addition to the tumour cells, PCNA-ir was observed in subpopulations of nestin-ir presumptive NPCs and in nestin-ir presumptive reactive astrocytes, as well as in unidentified non-nestin-ir cells dispersed lateral to the ventricle, in the corpus callosum, in the fimbria, and in the dentate gyrus. Our results suggest that the experimental glioma induced appearance of proliferating nestin-ir NPCs, nestin-ir reactive astrocytes, and other cells whose identity has not yet been determined. The position of the reactive astrocytes suggests that mechanical deformation may play an inductive role. NPCs near the meningeal layer or medial to the tumor may be responding to unidentified factors emerging from the tumor and/or from adjacent host cells. In neither case did it appear that the tumor environment was highly mitogenic for host cells, which suggests that host cells were not major contributors to the tumor burden.

70. TARGETED THERAPY WITH ANP IN CHILDREN LESS THAN 4 YEARS OLD WITH INOPERABLE BRAIN STEM GLIOMAS
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The purpose of the study is to evaluate the outcome of patients less than 4 years old with intrinsic diffuse brain stem gliomas (BSG) treated with ANP (antineoplastons A10 and AS2-1) in two FDA and Institutional Review Board monitored phase 2 trials. A total of 10 assessable patients who were less than 4 years old were among 25 participants of phase 2 trials (study and special exception patients): 2 with anaplastic astrocytomas, 1 with pilocytic astrocytoma, and 7 who had no biopsy because of a dangerous tumor location. Age ranged from 3 months to 3 years. Three patients failed prior radiation and chemotherapy, 1 had stable disease after radiation, 2 had tumor resection, and 4 were not treated prior to ANP. ANP was given intravenously daily through a subclavian venous catheter and a double channel infusion pump. The median duration of ANP administration was 19 months, and the average dosage of A10 was 12.0 g/kg/day and of AS2-1 was 0.41 g/kg/day. Responses were assessed by gadolinium-enhanced MRIs and confirmed by PET scans in some cases. The overall survival at 2 years was 30% and at 5 years 20%, and the maximum survival is 6.6 years. Median progression-free survival was 2 years and 2 months. Complete response was achieved in 30%, stable disease in 40%, and progressive disease in 30%. Serious toxicities included reversible anemia and hypokalemia. There were no chronic toxicities. ANP targets the AKT2, RAS, p53, and p21 pathways, and its administration results in substantial survival and response rates in a small group of young children who do not have a favorable prognosis for standard therapy.

71. INHIBITION OF INSULIN-LIKE GROWTH FACTOR I RECEPTOR SIGNALING INCREASES CHEMOSENSITIVITY OF PEDIATRIC CNS ATYPICAL TERATOID/RHABDOID TUMOR CELLS
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Central nervous system (CNS) atypical teratoid/rhabdoid tumors (ATT/Rt) are among the pediatric malignant tumors with the worst prognosis and fatal outcome. To date there are no explanations for their remarkable resistance to cytostatic drugs and radiotherapy. Insulin-like growth factor I receptor (IGF-IR) is upregulated in ATT/Rt and thus is a potential target for therapy. IGF-IR expression in various pediatric malignancies is associated with higher malignancy and poor survival, and has also been reported in several ATT/Rt cell lines. The presence of IGF-IR could make these cell lines sensitive to IGF-IR antagonists. For this purpose, we have established an in vitro model for the investigation of the antitumor properties of IGF-IR antagonists. We show in this work that the IGF-IR antagonists, which have been shown to inhibit tumor cell proliferation, also induce apoptosis in ATT/Rt cells. Our findings suggest that IGF-IR antagonists have therapeutic potential for the treatment of ATT/Rt.

72. HYPOXIA SENSITIZES MALIGNANT HUMAN GLIOMA CELLS TOWARD CD95L-INDUCED CELL DEATH
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Death ligands such as CD95 ligand (CD95L) have limited activity against glioma cells under normoxic conditions (Weller et al., J. Clin. Invest. 94, 954, 1994). However, many glioma cell lines can be sensitized toward death ligand–induced apoptosis by inhibition of epidermal growth factor receptor (EGFR) (Steinbach et al., Brain Pathol. 12, 12, 2002). Hypoxia is a critical aspect of the microenvironment of gliomas. We have established a paradigm for the investigation of hypoxia-induced cell death in glioma cells in vitro, which faithfully reproduces many aspects of human glioma pathology (Steinbach et al., Cell Death Differ. 10, 823, 2003). Here, we investigated the effect of co-treatment with hypoxia and CD95L in three human malignant glioma cell lines with different susceptibility to CD95L under normoxic conditions. Hypoxia sensitized all three cell lines toward CD95L–induced cell death. Co-exposure resulted in apoptotic changes in the early phase, with gradual conversion to secondary necrosis with increasing length of hypoxia. The mitochondrial injury induced by hypoxia was enhanced by co-treatment, and caspase cleavage became prominent. Inhibition of the EGFR, while sensitizing glioma cells to CD95L under normoxia, protects glioma cells from hypoxia by reducing energy consumption (Steinbach et al., Cancer Res. 64, 1575, 2004). However, the opposing effects of EGFR signaling on death induced by CD95L or hypoxia were neutralized by exposure to hypoxia and CD95L. Further, inhibition of protein synthesis by cycloheximide also reduced glucose consumption and conferred protection from hypoxia, but did not modulate CD95L–induced cell death under hypoxic conditions. These results suggest that death ligands may be useful to target but not to sensitize tumor cells resistant to conventional therapies or to complement strategies aiming at the induction of tumor hypoxia.

73. CONSTITUTIVE INTEGRIN ACTIVATION BY RAP GT PASE ON LEUKEMIC CELLS PROMOTES PROGRESSION OF LEPTOMENINGEAL LEUKEMIA
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Leptomeningeal metastases are a serious neurological complication in cancer patients and are associated with a dismal prognosis. Tumor cells that enter the subarachnoid space adhere to the leptomeninges and form tumor deposits. The role of integrins in tumor cell adhesion to leptomeninges is largely unknown. We studied the role of integrin expression and activation in the progression of leptomeningeal metastases. Therefore, we used a suspension (L1210-S) and an adherent (L1210-A) variant of a mouse acute lymphocytic leukemia cell line. Static adhesion levels of L1210-A cells on a 3-integrin ligand (vitronectin). Expression levels of these integrins and their activation state on the two cell lines is due to a difference in the activation of the small GTPase Rap, which is involved in integrin inside-out signaling. Rap activation on leukemic cells promotes leptomeningeal leukemia progression by increased adhesion to the leptomeninges via an aberrantly regulated Rap activation on death induced by CD95L or hypoxia were neutralized by exposure to hypoxia and CD95L. Further, inhibition of protein synthesis by cycloheximide also reduced glucose consumption and conferred protection from hypoxia, but did not modulate CD95L–induced cell death under hypoxic conditions. These results suggest that death ligands may be useful to target but not to sensitize tumor cells resistant to conventional therapies or to complement strategies aiming at the induction of tumor hypoxia.

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74. INHIBITION OF THE MAMMALLIAN TARGET OF RAPAMYCIN SENSITIZES GLIOMA CELLS TO ANTICANCER DRUGS
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The phosphatase and tensin homolog deleted from chromosome 10 (PTEN) functions as a tumor suppressor by negatively regulating the growth/survival signals of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. The PI3K/Akt pathway in PTEN-deficient tumors may be one of the key targets for anticancer therapy. Nevertheless, how PI3K/Akt pathway contributes to clinical drug resistance is unclear. The mammalian target of rapamycin (mTOR) is a serine-threonine kinase that functions downstream from Akt in PI3K/Akt/mTOR signaling pathway commonly activated in glioblastoma. In this study, we examined the effects of mTOR inhibitor rapamycin on apoptosis and cytotoxicity induced by several kinds of chemotherapeutic agents. Human malignant glioma cell lines T98G, U251MG (PTEN-deficient cells), and A172 (PTEN wild-type cells) were used for this study. We examined effects of the mTOR inhibitor rapamycin on apoptosis and cytotoxicity induced by chemotherapeutic agents including antimicrotubule agent vincristine, a topoisomerase II inhibitor etoposide, and a DNA cross-linking agent etoposide. As previously reported, early passages of this line were growth restricted, but later passages escaped the antiproliferative effects of TGFβ and developed a much greater proliferative potential. Both early and late passage glioma cultures expressing constitutively active TGFβ ligand expressed lower levels of the receptor SMAD3 (SMAD2 and 3), but the escape from TGFβ growth suppression was accompanied only by further SMAD3 expression loss in later passages. This finding is consistent with reports of decreased SMAD3 expression in human brain tumors. TGFβ exerts the major effects through interactions with the microenvironment. Therefore, we modified a previously developed transgenic glioma model in which an avian retroviral receptor is expressed in a tissue-specific manner [Nestin tv-a]. We interbred the Nestin tv-a mice with SMAD3 null mice to determine the impact of SMAD3 expression on glioma formation. Nestin tv-a SMAD3−/− mice were generated at less than Mendelian ratios but were viable. We generated tumors through intracranial implantation of retroviruses expressing PDGF or NGF at nearly all mice so treated. In the Nestin tv-a SMAD3−/− mice, there was no induction of nuclear SMAD3 expression in tumors with histopathology similar to human gliomas. The loss of SMAD3 did not impact the latency of tumor development. Despite the limited number of SMAD3−/− mice examined, the loss of SMAD3 was associated with an increased tumor grade relative to SMAD3+/+ and +/− mice. No SMAD3−/− mice lack tumors, whereas other genotypes lacked tumors in a fraction of mice. Tumor generation continues, and we are characterizing the impact of targeted disruption of SMAD3 on tumor angiogenesis and invasion. In conclusion, loss of SMAD3 may contribute to the shift of TGFβ from a tumor suppressor to tumor enhancer in gliomas in both human cellular and transgenic murine glioma models. This work was also supported by NIH grants R01 NS047409 J.N.R., and by Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

75. CASPASE-8 STATUS OF EX VIVO GLIOMAS
D. Ashley, C. Riffkin, A. Muscat, and C. Hawkins; Royal Children’s Hospital, Children’s Cancer Centre, Melbourne, Australia
We have identified that a significant proportion of glioma patient samples carry mutations in the caspase-8 gene, some of which act as dominantly interfering inhibitors. Malignant gliomas have a dismal prognosis, partly because of the inability of chemotherapy and radiotherapy to induce apoptosis in the tumor cells. Death ligands, including TRAIL/Apo2L, are attractive candidate therapeutic agents for unresponsive cancers like malignant glioma, as they induce apoptosis via a pathway that is distinct from that triggered by chemotherapy and irradiation. Caspase-8 is an apoptotic protease that plays a crucial role in apoptosis mediated by death ligands. Mutations or downregulation of caspase-8 have been reported in neuroblastoma and other tumor types, prompting its designation as a tumor suppressor gene. In this study, we analyzed the status of caspase-8, and other apoptosis pathway components, in ex vivo high-grade glioma specimens. Caspase-8 protein levels were frequently low or undetectable. We also amplified and sequenced the caspase-8 mRNA expressed by the tumors. A significant proportion of the gliomas bore mutations, some of which acted as dominantly negative inhibitors in death ligand apoptosis assays. These data have significant implications for our understanding of the process of gliomagenesis and the potential use of death ligands like TRAIL for treatment of patients with malignant glioma.

76. EXPRESSION LEVELS OF THE TRANSFORMING GROWTH FACTOR-BETA MEDIATOR SMAD3 DETERMINES GLIOMA PHENOTYPE IN A GENETICALLY DEFINED HUMAN GLIOMA MODEL AND A TRANSGENIC MURINE GLIOMA MODEL
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Transforming growth factor-β (TGFβ) plays an important role in gliomas through modulation of angiogenic factors, immune invasion, and immune escape. We previously demonstrated that TGFβ potently inhibits cellular proliferation of astrocytomas associated with a G1 cell cycle arrest and induction of p15/16/18, a cyclin-dependent kinase inhibitor expression. This process is dependent on SMAD3, as TGFβ largely failed to inhibit the growth of SMAD3−/− astrocytes or to induce p15/16/18. As most glioma cell lines resist TGFβ-mediated growth inhibition, we mimicked the activation of TGFβ signaling in brain tumors by expressing constitutively active TGFβ ligand in a genetically defined glioma model that we have previously reported. Early passages of this line were growth restricted, but later passages escaped the antiproliferative effects of TGFβ and developed a much greater proliferative potential. Both early and late passage glioma cultures expressing constitutively active TGFβ ligand expressed lower levels of the receptor SMAD3 (SMAD2 and 3), but the escape from TGFβ growth suppression was accompanied only by further SMAD3 expression loss in later passages. This finding is consistent with reports of decreased SMAD3 expression in human brain tumors. TGFβ exerts the major effects through interactions with the microenvironment. Therefore, we modified a previously developed transgenic glioma model in which an avian retroviral receptor is expressed in a tissue-specific manner (Nestin tv-a). We interbred the Nestin tv-a mice with SMAD3 null mice to determine the impact of SMAD3 expression on glioma formation. Nestin tv-a SMAD3−/− mice were generated at less than Mendelian ratios but were viable. We generated tumors through intracranial implantation of retroviruses expressing PDGF or NGF at nearly all mice so treated. In the Nestin tv-a SMAD3−/− mice, there was no induction of nuclear SMAD3 expression in tumors with histopathology similar to human gliomas. The loss of SMAD3 did not impact the latency of tumor development. Despite the limited number of SMAD3−/− mice examined, the loss of SMAD3 was associated with an increased tumor grade relative to SMAD3+/+ and +/− mice. No SMAD3−/− mice lack tumors, whereas other genotypes lacked tumors in a fraction of mice. Tumor generation continues, and we are characterizing the impact of targeted disruption of SMAD3 on tumor angiogenesis and invasion. In conclusion, loss of SMAD3 may contribute to the shift of TGFβ from a tumor suppressor to tumor enhancer in gliomas in both human cellular and transgenic murine glioma models. This work was also supported by NIH grants R01 NS047409 J.N.R., and by Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

77. TRAIL IS NONTOXIC TO NORMAL HUMAN ASTROCYTOCYTES
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Tumor necrosis factor (TNF) family members such as TNF and FasL can induce apoptosis in cancer cells; however, their toxicity to normal tissues has limited their use in clinical applications. TNF-related apoptosis-inducing ligand (TRAIL) is a new member of the TNF family, and its clinical application currently is under a similar debate. We have reported that the recombinant soluble TRAIL (amino acids 114-281) can induce apoptosis in human malignant glioma cells. Here, we report that TRAIL is nontoxic to normal human astrocytes. Normal human fetal astrocytes were prepared and cultured in RPMI-1640 medium supplemented with 10% FBS. Cell death was determined by crystal violet assay, and caspase cleavage was examined on Western blots. Small interfering RNA (siRNA) was generated to target specific genes. The normal human astrocytes were treated with FasL and TRAIL, and cell death analysis showed that the astrocytes are resistant to TRAIL and FasL-induced cell death. Treatment of the astrocytes with a pharmacologic inhibitor KN93 to calcium/calmodulin-dependent protein kinase II (CaMKII) sensitized the astrocytes to FasL-induced apoptosis through caspase-8-initiated caspase cascade, as evident by the cleavage of caspase-8, caspase-3 and DNA fragmentation factor 45 (DFF45). Treatment of the astrocytes with KN93 inhibited expression of cellular Fas-associate death domain-like, IL-1b-converting enzyme-inhibitory protein (c-FLIP) and phosphoprotein enriched in diabetes (PED) in human astrocytes. Transfection of siRNAs specifically targeting c-FLIP and PED gene sensitized the astrocytes to FasL-induced apoptosis. The results indicate that CaMKII-mediated c-FLIP and PED pathway modulates FasL signaling in human astrocytes. In contrast, neither KN93 nor siRNA targeting c-FLIP and PED sensitized the human astrocytes to TRAIL-induced apoptosis. Flow cytometry analysis revealed cell surface expression of Fas, but not DR4 and DR5 in the human astrocytes. The study identifies the different modulation mechanisms between TRAIL and FasL signaling pathways in normal human astrocytes. Lack of the expression of TRAIL receptors in human astrocytes contributes to TRAIL resistance.

78. STAGE-SPECIFIC EMBRYONAL ANTIGEN ANTIGEN EXPRESSION IN Glioblastoma Cells: Possible Role in Tumor Maintenance
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Stage-specific embryonal antigens (SSAs) are a group of carbohydrate molecules that are characteristically expressed on the surface of embryonic stem cells during development, or at the undifferentiated stage in vitro. They are rapidly downregulated at the blastocyst stage and upon ES cell differentiation. While their role is unclear, this temporal regulation is very
79. NEURAL STEM/PROGENITOR CELL MIGRATION AND GLIOMA TARGETING IS STIMULATED THROUGH THE EGF-PI3K SIGNALING CASCADE
S.E. Kendall, K.S. Abozy, M. Metz, J. Njapauer, E. Garcia, and C.A. Glackin; Departments of 1Molecular Medicine and 2Hematology/HCT and Neuroscience, Beckman Research Institute, City of Hope National Medical Center, Duarte, California, USA

Recent advances in neural stem/progenitor cell (NSPC) biology offer novel strategies to specifically target invasive gliomas through chemotactic migration. We have previously demonstrated the inherent tumor-propagating ability of transduced and untransduced NSPCs (Kendall et al., manuscript submitted). We are currently utilizing an immortalized human NSPC line, F3.C1 (obtained from Seung U. Kim MD, PhD [UBC]), which targets the invasiveness and immunophenotypic properties of transduced and untransduced NSPCs to several tumor types. Novel strategies to specifically target invasive gliomas through chemotactic migration and survival for neurosurgical patients undergoing surgery for a low-grade glioma under intraoperative MRI guidance. Further study within the context of a large, prospective, population-based project is needed to confirm these findings.

81. TEMPORAL TRENDS IN INCIDENCE OF PRIMARY GERM CELL TUMORS OF THE BRAIN AND CENTRAL NERVOUS SYSTEM IN THE UNITED STATES
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The objective of this study was to describe temporal trends in incidence of all primary (nonmalignant and malignant) germ cell tumors (GCTs) of the brain and central nervous system (CNS) in the United States (US) by using a large population-based series of tumors from the Central Brain Tumor Registry of the United States (CBTRUS). Brain and other CNS tumors have increased in the United States over the last two decades, both overall and for certain histologies. The observed increase is attributable in part to changes in diagnostic tools and tumor classification and coding. In the United States, the overall incidence rate for GCT of the brain/CNS is 0.08/100,000 person-years (py) (CBTRUS, 1997–2001). The rate is highest in young adults 20 to 29 years and lowest in those 80 years and older. We are currently utilizing an immortalized human NSPC line, F3.C1 (obtained from Seung U. Kim MD, PhD [UBC]), which targets the invasiveness and immunophenotypic properties of transduced and untransduced NSPCs (Kendall et al., manuscript submitted). We are currently utilizing an immortalized human NSPC line, F3.C1 (obtained from Seung U. Kim MD, PhD [UBC]), which targets the invasiveness and immunophenotypic properties of transduced and untransduced NSPCs to several tumor types. Novel strategies to specifically target invasive gliomas through chemotactic migration and survival for neurosurgical patients undergoing surgery for a low-grade glioma under intraoperative MRI guidance. Further study within the context of a large, prospective, population-based project is needed to confirm these findings.
82. PRIOR HOSPITALIZATION FOR EPILEPSY AND SUBSEQUENT RISK OF GLIOMA AND MENINGIOMA
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We conducted a case-control study to evaluate the preclinical association between epilepsy and primary adult brain tumors. We first identified all 1,501 low-grade glioma (LGG) and 4,878 high-grade gliomas (HGG) and 4,193 meningioma cases reported to the Swedish Cancer Registry from 1987 to 1999. Next, controls (137,485) were randomly selected from the continuously updated Swedish Population Registry and matched to cases diagnosed that year on age and sex. Finally, cases and controls were linked to the Swedish Hospital Discharge Registry (1969–1999). We found that eight or more years before HGG diagnosis (or control reference year) there was an elevated risk of HGG among people discharged with epilepsy (odds ratio (OR) = 3.01, 95% confidence interval [CI], 1.73–5.22). Two to three years before HGG diagnosis this risk increased (OR = 5.33, 95% CI, 3.58–7.93) and was especially strong among people under age 55 years (OR = 13.49, 95% CI, 6.99–25.94). During this two- to three-year pre-diagnostic period we also found an increased risk of HGG among people discharged with meningitis (OR = 3.02, 95% CI, 1.06–8.59) or viral encephalitis (OR = 12.64, 95% CI, 2.24–71.24). Results are similar for glioblastoma multiforme, LGG, and meningioma. The occurrence of excess epilepsy eight or more years before HGG diagnosis suggests a relatively long preclinical phase for these tumors.

83. RECORD OF CENTRAL NERVOUS SYSTEM PRIMARY TUMORS IN FRANCE: PRELIMINARY RESULTS
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This work aims at prospectively recording all primary tumors (PT) of the central nervous system (CNS) in France for which histological diagnosis is available. The main objectives are to create a national registry and a network in order to (1) perform epidemiological studies, (2) implement a new database and use it for setting up both clinical and basic research protocols, and (3) improve the health care system. All French neuropathology and neurosurgery departments have designated a correspondent to participate to the program. A data file is compiled by the clinician and the neuropathologist for every patient. The file contains socio-demographic, clinical, radiologic, and anatomicopathologic data. The Tumor Registry from Herault based in Montpellier (south of France), which is recording data recording, has extensive expertise in working with tumor data and holds the required authorizations for recording nominal data. Over the first year, 4094 cases have been recorded from 51 national and private institutions in France, which includes 53% women and 47% men. Tumor localizations were supratentorial (80%), infratentorial (14%), and spinal (7%). Surgical operations were tumor resections (76%) and biopsies (24%). Histology revealed glioma (48.4%), other neuroepithelial tumors (3.7%), meningioma (32%), neurinoma (7.8%), lymphoma (3.1%), and others (5%). Detailed results will be presented during the congress. Our objective of recording all primary CNS tumors cases nationwide is long and difficult. During the first year program, the number of recorded cases has increased. Preliminary results are encouraging and stimulating for the long-term goal of creating a National Registry (based on histological data at a first step) and a National Network for patients affected by CNS-PT.

84. DESCRIPTIVE EPIDEMIOLOGY OF PRIMARY OLIGODENDROGLIOMAS IN THE UNITED STATES
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The objective of this study was to estimate the incidence, to describe temporal trends in incidence, and to estimate survival rates for primary oligodendrogliomas (OLGs) in the United States. CBTRUS compiled population-based data on all primary brain and central nervous system (CNS) tumors diagnosed between 1997 and 2001 from 15 state cancer registries. Overall and sex-, race-, and age-specific incidence rates of OLG were estimated. Age-adjusted rates were standardized to the Year 2000 U.S. standard population. For the analysis of time trends in incidence, CBTRUS compiled population-based data from 6 state cancer registries for tumors diagnosed from 1985 to 1999. Multiplicative Poisson regression was used to calculate the average annual percent change (AAPC [95% CI]) in incidence rates over the time period while controlling for age, sex, race, and microscopic grade, and to statistically compare trends over time. Relative survival rates for primary OLG for cases diagnosed between 1973 and 2001 in nine Surveillance, Epidemiology, and End Results (SEER) Program areas were also estimated. Tumors of interest were OLG (ICD-3 morphology code 9450/3) and anaplastic OLG (9451/3, 9460/3). Overall incidence rate of OLG was 0.37/100 000 person-years (py) (CBTRUS, 1997–2001; N = 1385). Median age at diagnosis was 41 years. Rates were higher in males than in females (0.40 vs. 0.34/100 000 py) and in whites than in blacks (0.45 vs. 0.14/100 000 py). Rates were highest in the 55- to 64-year age group (0.08/100 000 py) and highest in the 35- to 44-year age group (0.66/100 000 py). Overall incidence rate of anaplastic OLG was 0.17/100 000 py (CBTRUS, 1997–2001; N = 738). Median age at diagnosis was 48 years. Rates were higher in males than in females (0.19 vs. 0.16/100 000 py) and in whites than in blacks (0.18 vs. 0.09/100 000 py). Rate was lowest in the 0 to 19-year and 85-year and older age groups (0.02/100 000 py) and highest in the 55- to 64-year age group (0.32/100 000 py). Incidence of OLG (N = 617) and anaplastic OLG (N = 153) increased over the time period 1985–1999 (AAPC = 6.9% [5.1%–8.8%] and 22.3% [17.7%–27.2%], respectively). One-, five-, and 10-year survival rates following diagnosis of OLG were 89%, 70%, and 52%, respectively (SEER, N = 1748), and for anaplastic OLG were 77%, 42%, and 30%, respectively (SEER; N = 340). Additional rates by age, sex, and race will be presented. These and further descriptive epidemiologic studies may facilitate the identification and elucidation of risk factors for these tumors.

85. RACIAL DISPARITIES IN PATIENT OUTCOME AFTER CRANIOTOMY FOR TUMORS IN THE UNITED STATES, 1988–2000
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Racially based disparities in American health care are well documented. We studied disparities in outcomes after 40,101 craniotomies for brain tumors (primary tumors, metastases, meningiomas, and acoustic neuromas) in the United States, 1988–2000. The following analytical methods were used: multivariate proportional-odds ordinal logistic regression corrects for clustering by hospital in random-effects model, 3 ana- lysing one-time and time-strapped heterogeneity estimation. Data source was the Nationwide Inpatient Sample (HCUP, AHRQ, Rockville, Maryland). Analyses adjusted for age, sex, insurance, income in zip code of residence, geographic region, admission type and source, medical comorbidity, and hospital volume of care. In-hospital mortality and adverse discharge discharge were both more likely in black patients than in others for all tumor types. Odds ratios for mortality ranged from 1.3 (primary tumors) to 8.5 (metastases); combined mortality OR for all tumor types was 1.70 (95% confidence interval [CI], 1.4–2.1, P < 0.001, no heterogeneity). Odds ratios for adverse discharge disposition ranged from 1.4 (primary tumors, acoustic) to 1.5 (meningiomas); combined adverse discharge disposition OR for all tumor types was 1.41 (95% CI, 1.3–1.6, P < 0.001, no heterogeneity). Adjusted for primary insurance and income in zip code of residence, black patients were more likely to present as emergency cases: OR, 1.7 (primary tumors) to 4.6 (acoustic neuromas); combined OR, 2.1, significant heterogeneity detected. Medical comorbidity was more severe in black patients: OR 1.3 (metastases) to 2.1 (acoustic neuromas); combined OR, 1.6, significant heterogeneity detected. Black patients were significantly more likely to suffer adverse outcomes after tumor craniotomy in adjusted analyses. Black patients were also more likely to present as emergency admissions and to have significant medical comorbidities than patients of other races, with the largest disparities observed for benign tumors.
86. PREVALENCE AND PROGNOSTIC SIGNIFICANCE OF POLYMORPHISMS AT THE GLUTATHIONE S-TRANSFERASE TOMAS.

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The glutathione S-transferase (GST) family of genes encodes proteins that metabolize and inactivate a wide variety of carcinogenic and anticancer agents and thus are potential determinants of individual risk to cancer caused by these agents and of the outcome of chemotherapy. This study examined the prevalence of polymorphisms at the loci of four GST genes, namely, GSTM1, GSTM3, GSTP1, and GSTT1, as a function of age, gender, and histological grade in astrocytomas patients and the relationship of these genetic polymorphisms with patient survival. Genomic DNA from peripheral blood of 334 patients with grades 1–IV astrocytomas was used to analyze the four GST genes for deletions and/or polymorphisms. Diagnostic and GST genotype were stratified by histological grade and correlated with patient survival/death. Of the 334 patients, 62% were male, and 38% female. Two thirds had glioblastoma multiforme, 16% anaplastic astrocytoma, less than 10% low-grade astrocytoma. Patients of age >50 years had a 2-fold higher rate of glioblastoma compared to those under 50 years, while higher grade tumors were more prevalent in the >50-year group. GSTM1 null and positive genotypes were present in equal proportions. Among the GSTM1+ve patients, the GSTM1A allele was 4-fold more frequent than the M3B allele. The GSTP1A allele (Ile104/Va113) was present at 66%, GSTP1B (Ile104/Ala114) at 29%, and GSTP1C (Val104/Val113) at 9%. Despite its low frequency, the GSTP1C allele was present at 66%, GSTP1B (Ile104/Ala114) at 29%, and GSTP1C (Val104/Val113) at 9%. Despite its low frequency, the GSTP1C allele was present at 9%. GSTP1B (Ile104/Ala114) was associated with decreased survival, while GSTP1C (Val104/Val113) was associated with early death. We conclude that significant differences exist in the prevalence of GST alleles and genotypes in different histological grades of astrocytomas and that the GSTP1C and GSTT1 null genotypes are associated with decreased survival.

87. IS ANDROPAUSE PROTECTIVE IN THE DEVELOPMENT OF MENINGIOMAS IN MEN?

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Clinical, epidemiological, and hormonal receptor data imply a link between meningiomas and ovarian hormones. The fact that a third of meningiomas occur in men and active androgen receptors are found in nearly 70% of tumors arising in males suggests that there may be additional hormonal influences. We indirectly investigated the protective effect of andropause on the age-specific incidence of meningiomas arising within the central hemispheres and spine by evaluating the relationship between age at diagnosis and meningioma. Population-based incidence data from the Central Brain Tumor Registry of the United States (CBTRUS) was obtained, and linear regression methods were applied. The rate of increase in incidence rates accelerated in males after age fifty-five. Age-specific linear regressions found that the slope of the line under age fifty-five was 2.5 and for those over age fifty-five was 3.8. Considering tumor location, there was no exponential increase in spinal meningioma rates until age 50 where, up to age 75, the slope was 5.2. The risk of male meningioma also accelerates after age fifty in the cerebral hemispheres. These male increases, rather than a female decrease, account for the declining female/male ratios in patients over 55 years of age. These results suggest the existence of multiple genetic/ectopic pathways leading to the development of meningiomas. In addition, the etiology of meningiomas may be contingent upon gender and anatomical location.

88. THE ROLE OF ENVIRONMENTAL AND HORMONAL FACTORS IN THE ETIOLOGY OF RADIATION-ASSOCIATED AND SPORADIC MENINGIOMAS

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Current knowledge about the etiology of meningiomas is sparse, with ionizing radiation being the only well-established risk factor. The aim of this study was to comprehensively assess the contribution of environmental and hormonal risk factors in radiation-associated meningiomas (RAM) and sporadic meningiomas. Between 1948 and 1960 about 20,000 children in Israel were treated with ionizing radiation to the scalp for tinea capitis (TC). An additional unknown number of children were treated abroad. This research was designed as a nested 4+4 case-control study balanced for estimation of the impact of the expected risk factor as well as its interaction with radiation. The total study population included 526 subjects: 161 RAM cases that were irradiated for TC in childhood and subsequently developed meningioma, 85 sporadic cases, 145 irradiated controls, and 135 nonirradiated controls. The latter 3 groups were individually matched to the RAM cases by gender, year of birth, and continent of origin. Data on smoking, alcohol consumption, history of head trauma and asthma, parity, age at menarche and menopause, and use of exogenous hormones was collected by using a personal interview. In a multivariate analysis of risk factors in men, smoking was associated with a significant increased risk for meningioma (OR = 2.50; 95% CI, 1.30–4.77), while alcohol consumption decreased the risk (OR = 0.59; 95% CI, 0.02–0.87). For both of these factors a dose-response association was found. In women, a significant interaction between active smoking and radiation was observed. In the subgroup of nonirradiated women, smoking was associated with a decreased risk for meningioma (OR = 0.34; 95% CI, 0.13–0.88). The risk decreased with increasing smoking pack-years (P for trend = 0.003). This protective effect was not seen among irradiated women. In the analysis of hormonal factors, we observed a protective effect for hormones that reflect a reduced exposure to endogenous and exogenous feminine hormones. This study design enabled the assessment of risk factors (other than radiation) for meningiomas and the evaluation of interactions between radiation and other determinants in the development of this tumor. The dose-response effect observed for both smoking and alcohol in men supports the causal interpretation of these results. The protective effect of smoking in nonirradiated women may be explained by the antiestrogenic effect of smoking. The negative association found between exposure to exogenous hormonestransferases and meningiomas is biologically plausible, considering the higher incidence of this tumor in women and the existence of steroid hormone receptors in the tumor tissue. As more mechanistic information on susceptibility and etiology of the disease become available, risk assessment and possible primary and secondary prevention could be considerably improved.

89. POLYMORPHISMS ASSOCIATED WITH ASTHMA ARE INVERSELY RELATED TO GLOIOBLASTOMA MULTIFORME RISK

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A reduced risk of primary adult brain tumors is observed among people reporting asthma, hayfever, and other allergic conditions; however, findings may be attributable to prediagnostic effects of tumors or recall bias. To determine whether asthma and allergic condition polymorphisms are inversely related to glioblastoma multiforme (GBM) risk, we conducted a population-based case-control study of 111 GBM patients and 422 controls. We identified five single nucleotide polymorphisms (SNPs) on three genes previously associated with asthma (interleukin [IL] 4R, IL13, ADAM33) and one gene associated with inflammation (COX2). Confirming previous literature, we found that self-reported asthma, hayfever, and eczema reduce GBM risk (e.g., asthma and hayfever at time of interview, odds ratio [OR] = 0.38, 95% confidence interval [CI], 0.18–0.83). In addition, IL4RA -4739 pro/T, CC, and IL4RA g3551 arg/AG, AA are associated with increased GBM risk (OR = 1.64, 95% CI, 1.05–2.55, and 1.61; 95% CI, 1.05–2.47) while IL13 -1112 CT, TT is associated with decreased GBM risk (0.56, 95% CI, 0.33–0.96). Each of these polymorphisms-GBM association was in the opposite direction of a corresponding polymorphism-asthma association, thereby confirming previous findings that asthmatics and people with allergic conditions have lower GBM risk than do people without these conditions. Because we used germline polymorphisms as biomarkers.
for asthma and allergic conditions, our results cannot be attributed to recall bias or effects of GBM on the immune system. Our findings have implications for asthma or allergy treatments that inhibit IL13 production.

90. FEASIBILITY OF WEB-BASED VERSUS TELEPHONE INTERVIEWS AS A DATA COLLECTION METHOD IN EPIDEMIOLOGY STUDIES OF BRAIN TUMOR PATIENTS

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As part of a pilot study conducted for a large case-control study of neurocarcinogens, gene polymorphisms, and adult brain cancer, we have developed a Web-based data collection instrument intended to allow patients to participate at their own pace in a comfortable environment, while minimizing study data collection effort. Among 223 respondents who completed the Web-based survey and 67 respondents who completed a telephone survey, we examined characteristics associated with the choice of survey mode. We also examined the reliability of responses for 140 respondents who completed both the Web-based survey and a short Web-based resurvey, as well as the reliability of responses for 47 respondents who completed both a phone survey and phone resurvey. Compared to telephone survey participants, Web-based survey participants were less likely to be divorced or widowed (6% vs. 21%, respectively), more likely to have a family income above $75,000 (40% vs. 23%), and less likely to have never searched the Internet (4% vs. 24%). Individuals completing the Web-based survey, however, were also slightly more likely to perceive the interview as difficult to complete (5% vs. 1%). The reliability study (Kappa or Spearman rank correlations) for responses to 5 questions on history of smoking and oral contraceptive use ranged from 0.84 to 0.97 for Web responses and 0.56 to 0.92 for phone responses. Responses to questions intended for use in constructing variates were expected to be more reliable. Data collected on living arrangements (heating sources, drinking water sources, dwelling type, living in a farm, daycare as a child), methods of food preparation, intake of fresh fruits and vegetables, and use of indoor pesticides. Reliability for the 23 exposure variables examined ranged from 0.17 to 0.78 (median, 0.54) for Web responses and from 0.02 to 1.0 (median, 0.61) for phone responses. Phone respondents answered a median of 8 of 35 questions with concordance at both surveys, whereas Web respondents answered a median of 23 of 35 questions with concordance at both surveys. Results will be presented by case and control status and controlled by age. Preliminary results suggest that a Web-based survey format is feasible in some research settings and that it has some advantages over traditional formats. However, to obtain information on all demographic subgroups and to minimize biases in studies, more than one mode of data collection may be optimal.

91. AGE-RELATED PATTERNS IN CNS TUMOR INCIDENCE WITH A FOCUS ON THE ADOLESCENT AND YOUNG ADULT POPULATION

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Brain tumor incidence data were analyzed from two large population-based databases, the Surveillance, Epidemiology, and End Results Program (SEER) and the Central Brain Tumor Registry of the United States (CBTRUS), to determine the incidence of brain tumors according to histological subtype and age at diagnosis. The focus of the study was to analyze incidences of glioma and PNET among young adult (15-29 year) patients with brain tumor and to look for any patterns of incidence that might explain mechanisms of tumorigenesis. Incidence of CNS tumors during 1975 to 1998 in SEER registries was determined for each 5-year age group from 0 to 44 years. SEER was divided into broad histological categories: astrocytomas (grade I–IV), other gliomas (oligoastrocytoma and oligodendroglioma), PNET, ependymoma and germ cell tumors. Additional incidence data were obtained from the published 1985–1999 statistical report of the CBTRUS, to supplement the SEER data, and to provide age-related incidence data for specific histological subtypes, including meningioma, craniopharyngioma, mixed glioma, and each grade of astrocytoma and oligodendroglioma. Four patterns of tumor incidence were recognized from the SEER and CBTRUS databases: (1) peak incidence in the 15-19 year age group (oligoastrocytoma), (2) falling incidence with aging (grade I astrocytoma and PNET), (3) rising incidence with aging (grade II–IV astrocytoma, oligodendroglioma, oligoastrocytoma and meningioma), and (4) stable incidence with aging (craniopharyngioma and ependymoma). The patterns of age-related incidence of CNS tumors suggests that their development is driven, in part, by factors linked to the completion of the brain’s growth and development, by hormones key to sexual maturation, and by factors that influence adult aging. The peak incidence of germ cell tumors in the AYA years justifies them being taken as the model tumor for AYA neuro-oncology. Their rarity justifies a worldwide clinical trial strategy building upon evidence of rising survival rates (germinoma >90%, nongerminomatous germ cell tumor 50–80 year). Quality of survival is widely recognized as a significant outcome, although it has not yet been selected as a primary outcome measure. We propose a worldwide, combined age approach to the investigation of CNS germ cell tumors aimed at optimizing quality of survival.

92. EPIDEMIOLOGICAL AND CYTOGENETICAL INVESTIGATIONS IN THE GROUP OF THE CHERNOBYL CLEAN-UP WORKERS WITH THE DISEASES OF NERVOUS SYSTEM

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The increase of the rate of malignant pathologies in Ukraine is significantly connected with the rise of the carcinogenic loading on the population. Special attention to the carcinogenic effects of radiation was given after the accident in Chernobyl. The purpose of the present investigation was to study the frequency of the diseases of nervous system (cancer and noncancer pathologies), peculiarities of cytogenetical effects in blood lymphocytes of clean-up workers of the Chernobyl catastrophe, and their dependence on the dose of radiation exposure. Epidemiological studies (more than 20,000 clean-up workers with documented doses of exposure) and metaphase analyses of radiation-induced chromosome aberrations in peripheral blood lymphocytes (cytogenetical investigation of 2000 clean-up workers with documented doses of irradiation) were conducted. It was established that diseases of nervous system took the first place in the structure of the development of diseases of the clean-up workers of the Chernobyl catastrophe during all years of our investigation (1990–2002). Statistically significant tendency of the increase of the diseases of nervous system ( unstochastic effects) with the dose of irradiation (1.85 cGy) was observed irrespectively of the age of clean-up workers. As for neuro-oncological pathologies (stochastic effects), their highest frequency was registered in the group of the clean-up workers irradiated in a range of low doses (1–5 cGy). Dose dependence of the frequency of radiation markers (dicentric chromosomes) in the lymphocytes of peripheral blood was observed only in a group of clean-up workers with neuro-oncological pathologies, when the lack of anticancer protection of the organism was caused by the radiation injury in low doses. Cytogenetical criteria for the formation of the groups with the increased neuro-oncological risk and the improvement of the monitoring of the health status of clean-up workers were determined. Low doses of ionizing radiation are statistically significant factors of cancer risk, including development of neuro-oncological pathologies.

93. THE RISK FOR MALIGNANT PRIMARY ADULT-ONSET GLIOMA IN A LARGE, MULTI-ETHNIC, MANAGED CARE COHORT: CIGARETTE SMOKING AND OTHER LIFESTYLE BEHAVIORS

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The purpose of this study was to determine the risk for malignant primary adult-onset glioma in a large, multi-ethnic, managed-care cohort. The study population included a cohort of 133,811 subscribers to the Kaiser Permanente Medical Care Program of Northern California (KPMPC-NC) who had received a multiphasic health checkup and questionnaire (MHC) between 1977 and 1985, were at least 25 years old at their start of follow-up, and had no prior history of benign or malignant brain tumors. In this cohort, patients were followed for up to 21 years for the development of MPA. Risk for MPAG among women increased with increasing packs of cigarettes smoked per day (P trend = 0.04), adjusting for cigar and pipe smoking, patient age, sex, race, education, alcohol use, and coffee consumption. A similar pattern was not observed for men. Individuals who smoked marijuana at least once a month, adjusting for cigarette smoking (packs smoked per day) and for the factors noted above, had a 2.8 (CI, 1.3–6.2)-fold increased risk for MPAG. Relative risk for MPAG increased with increasing consumption of coffee (P for trend = 0.05). Cigarette smoking was associated with an increased risk for MPAG among women but...
94. MATERNAL DIET DURING PREGNANCY AND ITS ASSOCIATION WITH MEDULLOBLASTOMA
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not among men. Individuals who smoked marijuana at least once a month had an increased risk for MPAG, although no dose-response relation was observed. Drinkers of >7 cups of coffee per day had a 70% increased risk for MPAG, with an even higher risk elevation for power consumption. Alcohol use was not associated with an increased risk for MPAG.

95. NEURAL AND GLIAL DIFFERENTIATION OF ASTROCYTES FROM EMBRYONIC STEM CELLS IN VITRO
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The pluriplombency of embryonic stem (ES) cells is determined by the ability to differentiate into derivatives of the three germ layers. We have determined an optimal protocol for the in vitro synthesis of astrocytes from murine ES cells harboring wild-type (wt) and p53 (±) genetic backgrounds and undertaken expression and tumorigenicity studies. Astrocytic differentiation of the ES cells, with GFAP positive and negative OLG2, RESTIN plus OCT2 expression, was found in more than 90% of cells. RT-PCR for these and other neural-glial lineage markers further supported that these were synthesized derived astrocytes from the ES cells. The majority of the synthetic astrocytes from wt-ES cells displayed morphology similar to Type-1 astrocytes. cDNA microarray analyses on Affymetrix arrays were performed. After normalizing and scaling of the data, the transcriptomes of synthetic astrocytes with wt or p53 (±) genetic backgrounds were found to be very similar (r = 0.89). However, several genes were differentially expressed (P < 0.001), most likely representing p53 response genes. For some of these differentially expressed genes, verification was obtained by real-time PCR. The transcriptomes of the synthetic astrocytes were compared to the published transcriptomes of in vivo astrocyte cultures established from various murine embryonic, postnatal, and adult brain sections (P < 0.001). In contrast to the extent of similarity coefficient (r, P < 0.001) with possible astrocyte specific genes (~325 transcripts) identified by having an expression pattern similar to GFAP, using a reverse-supervised machine (R-SVM) class prediction analysis. We found most synthetic astrocytes were four times more similar to astrocytes from the adult cortex (r = 0.281), while synthetic p53 (±) astrocytes were most similar to astrocytes from the cortex (P2) (r = 0.212). By comparison, using 156 astrocyte-specific markers identified by R-SVM analysis, both wt- and p53 (±) synthetic astrocytes were four times more similar to astrocytes from the cortex (P2) (r = 0.287, r = 0.315). Finally, both wt- and p53 (±) synthetic astrocytes were tested for in vivo intracranial transformation in Nod-Scid mice. Intracranial injections into these mice with wt or p53 (±) ES cells resulted in tumors within 3 to 5 weeks. No tumors developed with wt or p53 (±) synthetic astrocytes. Study is ongoing with synthetic derived astrocytes from ES cells, of varying glioma relevant genetic backgrounds, which we believe provide a relatively quick and easy way to understand the roles of these genetic alterations in glial differentiation and transformation.

96. ORGANO TYPIC SPHEROIDS OF GLO B L O S TA M OA: AN IN VITRO HIGH-THROUGHPUT DRUG SCREENING SYSTEM
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The plethora of newly developed potential therapeutics for glioblastoma requires a test model that adequately quantifies response and is biologically valid. Historically, monolayer cultures and commercially available cell lines are used for this purpose. However, there is marked discrepancy in responses of these widely used test systems and human tumor responses, which illustrates the biological invalidity of these cell-suspension test models. This is partially explained by clonal selection and loss of intercellular cross-talk. To circumvent these problems, surgical specimens are cultured in medium as explants on the short term and grown as organotypic spheroids as previously described by Bjerkvig et al (J. Neurosurg. 72, 463, 1990). Limitations for use in drug experiments were based on (1) lack of quantification of response using only qualitative morphological assessment, (2) heterogeneity of spheroids with undetermined implications for design of experiments, and (3) only hypothesized superior biological validity as compared with widely used and easily quantitated cell-suspension models. First, therefore, a semiautomated method has been developed that facilitates quantification of viability, proliferation, and apoptosis, and this appears to be a valid quantitative approach to determine response. Images of enzyme-histochemically and immunohistochemically stained cryostat sections of spheroids are digitally captured and analyzed using automated image cytometry. Second, the natural variance of mass to spheroids the number of spheroids to be used in experiments. This is shown to be within ranges that allow for high-throughput screening. Third, to demonstrate the biological validity of the spheroid test model, DNA microarrays experiments are performed to compare the genetic profile of the original tumor with both monolayer culture and organotypic spheroids from the same material. It is hypothesized that the organotypic spheroids show good correlation with the original tumor, in contrast to the monolayer culture. In conclusion, an organotypic spheroid test system in malignant glioma has been developed that rapidly quantifies response, allows for high-throughput screening, and appears to be superior to cell-suspension models in genetic profile as compared with the original tumor.

97. MOLECULAR PROFILING AND CHARACTERIZATION OF SYNTHETIC ASTROCYTES, DIFFERENTIATED FROM EMBRYONIC STEM CELLS IN VITRO
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Dimensionality plays a major role in cellular behavior, and in vitro tumor invasion studies have been hampered by the lack of suitable 3D models of tumor/host brain interaction. Allowing enzyme dissociated cells to reform into 3D spheroids has provided the means of developing such a model. Spheroid cultures of enzymatically dissociated fetal rat cerebellum, brainstem, and cerebral cortex (E18) were morphologically and immunohistochemically characterized at 3, 5, and 10 days in vitro. Comparisons between 3D spheroids, 2D monolayer, and undissociated brain tissue demonstrated that spheroid cultures begin to differentiate and express neurofilament proteins and glial fibrillary associated proteins (GFAPs) in addition to forming synaptic contacts and extracellular collagen. Neural cells appear to differentiate at a higher rate than glial. The developmental profile of neural and glial in the 3D regional brain spheroids mimics the in vivo cytoarchitecture to a greater extent than 2D brain cell cultures. The level of GFAP immunoreactivity (IR) is significantly greater than neurofilament (NF) immunoreactivity (IR) in all monolayer cultures (50%), compared to 8–10% in vitro. Levels do not significantly change over time in vitro. NF IR reaches 97% and GFAP 65%. Adult rat brain tissue has a significantly greater IR to NF (88%) than GFAP (51%). Therefore, the ratio of NF:GFAP IR in regional brain spheroid approaches that found in adult rat brain. The 3D spheroid microenvironment results in cellular selection that more closely reflects adult tissue cellular composition. By using a 3D spheroid co-culture model of host brain/Daoy cells (medulloblastoma cell line), it was found that the Daoy cells preferentially invaded cerebellum and brainstem, and not the cerebral cortex, possibly reflecting differences in cellular microenvironments. Therefore, this 3D model appears to mimic in situ medulloblastoma behavior. The viability and reproducibility of these regional brain spheroid cultures make them a useful tool for further inves...
99. HEMATOPOIETIC STEM CELLS HOME TO MALIGNANT GLIOMAS

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Stem and progenitor cells (PCs) of various lineages have become attractive vehicles to improve therapeutic gene delivery to cancers, notably glioblastoma. Here we report that adult human and murine hematopoietic PCs, and display a tropism for intracerebral gliomas but not for normal brain tissue in mice. Organotypic hippocampal slice culture and spheroid confrontation assays confirm a directed PC migration toward glioma cells ex vivo and in vitro. RNA interference-mediated disruption of transforming growth factor beta (TGF-b) synthesis by the glioma cells strongly inhibits PC migration. We delineate a CXC chemokine ligand (CXCL1) 12-dependent pathway of TGF-b-induced PC migration that is facilitated by MMP-9-mediated cleavage of the cell surface factor (SCF). Moreover, neutralizing antibodies to CXCL12 strongly reduce PC homing to experimental gliomas in vivo. Thus, we define here the molecular mechanism underlying the glioma tropism of the probably most easily accessible PC population suitable for cancer gene therapy, that is, adult hematopoietic PC. This work was supported by the Landesstiftung Baden-Wuerttemberg, State of Baden-Wuerttemberg, Germany (P-LS-AS/HSPA7-12).

100. ACTIVATION OF PROTEOLYTIC PATHWAYS BY POLYPHENOLIC PHYTOCHEMICALS FOR APOPTOSIS IN HUMAN GliOBlastOMA T9G CELLS

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Glioblastoma, the deadliest and most prevalent human brain tumor, still remains refractory to almost all conventional chemotherapeutic agents. Therefore, alternative and innovative therapeutic strategies need to be developed for treating this deadly disease in humans. Extensive research in the last few years has revealed that regular consumption of certain fruits and vegetables can reduce the risk of acquiring specific cancers. Flavonoids such as apigenin (APG), (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), and genistein (GST) are polyphenolic phytochemicals, which ubiquitously occur in fruits and vegetables. Flavonoids have been shown to suppress proliferation of various cancer cells, inhibit growth factor signaling pathways, suppress expression of antiapoptotic proteins, and also induce apoptosis, indicating that they may have untapped therapeutical value. Very recently, these phytochemicals have also been found to reverse both radioresistance and chemoresistance in patients undergoing glioblastoma treatment. In the current investigation, we have examined the molecular events relating to mitochondria-mediated apoptosis in human glioblastoma T9G cells following exposure to APG, EGC, EGCG, and GST. We used trypsin blue dye exclusion test for assessing cell viability, Wright staining for determining distinct morphological characteristics of apoptosis, modified version of the telomerase repeat amplification protocol (TRAP) assay for detecting telomerase activity, fura-2 assay for quantifying intracellular free [Ca2+]i, Western blotting for examination of expression of specific proteins, and also a colorimetric assay for estimating caspase-3 activity in 9T G cells following exposure to the flavonoids. Trypan blue dye exclusion test showed that the number of viable T9 G cells was decreased in a dose-dependent manner when T9 G cells were exposed to these phytochemicals. Wright staining indicated predominantly apoptotic morphology in T9G cells exposed to 100 mM APG, 50 mM EGC, 50 mM EGCG, or 100 mM GST for 24 h. We applied a modified version of the TRAP assay to phytochemical treated T9G cells to determine any downregulation in the activity of telomerase, an enzyme responsible for lending an unlimited capability of proliferation to the cancers including glioblastoma. Apoptosis in T9G cells following exposure to these phytochemicals was associated with an increase in intracellular free [Ca2+]i, as determined by fura-2 assay. Western blot analyses showed an increase in Bax:Bcl-2 ratio, as well as overexpression of calpain and caspase-3 that were also activated to cleave 20 kDa-α-spectrin and 22 kDa specific sites for generation of breakdown product (SBDP) and 120 kDa SBDP, respectively. Further, colorimetric assay using a synthetic substrate specific for caspase-3 confirmed activation of caspase-3 in apoptotic T9G cells following exposure to 100 mM APG,

50 mM EGC, 50 mM EGCG, or 100 mM GST for 24 h. Taken together, these results strongly suggest that selective polyphenolic phytochemicals can be used for activation of proteolytic activities of calpain and caspase-3 for apoptosis in human glioblastoma T9G cells. This investigation was supported in part by the R01 grants from the NCI and NINDS of the NIH and also a grant from the State of South Carolina.

101. COMBINATION OF HISTONE DEACETYLASE INHIBITORS AND RADIATION OFFERS A PROMISING THERAPY FOR NEUROBLASTOMA

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Neuroblastoma, a malignancy arising from neural crest cells, exhibits resistance to current therapies. HDAC inhibitors have emerged as exciting new cancer therapies and have demonstrated antineoplastic effects against neuroblastoma. Pivaloyloxymethyl butyrate (AN-9, Pivanex) was derived from the HDACI butyric acid (BA) and shows more efficient delivery and higher intracellular concentrations in comparison with BA. We sought to test the combination of the AN-9 and radiation in the treatment of neuroblastoma in vitro. The neuroblastoma cell line SHEP21, constructed to contain inducible MYCN, was treated with different doses of radiation and AN-9. Effects on cell viability and apoptosis were assessed. This work was supported by a grant from the State of South Carolina.

In order to study brain tumor invasion in vitro, previous three-dimensional model systems have utilized pre-cultured chick heart fragment and brain tissues as targets in co-culture with human brain tumor. We have now succeeded in establishing a novel three-dimensional model system of human neuroblastoma. In this model, non-neoplastic human brain tissue was fixed with formalin, cut into cubes, and embedded in a collagen matrix. The spheroids are juxtaposed with spheroids derived from human neuroblastoma. Spheroids are derived from different established glioma-derived cell lines or from primary cultures of glioma biopsy as well as brain resected from patients who have undergone surgery for epilepsy, short-term astrocyte-rich cell cultures derived therefrom. Using the “hanging drop” method, 45,000 cells were inversely suspended from a petri dish in 20 ml of DMEM. After 24 h (fast-growing cells) or 48 h (slower growing cells) in their gravity pools, spheroids are transferred to agar-coated petri dishes for a further 24 h prior to confrontation. Cells were tracked with the use of fluorescent cell trackers; progress was recorded every 3 days for up to 15 days. Fluorescent cell trackers were also employed for time-lapse video microscopy in the most invasive combinations over 5 days. We are further developing the model in utilizing human serum instead of fetal calf serum, which has been shown to alter antiangiogenic properties and expression of human glioma cells in two-dimensional culture. The effects of various agents and combinations thereof were tested in high and low M cyclin-expressing cells by using MTS assays and FACS analyses. The effect of AN-9 on the M cyclin expression was examined by Western blot. We also addressed the influence of the order of administration of AN-9 and radiation on antineoplastic activity. Consistent with published literature, AN-9 inhibited M cyclin expression. Combinations of AN-9 and radiation were more effective than either treatment alone. The combined effect was additive in MTS cell viability assays. FACS analyses demonstrated that high-M cyclin-expressing cells were markedly more susceptible to apoptosis than their low-M cyclin-expressing counterparts. The order of administration significantly influenced antineoplastic efficacy, as exposure to AN-9 after radiation was more effective than the reverse order. AN-9 and radiation exhibit additive antineoplastic effects in neuroblastoma, a promising clinical approach, as these two therapies have nonoverlapping toxicities. Although high-M cyclin-expressing neuroblastoma cells were much more sensitive to radiation-induced apoptosis, AN-9 inhibited M cyclin expression. It follows that delivering radiation prior to AN-9 would maximize the efficacy of combined HDACI and radiation treatments. Combination therapy using HDACIs and radiation represents an exciting new therapeutic approach to neuroblastoma.
103. AN IN VITRO DEMONSTRATION OF SYNERGISTIC GLIOMA CELL KILL BY THE SELECTIVELY REPLICATION COMPETENT ICP34.5-NULL HSV MUTANT, HSV1716, AND IONIZING RADIATION

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Here we demonstrate, in vitro, in the human glioma cell line U373, a synergistic relationship between cell kill from the oncolytic ICP34.5 null herpes simplex virus mutant, HSV1716, and ionizing radiation. Additive cell kill is shown in another human glioma cell line, MOG. Cell kill from HSV1716 and ionizing radiation was investigated in vitro using the MTS assay, a colorimetric method of determining the number of viable cells. HSV1716 and ionizing radiation were combined to determine cell kill in the human glioma MOG and U373 cell lines. Experiments combining HSV1716 and ionizing radiation indicated additional cell kill in U373 cells by day 6 compared to either modality in isolation. Isobologram analysis confirmed a synergistic relationship between ionizing radiation and HSV1716 when the U373 cells were irradiated one hour prior to virus inoculation. In the MOG cell line the relationship in terms of cell kill appears to be additive. Phase 1 studies in glioma patients have failed to demonstrate toxicity when HSV1716 is injected into tumor or normal brain, nor when they proceeded to second and third courses. Our results demonstrate that the additional cell kill demonstrated by various oncolytic HSV null mutants when combined with radiation is due to the upregulation by radiation of certain cellular proteins such as mammalian ribonucleotide reductase or GADD34, which complement the missing gene in the attenuated virus. Multicycle growth experiments showed that HSV1716 fails to replicate as efficiently as wild-type HSV in U373 cells. However, pre-irradiating U373 cells failed to result in enhanced viral replication, suggesting that increased viral replication might not be the reason for the increased cell kill. The demonstration of a synergistic relationship between ionizing radiation and HSV1716 in vitro is of interest if these modalities are to be combined in clinical practice and justifies further investigation of the mechanisms involved.

104. PHYSICAL AND NUMERICAL MODELING OF CEREBROSPINAL FLUID BEHAVIOR FOR DRUG DELIVERY TO THE LEPTOMENINGS

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Leptomeningeal dissemination (LMD) of tumor is a high risk in hematological malignancies, primary brain tumors and parameningeal tumors of childhood and adolescence, justifying either prophylactic or radical CNS directed chemoradiotherapy. In leukemias, combined systemic and intrathecal therapy has replaced cranial radiotherapy as prophylaxis; in primary brain tumors, reducing doses of craniospinal or focal radiotherapy in combination with systemic chemotherapy is being explored. In this latter group, intrathecal therapy has not been extensively studied because of uncertainties of timing and treatments. A multi-disciplinary study at the University of Nottingham is to reconsider the design of intrathecal drug delivery methods for prevention of LMD. An MRI scan of the entire CNS was obtained and data used to create 3D models using commercial engineering packages (Fluent). The existing physical model is constructed from thin-walled acrylic sheet; it contains the ventricles, a simplified brain surface, and spinal cord and the boundary of the spine and skull. It is suspended in a transparent tank on a mobile frame allowing rotation of the model and visual inspection of flow tracers. The CFD model solves fluid flow equations and can be programmed to induce motion of the model boundaries, thus allowing investigation of arterial pulsation of the brain and respiratory effects in the spine. For effective intrathecal delivery, the flow induced by CSF production needs to be sufficient, and the flow emitted from the foramina of Luschka and Magendie is transported mainly around the cranial SAS; diffusion is much less than the likely convection of fluid from the foramina to the arachnoid villi. Computational work shows flows and mixed boundary layers driven by physiology external to the SAS. Experimenting with models is a relatively cheap technique for illuminating the likely behavior of highly complex fluid flow in this nearly inaccessible region of the human body. The models will provide insight into current drug delivery behavior and potential new delivery methods.

105. LACTACYSTIN EXHIBITS POTENT ANTITUMOR ACTIVITY IN AN ANIMAL MODEL OF MALIGNANT GLIOMA WHEN ADMINISTIERED VIA CONTROLLED-RELEASE POLYMERS

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Lactacystin, a proteasome-inhibitor, has been shown to induce apoptosis of experimental gliomas in vitro. However, its systemic toxicity prevents further clinical use. To circumvent this problem, lactacystin can be delivered locally to the tumor site. We tested the efficacy of lactacystin incorporated into controlled-release polymers for treating experimental gliomas in vitro and in vivo. 9L-Gliosarcoma and F98-glioma cell lines were treated with lactacystin (10–100 μg/ml) for 72 h in vitro. Cell viability was measured with MTT assay, which is sensitive to the growth of the cells. In the 9L cell line, treatment with lactacystin resulted in a 50% reduction in cell viability at 10 μg/ml. Similar data was observed in the F98 cell line. In vivo, lactacystin was tested in a murine glioma model, where the tumor was implanted subcutaneously in the back of the mouse. Treatment with lactacystin/polycaprolactone (PCL) PLGA polymers was established in vivo by using Fischer-344 rats that were intracranially implanted with lactacystin polymers loaded from 0.1% up to 2% lactacystin by weight (w/w). The efficacy of 1, 1.3, 1.5, and 1.7% lactacystin/polycaprolactone (PCL)-PLGA polymers was determined. In vivo, lactacystin inhibited growth of F98 by 18 ± 8% at 10 μg/ml and 74 ± 2% at 100 μg/ml in vitro. Polymers released lactacystin for 21 days and intracranial implantation in rats did not generate local nor systemic toxicity at doses lower than 2% (w/w). Treatment with lactacystin/polycaprolactone (PCL) polymers loaded with concentrations of 1.0, 1.3, and 1.5% prolonged survival of animals intracranially challenged with 9L when polymers were inserted in the day of tumor implantation. Lactacystin exhibits potent cytotoxic activity against 9L and F98 in vitro. Furthermore, lactacystin can be efficiently incorporated and delivered using controlled-release polymers, and at the proposed concentrations, lactacystin polymers are safe for CNS delivery and prolong survival in the 9L model. These findings support the role of the proteasome inhibitors in the treatment of malignant gliomas when administered using local drug delivery.
though GBMs consist of heterogeneous cell populations, only a small fraction in this tumor system. Taken together, our data suggest that even though GBMs consist of heterogeneous cell populations, only a small fraction of GBM cells were specifically downregulated by a 6-day cyclopamine treatment. Concomitantly, a 3.5-fold reduction of cell proliferation (p < 0.01, n = 3) was observed at 10 mM cyclopamine treatment. Thus, constitutively active sonic hedgehog signaling critically contributes to GBM cell proliferation via PDGFRα expression in this tumor system. Taken together, our data suggest that even though GBMs consist of heterogeneous cell populations, only a small fraction of these cells is responsible for tumor cell reorganization.

107. COMPUTERIZED TIME-LAPSE MICROSCOPY OF HUMAN GLIOMA INVASION IN ORGANOTYPIC BRAIN SLICE CULTURES
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The purpose of this study was to establish a computerized time-lapse microscopy assay in order to monitor and characterize the growth of human gliomas in organotypic brain slice cultures. Recent results have shown that human glioma invasion in such cultures depends on the histological typing of the gliomas, suggesting that this model might be of considerable value in studying the mechanisms of brain tumor invasion. Human glioma biopsy specimens were produced from freshly resected grade II–IV gliomas and incubated with the fluorescent dye DiI for live cell labeling. Then the specimens were implanted in organotypic rat brain slice cultures grown on semiporous membranes for 1 to 2 weeks according to standard protocols. The slice cultures with implanted glioma biopsy specimens (n = 5–10 per case) were thereafter transferred for time-lapse fluorescence microscopy by using an automatic inverted fluorescence microscope placed in a specially constructed CO2 incubator. Controlled by a computer program developed locally by Bonde et al., the cultures were photographed by fluorescence microscopy and phase contrast microscopy every 30 min for up to 2 weeks, only interrupted by change of culture medium. Larger series of slice cultures with implanted glioma tissue (n = 12–36 per case) were photographed by conventional inverted fluorescence and phase contrast microscopy with 4- to 6-day intervals during 2 weeks. The results show that the invasion of human glioma cells into organotypic brain slice cultures can be monitored for up to 2 weeks by conventional and time-lapse microscopy in the present setup, demonstrating how both glioma cell density and distance of invasion increased with time. In contrast, when glioma biopsy specimens were placed directly on semiporous membranes, no outgrowth of glioma cells on the membrane was seen. The present study shows that computerized time-lapse microscopy of fluorescently labeled glioma cells implanted in rat brain slice cultures provides a model for monitoring quantitative and qualitative growth characteristics. This work was supported by the Danish MRC, the Danish Cancer Research Foundation, Foundation in the memory of Alice Brenå, Grant in the memory of Einar Willumsen, Anniversay Grant of King Christian IX and Queen Louise, King Christian X Foundation, Simon Fougner Hartmanns Foundation and EU 5th FP (QLK3-CT-2001-00407).

108. HUMAN GlioBLASToma T98G CELLS XENOgRAFTED IN ATHYMIC NUDE MICE OVERWHELMINGLY COMMITTED APOPTOSIS AFTER TREATMENT WITH all-trans-RETInOIC ACID AND TaxOL
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Glioblastoma is the most prevalent and highly malignant brain tumor in humans. It is not yet amenable to any currently available therapeutic strategy and thus remains as a challenge both to basic scientists and to physicians. Therefore, new therapeutic approaches need to be explored for effective treatment of glioblastoma. We used all-trans-retinoic acid (ATRA) and taxol (TXL) alone and also in combination for controlling the growth of human malignant glioblastoma T98G cells xenografted in athymic nude mice. For xenotransplantation of glioblastoma, 6-week-old athymic nude mice (Charles Rivers) were subcutaneously (sc) injected with a (1:1) mixture of exponentially growing T98G (6 million cells/mouse) and Matrigel. Animals with 3-week-old glioblastoma xenografts were randomly divided into 4 groups: control, ATRA, TXL, and ATRA plus TXL. Animals in the control group did not receive any therapy. Each animal in the other groups received intraperitoneally (ip) a daily dose of ATRA (1.5 mg/kg), or TXL (45 μg/kg), or ATRA (1.5 mg/kg) plus 4 h later TXL (45 μg/kg) for 7 days. Histopathological and biochemical examinations were conducted to evaluate the efficacy of different treatments. Histopathological examination of H&E stained tumour sections revealed that control tumors had a histodifferentiated growth of glioblastoma. ATRA alone inhibited tumor cell proliferation and caused astrocytic differentiation, TXL alone induced apoptosis to some extent, and ATRA plus TXL caused significant amounts of apoptosis in different cell subtypes. Differentiation of cells was associated with the upregulation of telomerase activity. In situ immunofluorescent staining showed calpain overexpression in apoptotic cells, suggesting a role for calpain in mediation of apoptosis. Further, in situ TUNEL and double immunofluorescent labeling confirmed cell death with an increase in calpain expression in tumor sections treated with TXL, or ATRA plus TXL. Western blot analyses showed changes in expression of Bax and Bcl-2 proteins leading to increased Bax/Bcl-2 ratio, cytosolic release of cytochrome c, activation of calpain and caspase-3 for degradation of 270 kDa α-spectrin at the specific sites to generate 145 kD spectrin breakdown product (SBDP) and 120 kD SBDP, respectively, in the course of apoptosis. Our investigation in an animal model revealed that xenografted glioblastoma cells with ATRA plus TXL induced differentiation and apoptosis for controlling malignant growth.

109. INTERACTIONS OF NEURAL STEM CELLS AND GLIOMA CELLS IN A THREE-DIMENSIONAL ORGANOTYPIC BRAIN SLICE CO-CULTURE SYSTEM
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Various in vivo studies have demonstrated a migration trend of neural stem cells (NSCs) toward intracranial gliomas, making these cells a potential carrier for delivery of therapeutic genes to disseminated gliomas. With this in mind, we investigated the response of glioma cells in an organotypic brain slice model in order to mimic the in vivo microenvironment including the three-dimensional architecture of murine brain tissue. The chemotactic effects of NSCs on glioma cell lines and their responding conditioned media on the murine NSC line C17.2 in a co-culture organotypic brain slice system were assessed. NSCs and glioma cells were identified inside the standardized murine brain slice by pre-implantation staining with Dil and DiD. Migration of NSCs and glioma cells inside the brain slice was characterized and quantified by using a confocal laser microscope on days 2, 6, and 12. C17.2 NSCs migrate inside the brain slices and seem to follow preserved anatomic structures. Migration of the NSCs was modified by conditioned media of glioma cells. Conditioned media of two glioma cell lines augmented migration of NSCs up to 50% compared to controls. In two gliomas, conditioned media stimulation was only moderate (20%). Conditioned media of one cell line produced inhibition of NSC migration. Co-inoculation of NSCs and glioma cells inside the brain slices resulted in a directed migration of both cell types toward each other in 3 of 5 glioma cell lines. The organotypic brain slice model displays several advantages over less complex in vitro migration models since a physiologic microenvironment and brain tissue architecture are preserved. Migration of NSCs towards gliomas in our assay system seems to depend on individual phenotypic characteristics and growth factor release patterns of the target tissue.
110. INTERSTITIAL PHOTODYNAMIC THERAPY OF RECURRENT MALIGNANT GLIOMAS USING 5-AMINOLEVULINIC ACID (5-ALA)

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Photodynamic therapy (PDT) might have the potential to improve local tumor control in selected patients: In PDT a photosensitizer (PS) is transferred selectively in tumor cells and activated by light of an appropriate wavelength, which leads to cytotoxic reactions. However, uncertainties concerning dosimetry and PS-distribution as well as therapy-related side effects have limited the clinical impact of PDT. These disadvantages might be overcome by the concept of stereotactic interstitial PDT (iPDT) using 5-ALA as PS. iPDT was considered to be indicated for patients with a minimum Karnofsky performance status (KPS) of 70 with a “circumscribed” recurrence of a malignant glioma after prior multimodal therapy with a maximum diameter of 3 cm. All operations were performed under general anesthesia. Patients received 20 mg/kg 5-ALA 1 h preoperatively. After tumor histology had been verified by stereotactic biopsy, 3-D-treatment planning (CT, MRT, FET-PET) followed by stereotactic implantation of up to 6 laser-probes was performed. Irradiation time was 60 min (Cerasal PDT Diode Laser; wavelength 633 nm, power 200 mW/cm 2). Therapy effects were documented by MRI (T1, T2-contrast) 24 h, 4 weeks, and then in 3-month-intervals postoperatively. Between October 2002 and December 2003, 10 adult patients (mean age 54, range 31–72 years; mean tumor volume 7.9 ml, range 2.1–26 ml) were included. The applied volume-dose was in the range of 1000 to 1500 J/cm 2. Early MRI follow-up within 24 h post-treatment showed a complete remission rate was 10% (3 patients had died on last follow-up). There was no surgery- or treatment-related morbidity or mortality. Stereotactic i PDT using 5-ALA is a minimally invasive and low-risk therapy. The multimodal 3-D treatment planning for the first time allows for an exact three-dimensional dosimetry and irradiation of a defined tumor volume.

111. HSV1716 INJECTION INTO BRAIN ADJACENT TO TUMOR FOLLOWING SURGICAL RESECTION OF HIGH-GR ANDE GLIOMA: SAFETY DATA AND LONG-TERM SURVIVAL

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In this study the authors have demonstrated that herpes simplex virus 1716 (HSV1716) is safe following injection into brain adjacent to excised high-grade glioma in patients who proceeded to receive further treatment: a total dose of 0.2 ml of HSV1716 was injected into the brain adjacent to tumor. Aliquots of 0.1 to 0.2 ml of HSV1716, were injected into eight to ten sites of the brain adjacent to tumor with the intent of infecting residual tumor cells. Patients were reviewed daily in the first week, weekly for six weeks, then twice monthly for the first year, and then on a six-month basis. Clinical and neurological parameters were recorded and blood samples were obtained for hematological, biochemical, and serological assessment. In addition, MRI, or CT if MRI was not tolerated, with and without contrast, was performed pre- and postoperatively, and then in line with the clinical assessment review schedule. Patients also underwent SPECT (single photon emission computed tomography) with thallium-201 used to identify areas of high cellular metabolic activity reflecting high-grade tumor growth. Patients were reviewed imaging with protocol when clinically indicated. There was no clinical evidence of toxicity during the period of formal follow-up or during the period associated with the administration of HSV1716. Longitudinal follow-up, until February 2003, allowed assessment of overall survival compared to that of similar patients not treated with HSV1716. Three patients remain alive and clinically stable at 15, 18, and 22 months post-surgery and HSV1716 injection. Remarkably, the first patient in this trial to have invasive recurrent disease at 22 months following injection of HSV1716 and 29 months following first diagnosis, Imaging demonstrated a reduction of residual tumor over the 22-month period despite no further medical intervention since surgery and HSV1716 injection. In this study we demonstrate that on the basis of clinical observations, there has been no toxicity following the administration of HSV1716 into the resection cavity rim in patients with high-grade glioma. This is a novel and imaging tool for which the authors recommend to proceed to a clinical trial to demonstrate efficacy of HSV1716 in glioma patients.

112. TEMOZOLOMIDE/PEGYLATED LIPOSOMAL DOXORUBICIN (PLD) IN PROGRESSIVE GliOMA MultIfORMe (GBM)

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GBM still is one of the most aggressive malignancies, with a median survival of 1 year. Two-year progression-free survival is about 11% in newly diagnosed cases, and 5-year overall survival less than 5%, so most of the patients (pts) will experience a progression within the first 2 years. Although magnetic resonance imaging (MRI) seems to indicate deficiency in the blood-brain barrier (BBB), only drugs penetrating the intact BBB have in vitro efficacy in GBM, with a median survival benefit of about 3 months only. Temozolomide (T) is one the most effective drugs in progressive GBM. Long-term survival, however, remains limited. Doxorubicin, a very effective drug in GBM in vitro, does not penetrate the BBB and thus lacks efficacy. PEGylated liposomal doxorubicin (PLD) is able to penetrate the BBB and has shown modest activity in temozolomide refractory GBM. The drug profiles of T and PLD highly suggest additive activity with overlapping toxicity. From August 2001 to May 2004, 40 pretreated GBM pts (31 progressive, 9 recurrent) were treated with T/PLD for progression. T was given on days 1, 5, 28, 35–39 as a 300 mg/m 2 body weight dose over 30 min by intravenous injection. PLD was infused in a rate of 0.3 ml/h for 5 days, resulting in a total dose of 9 to 10 mg/m 2 body weight. Treatment was continued until progression or severe side effects occurred. To date 28/40 pts have developed signs of progression. In 22-month period despite no further medical intervention since surgery and
of paclitaxel is a very effective therapy for patients with recurrent glioblastoma multiforme with an acceptable safety profile. Long-term observations with a larger number of patients will follow.

114. PERITUMORAL CONVECTION-ENHANCED DELIVERY (CED) OF IL13-PES3QR (IL13PE): RESULTS OF MULTICENTER PHASE 1 STUDIES IN RECURRENT HIGH GRADE GLIOMA (HGG)

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IL13PE is a recombinant protein consisting of IL13 and truncated Pseudomonas exotoxin that has selective antitumor activity against malignant glioma cells overexpressing the IL13 receptor. We assessed the safety and efficacy of peritumoral CED of IL13PE in patients with recurrent HGG. Three Phase 1 studies evaluated peritumoral CED of IL13PE following tumor resection. Safety criteria included age ≥18, KPS ≥70, and no tumor amenable to resection. The first study (002) determined the maximum tolerated dose of IL13PE, using a dose escalation design. Infusion duration and feasibility and accuracy of postresection catheter placement were also investigated. A second study (018) expanded the patient population treated at the MTD, and a third study (103) further assessed drug distribution at the MTD. Patients underwent tumor resection followed by a single 4-6 day infusion of 0.25-1.0 μg/ml of IL13PE (dose = 18-72 μg) and were followed with serial MRI scans. Enrollment is complete; 51 patients received study drug via peritumoral infusion. Forty-five patients had histologically confirmed recurrent glioblastoma multiforme (GBM; study 002, n = 38; 103B02, n = 3; 105, n = 4). Maximum tolerated infusion concentration was 0.5 μg/ml. Most frequent adverse events (AEs) were headaches and hemiparesis. There were 2 grade 4 toxicities at the highest concentration, and 9 grade 3 AEs reported in the study group. Concentration-dependent imaging changes related to study drug were also observed. Median overall survival (OS) for GBM patients was 45.9 weeks. Median OS for GBM patients with peritumoral infusion concentration of 0.5 μg/ml (n = 24) is currently 70.3 weeks. Eight patients remain progression free (24+ to 174+ weeks, median = 69 weeks). Initially, 50% of intraoperatively placed catheters were positioned accurately; accuracy increased to 90% when postresection stereotaxis was utilized. IL13PE appears to have a favorable risk-benefit profile, and prolonged overall survival has been observed. Postresection catheter placement appears to enhance positioning accuracy and, potentially, drug distribution and OS. Design of the ongoing international phase 3 trial to determine efficacy and safety of IL13PE in first relapse GBM is based on these findings.

115. INDIVIDUALIZED CHEMOTHERAPY FOR MALIGNANT ASTROCYTOMAS BASED ON O6-METHYL GUANINE-DNA METHYLTRANSFERASE METHYLATION ANALYSIS

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The correlation between O6-methylguanine-DNA methyltransferase (MGMT) methylation and responsiveness to nitrosourea chemotherapy observed in recent clinical studies suggests that use of this alkylating agent should be reserved for MGMT-methylated tumors. MGMT appears not to be linked to platinum resistance, which makes platinum chemotherapy a good candidate for the treatment of MGMT-unmethylated tumors. We instituted a preliminary trial of individualized chemotherapy based on MGMT methylation status combining interferon and radiation therapy for newly diagnosed malignant astrocytomas. Ninety-three percent received prior external beam radiation, and 51% were treated with prior chemotherapy. The median age was 54.5 years (range, 26-77) and 63% were males. All patients received 100 mg/m² of TMZ, and 73% of patients achieved an MTD of 75 mg/m². For patients with MGMT-unmethylated tumors treated with the carboplatin and etoposide (CE) regimen, only one (14%) of 7 patients with assessable disease partially responded to the CE therapy. Three patients were free from progression at 11, 14, and 20 months, whereas the remaining 10 patients progressed early following combination chemotherapy. Our results provide support for the responsiveness of MGMT-methylated malignant astrocytomas to the PAV therapy, but do not justify the usefulness of the CE regimen in unmethylated tumors.

116. A PHASE 1/2 TRIAL OF TEMOZOLOMIDE AND VINORELBINE IN PATIENTS WITH RECURRENT BRAIN METASTASES

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Treatment options for recurrent brain metastasis (BM) are limited. Temozolomide (TMZ) is an alkylating agent with efficacy in BM. Vinorelbine is a lipophilic agent with efficacy in a variety of solid tumors and can potentially cross the blood-brain barrier. We designed a phase 1/2 trial for recurrent BM utilizing vinorelbine in combination with TMZ in a scheduled manner. Patients with solid tumors and recurrent or progressive BM were eligible. One cycle was defined as 28 days. TMZ was given on days 1 to 15 at a dose of 150 mg/m² on days 1 to 5 and 15 to 19. Temozolomide was given on days 1 and 8 at escalating doses for the phase 1, with a starting dose of 15 mg/m² and increments of 5 mg/m² for each cohort of 3 patients, until maximum tolerated dose (MTD) or a dose of 30 mg/m² was reached. For the phase 2, 20 pts would be treated at the MTD; if two or more major responses were seen, sample size would be increased to 32. Twenty-seven pts have been enrolled (phase 1, 18 pts; phase 2, 9 pts). Median age was 60 (40-76); 11 pts were men, median KPS was 80 (70-100). Primary cancer was lung in 16 pts (non-small cell, 13 pts), breast in 8, renal in 1, endome- trium in 1, and ovary in 1. Temozolomide was given on days 1 and 8; vinorelbine was observed at phase 2 dose of vinorelbine has been 30 mg/m². Myelotoxicity has been the main adverse event, with grades 3 or 4 neutropenia in 7 pts, lymphopenia in 13, anemia in 2, and thrombocytopenia in 3. Response has been evaluated in 18 pts (complete response, 1 partial response, 1 minor response, 1 stable disease, 6 progressive disease, 10). Median time to progression was 2 (1-6) months. This regimen is well tolerated, and preliminary results suggest promising efficacy. The phase 2 sample size will be increased to 35 pts. Updated results will be presented.

117. AN UPDATE OF PHASE 2 TRIAL RESULTS: PATIENTS WITH RECURRENT MALIGNANT GLIOMA TREATED WITH IOIDINE 131-LABELED MURINE ANTITENASCIN MONOCLONAL ANTIBODY 81C6 INTO THE RESECTION CAVITY

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We previously established the maximum tolerated dose of iodine 131-labeled murine antitenascin antibody 81C6 (131I-81C6) administered into a surgically created resection cavity (SCRC) for the treatment of recurrent malignant glioma in adult patients to be 100 μCi. The current phase 2 study is designed to evaluate the clinical activity of a 100-μCi dose of 131I-81C6 administered to patients with recurrent malignant glioma and to further evaluate the safety and toxicity of this approach. Eligibility criteria included the following: adults with recurrent malignant CNS tumor; gross total resection; lack of communication between the resection cavity and the ventricular system; KPS greater than 60%; and adequate bone marrow, hepatic, and renal function. To date 43 patients have been enrolled, including 34 with either GBM or gliosarcoma, 6 with AA, 2 with AO, and 1 with metastatic adenocarcinoma. Ninety-three percent received prior external beam radiation, and 51% were treated with prior chemotherapy. The median age was 54.5 years (range, 26-77) and 63% were males. All patients received 100 μCi except for two patients who received 67 μCi and 73 μCi respectively because of the limited size of the SCRC. Reversible hematologic toxicity occurred in 27% of patients, and 5 patients (15%) developed delayed neurotoxicity. However, only one patient required reoperation for symptomatic radionecrosis. The median survival of all patients and of those with GBM was 67.5 and 63.2 weeks, respectively. Four patients died on or before day 30, and 31 patients were evaluable. Of these, 12 (39%) responded, 22 (68%) stabilized, and 7 (22%) progressed. The probability of 1-year survival is 0.56 (95% CI, 0.41-0.78). These encouraging results suggest that further study of 131I-81C6 for patients with recurrent CNS tumors is warranted.
118. PROGNOSTIC RELEVANCE OF CLINICAL AND THERAPEUTICAL ASPECTS IN PATIENTS WITH GLIOBLASTOMA MULTIFORME. ANALYSIS OF 99 PATIENTS
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Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor in adults. Despite advances in research and treatment, the prognosis for these patients remains poor, with a median survival of 1 year. Current management strategies used for GBM include surgery followed by adjunctive radiation therapy and chemotherapy. There is controversy among the neurosurgeons regarding surgical strategies. The purpose of this study was to identify clinical and therapeutic predictors of survival. We retrospectively analyzed 99 consecutive patients with primary supratentorial GBM who underwent tumor removal at our institution between 1999 and 2003. A surgery patients were treated with adjunct therapy: radiotherapy, chemotherapy, radioimmunotherapy. Of 99, 23 underwent a reintervention after recurrence. Based on different therapeutic strategies and clinical aspects different subgroups have been defined. We determined the median survival of each one using the Kaplan-Meier method. In concordance with literature we have obtained that age and total removal are predictors of survival. Other data differ from literature: survival according to Karnofsky Performance Scale score, epilepsy as first symptom, and functional location and presence of central nervous were not statistically significant. The most interesting results come from therapeutic. Patients who underwent total en bloc resection had a survival of 19 months, while total inside-out resection group had a survival of 12 months (P < 0.0005). Patients who underwent one surgical treatment had a survival of 10.5 months, and patients who underwent more surgical treatments had a survival of 22 months (P = 0.002). Survival according to adjuvant therapy has been 23 months (radiotherapy, chemotherapy, radioimmunotherapy), 18 months (radiotherapy, chemotherapy), and 14 months (radiotherapy) (P < 0.0002). In our series the “en bloc” resection represents an important prognostic variable. When tumor recurs, a re-intervention has to be considered. The treatment of GBM remains multidisciplinary. In our experience the best adjuvant treatment is radio + chemo + radioimmunotherapy.

119. PHASE 1 STUDY RESULTS OF GEFITINIB (IRESSA; ZD1839) PLUS RAPAMYCIN IN THE TREATMENT OF PATIENTS WITH RECURRENT MALIGNANT GLIOMA
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Aberant signaling of the PI3K/Akt pathway is common in malignant glioma. The primary objective of this phase 1 study is to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of Gefitinib, and the safety profile of Gefitinib and rapamycin in combination. Gefitinib, a macrolide antibiotic capable of inhibiting mTOR, is a critical downstream regulator of PI3K/Akt signaling, among patients with recurrent malignant glioma. Eligibility criteria include the following: recurrent malignant glioma; age greater than 18 years of age; KPS greater than 60%; adequate hepatic, renal, pulmonary and bone marrow function. Patients previously treated with EGFR or mTOR-directed therapies are excluded. Patients are stratified based on concurrent use of enzyme-inducing anticonvulsants (ELAC: phenytoin, carbamazepine, and phenobarbital). A standard “3+3” dose escalation design was employed with both strata independently escalated. Glioblastin and rapamycin are administered on a continuous daily dosing regimen. Each treatment cycle is 28 days, and patients are evaluated for response every other cycle. Twenty-three patients have been enrolled to date, including 20 with recurrent GBM and 3 with recurrent AA. The median age is 51 (range, 33–66); 65% are male, and 52% are on ELAC. Accrual and dose escalation are ongoing, and the MTD has yet to be defined for either stratum. The only DLT to date was an episode of grade 3 mucositis. Pharmacokinetic sampling has been collected in approximately half of patients. Six patients have discontinued therapy due to progressive disease, while 17 continue on study having received 1 to 3 cycles of therapy. One marked radiographic response has been observed to date. An update of the outcome and toxicity of Glioblastin plus rapamycin for patients with recurrent malignant glioma will be presented based on additional follow-up and enrollment.

120. ENHANCED DELIVERY OF CHEMOTHERAPY BY OSMOTIC BLOOD-BRAIN BARRIER DISRUPTION (BBBD) WITH DEFERRED RADIOTHERAPY FOR TREATMENT OF PRIMARY CNS LYMPHOMA (PCNSL): ANALYSIS OF 33 PATIENTS
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The optimal therapy for PCNSL has not yet been established. The addition of high-dose chemotherapy (chTx) to radiotherapy substantially improves median survival, but the combined therapy carries a high risk of severe treatment-induced delayed neurotoxicity. Several studies tried to use intensive chTx as the sole treatment for PCNSL but their limitations proved to be either a low response rate, short duration of response, or a high rate of treatment-related death (10%). A single institution’s experience with delivery of chTx in conjunction with osmotic BBBD, without subsequent radiotherapy, was reported to yield a survival rate similar to that observed in standard regimens that use both chTx and radiation therapy. This BBBD-enhanced therapy was not associated with delayed neurotoxicity. We report the experience at Hadassah University Hospital with BBBD-enhanced chTx without radiotherapy in PCNSL. Pts with non-AIDS related PCNSL were prospectively treated with methotrexate-based chTx as first-line treatment or with carboplatin-based therapy if they failed any previous methotroxate treatment. Both regimens were given in conjunction with BBBD and with no sign of other radiation therapy or chTx. A complete response was achieved in 23 pts (70%). Median follow-up was 27 months (range, 5–94), and the median survival is 39 months with a 5-year survival rate of 41%. Three pts died of causes other than disease progression; one was treatment related. Other procedure-related complications included 2 minor strokes, 2 arterial injuries successfully repaired by stenting, and one reversible brainstem lesion induced by carboplatin. The median time to tumor progression has not yet been reached (13/33 progressed) with 24- and 36-month PFS rates of 69% and 57%, respectively. Of 75% of cycles based on carboplatin with a total of 474 BBBD procedures. A complete response was achieved in 23 pts (70%). Median follow-up was 27 months (range, 5–94), and the median survival is 39 months with a 5-year survival rate of 41%. Three pts died of causes other than disease progression; one was treatment related. Other procedure-related complications included 2 minor strokes, 2 arterial injuries successfully repaired by stenting, and one reversible brainstem lesion induced by carboplatin. The median time to tumor progression has not yet been reached (13/33 progressed) with 24- and 36-month PFS rates of 69% and 57%, respectively. Of 75% of cycles based on carboplatin with a total of 474 BBBD procedures.
122. MODERATE HEMATOLOGICAL TOXICITY OF DOSE-INTENSIFIED TEMOZOLOMIDE (TMZ) IN PATIENTS WITH RECURRENT GLOBLASTOMA

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A one-week-on/one-week-off TMZ schedule had shown clinical efficacy and moderate hematological toxicity in 21 patients with recurrent glioblastoma. In a reassessment of our database, 39 patients with recurrent or progressive glioblastoma treated with the one-week-on/one-week-off regimen of TMZ, starting at 150 mg/m² with dose adaptation in 25 mg/m² steps, have been evaluated according to leucocyte and platelet counts which were determined in weekly intervals. The analysis similarly to the published data demonstrated a promising progression-free survival rate of 36% at 6 months. The overall response rate at 12 months from recurrence was 21%. Hematological toxicity in a total of 382 treatment weeks was low. Thirty-two of 39 patients never experienced leucocyte counts below 3,000/µl or platelet counts below 100,000/µl. Thrombomnesia necessitated platelet transfusions in 5 patients. Two patients experienced grade 3/4 hematological toxicity according to the common terminology criteria for adverse events later than 3 months after they started on TMZ. All other patients suffered such toxicity within the first weeks of TMZ. None of the 13 patients receiving TMZ for more than 6 months experienced grade 3/4 hematological toxicity. Our present extended analysis of the continuous one week on/one week off schedule of TMZ confirms the safety and efficacy of this regimen in recurrent or progressive glioblastoma.

123. LOOK FOR ANXIETY IN THE YOUNG AND DEPRESSION IN THE PREVIOUSLY DEPRESSED IN POSTOPERATIVE PATIENTS WITH BRAIN TUMORS

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Anxiety and depression can be symptoms that limit the quality of life of patients if not adequately diagnosed or treated. The period between surgery and radiotherapy for patients with brain tumors is a period where anxiety and depression commonly occur. We prospectively studied patients at 3 time points after surgery to study the frequency of anxiety and depression, the most likely time point for developing symptoms and any features that might act as a predictor of anxiety or depression. Fifty-one consecutive patients with intrinsic brain tumors gave consent to be involved in the study. Only 34/51 completed the Hospital Anxiety and Depression (HAD) scale at three points. A full history was taken, and a HAD scale score was obtained post-surgery, three weeks post-surgery, and pre-radiotherapy. A HAD score of 11 was considered abnormal. Thirty-eight patients (74%) completed the first HAD questionnaire, 34 (67%) completed the second, and 34 (67%) completed the third. Of 37 patients, 23 (62%) improved in functional improvement over the study period, and 5 patients (13%) deteriorated. Six of 11 of the patients (55%) who functionally deteriorated through the study did not complete the HAD scale. Five of 38 patients (13%) were anxious post-surgery, and four of these patients continued to be anxious throughout the study period. Eight of 34 (24%) were anxious pre-radiotherapy. All patients reporting heightened levels of anxiety were aged 65, and the anxiety levels were not related to initial functional impairment or change in function. Five patients had a significant depression at one or more time points between surgery and radiotherapy. Four of the 5 patients with depression had a past history of depression. Anxiety was more common in younger patients. Anxiety was slightly more frequent in patients if not adequately diagnosed or treated. The period between surgery and radiotherapy for patients with brain tumors is a period where anxiety and depression commonly occur. We prospectively studied patients at 3 time points after surgery to study the frequency of anxiety and depression, the most likely time point for developing symptoms and any features that might act as a predictor of anxiety or depression. Fifty-one consecutive patients with intrinsic brain tumors gave consent to be involved in the study. Only 34/51 completed the Hospital Anxiety and Depression (HAD) scale at three points. A full history was taken, and a HAD scale score was obtained post-surgery, three weeks post-surgery, and pre-radiotherapy. A HAD score of 11 was considered abnormal. Thirty-eight patients (74%) completed the first HAD questionnaire, 34 (67%) completed the second, and 34 (67%) completed the third. Of 37 patients, 23 (62%) improved in functional improvement over the study period, and 5 patients (13%) deteriorated. Six of 11 of the patients (55%) who functionally deteriorated through the study did not complete the HAD scale. Five of 38 patients (13%) were anxious post-surgery, and four of these patients continued to be anxious throughout the study period. Eight of 34 (24%) were anxious pre-radiotherapy. All patients reporting heightened levels of anxiety were aged 65, and the anxiety levels were not related to initial functional impairment or change in function. Five patients had a significant depression at one or more time points between surgery and radiotherapy. Four of the 5 patients with depression had a past history of depression. Anxiety was more common in younger patients. Anxiety was slightly more frequent pre-radiotherapy. A past medical history of depression is a predictor of significant depression in the postoperative period.

124. ADVERSE EFFECTS OF ANTIEPILEPTIC DRUGS IN BRAIN TUMORS: PRELIMINARY DATA OF A MULTI-INSTITUTIONAL SURVEY. ARE NEW ANTIEPILEPTIC DRUGS LESS TOXIC?

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Adverse effects of antiepileptic drugs (AEDs) in patients affected by brain tumors are frequent than in nonneurologically epileptic patients (2% vs. 12%, respectively, Glantz et al). Several classical AEDs (phenobarbital, phenytoin, and carbamazepine) are potent enzyme-inducing drugs, have serious CNS side effects that may alter quality of life and interfere with anticancer treatments, and may presents hematological and systemic toxicities.

125. PHASE 2 STUDY OF TOPOTECAN IN COMBINATION WITH CONCURRENT RADIOTHERAPY IN ADULTS WITH GLIOBLASTOMA WITH COMPLETE RESECTION

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In a previous phase 1 study of topotecan (TPT) administered as a continuous infusion (CI) for 5 days every 2 weeks in combination with concurrent radiotherapy (RT), the recommended dose of TPT was 0.9 mg/m²/day (Lesimple et al., J. Neurooncol. 65, 2003). The antitumor activity (measured by 12-month overall survival [OS]) and the safety of TPT were assessed in patients (pts) with histologically proven and previously untreated glioblastoma multiforme (GBM): After partial resection or stereotactic biopsy, pts received cranial RT (60 Gy/30 fractions/40 days) and 3 cycles of 0.9 mg/m²/day of TPT (Hycamtin, GlaxoSmithKline, Marly le Roi, France) as CI from day 1 to 5 weeks 1, 3, and 5 during RT. A total of 50 pts were entered, and 37 pts (M/F: 24/13; median age: 59; range: 42-69; PS 0/1-2: 17/20) were analyzed here. Twenty-one pts had stereotactic biopsy and 16 had partial resection. Grade 3–4 hematological toxicity was observed in 15 patients: neutropenia (16%, associated with fever in 4%), lymphopenia (15%), thrombocytopenia (5%) and anemia (3%). Grade 3–4 nonhematological toxicity consisted of hyperglycemia (4 pts); vomiting, diarrhea, hypokalemia (2 pts); and hyponatremia, GGT elevation, catheter-related infection, and thrombocytopenia (1 pt each). One patient experienced a partial response (3.7% of 27 evaluable pts for response) and 11 pts (40.7%) had stabilization, with a time to progression of 12 weeks. One-year OS rate was 41.7% for the 37 pts (median OS, 41 weeks). TPT in combination with RT was well tolerated but had modest activity in partially resected GBM in terms of response rate and 12-month OS.

126. TREATMENT WITH 3-WEEK COURSES OF DAILY TEMOZOLOMIDE AS SECOND-LINE CHEMOTHERAPY OF RECURRENT HIGH-GRADE GLIOMAS

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Several studies suggest that continuous treatment with temozolomide (TMZ) may be more effective than the standard 5-day schedule. We present our results with 3-week courses of daily TMZ, in recurrent high-grade gliomas (HGG). Between March 2003 and September 2004, 17 patients (pts) were included. Patient characteristics were as follows: 9 males, 8 females; median age, 39 years (range, 18–59); histology: 7 glioblastomas, 4 anaplastic astrocytomas, 1 anaplastic ependymoma, and 3 suspected HGG by neuro-imaging and clinical criteria. Previous treatments of recurrence were as follows: TMZ (200 mg/m² for 5 d every 28 d) in 13 cases; CPT-11 (125 mg/m² d, 1, 8, 15, and 22) and 29 d every 28 d) in 3 cases; PCV in 1 case. As progression, pts were treated with TMZ, 75 to 80 mg/m²/d for 21 d every 4
127. PREDICTORS OF SURVIVAL AND TUMOR RECURRENCE IN A COHORT OF 530 PATIENTS WITH GLIOBLASTOMA MULTIFORME: A REPORT FROM THE BRAIN CANCER REGISTRY OF THE NATIONAL NEUROLOGICAL INSTITUTE CARLO BESTA, ITALY, 1997 TO 2002

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Glioblastoma multiforme (GBM) is the most frequent brain tumor and accounts for approximately 12%-15% of all intracranial neoplasms and 50%-60% of all glial tumors. Aims of our study were to examine if resection extent, biopsy type, to verify the role of surgery, to determine the presentation, the incidence, and the severity of epileptic seizures, and to evaluate the added benefit of radiotherapy and chemotherapy. All patients were consecutively registered from January 1997 to December 2002 in the Brain Cancer Registry of the National Neurological Institute Carlo Besta, Milan. During this period, all adult patients newly diagnosed with histologically verified cerebral GBM were included and followed up until December 2003. Data from 1997 to 2002 were analyzed. Analysis was done using both Cox and logistic regression models. Five hundred thirty patients (60% males, 40% females) were included; mean age at intervention was 56 years (16-81 years). Median clinical follow-up time was 48 weeks. Median survival time was 58 wks (95% CI, 54–62). Survival probability was 55% at 1 year and 7% at 3 years. In multivariate analysis, age resulted as one of the most significant prognostic factors for survival of pts with GBM, with a hazard ratio (HR) of 1.32 (95% CI, 1.04–1.68) for age 63 to 63 and 1.83 (95% CI, 1.40–2.39) for age >61 years compared with 15 to 32 years (reference category). The intervention most significantly associated with survival was radiotherapy with HR = 4.17 (95% CI, 2.98–5.85) for no radiotherapy. With respect to extent of resection, HR was 2.03 (95% CI, 1.36–3.09) for biopsy and 1.25 (95% CI, 0.90–1.74) for partial resection (reference category: total resection). Median time to recurrence/progression (TTP) was 24 weeks (95% CI, 22–26). In multivariate analysis, the most significant prognostic factor for TTP was radiotherapy with HR = 3.86 (95% CI, 2.85–5.22) for no radiotherapy. Biopsy, compared to surgical intervention, was significantly associated with the following: sex (females vs. males), with a relative risk (RR) of 0.92; with age (95% CI, 0.80–1.06); number of lesions (multiple vs. single), with RR = 4.04 (95% CI, 2.03–8.05); tumor size (overlapping lesion vs. single lesion), with RR = 3.66 (95% CI, 1.88–7.12); and side (bilateral/median vs. unilateral), with RR = 7.92 (95% CI, 3.36–18.65). Partial resection, compared to a total one, was significantly associated with KPS (<70 vs. >70), with RR = 2.34 (95% CI, 1.15–4.79), and tumor size (overlapping lesion vs. single lesion), with RR = 1.97 (95% CI, 0.98–3.96). We analyzed data from the largest data set on GBL in Italy. Radiotherapy and total surgical resection were significant independent predictors of survival of patients with GBM, but measurements of morbidity and quality of life associated with treatments are now critical issues for these patients.

128. EPILEPTIC SEIZURES DURING FOLLOW-UP OF PATIENTS TREATED FOR PRIMARY BRAIN GLIOMA

129. IMAGING RESPONSE IN PHASE 2 TEMOZOLOMIDE TRIALS—REPORT INITIAL TUMOR SIZE

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Imaging response is an important measure in most trials of malignant glioma. In a previous paper of RMP-7 and carboplatin, we found age was related to tumor grade and initial size of tumor in glioblastoma (GBM), but not anaplastic astrocytoma (AA). Initial tumor size was important for response in GBM, and age was linked to speed of response in AAs, but numbers were too small in the GBM group to allow analysis. This study analyzes imaging response data from three trials of temozolomide. Imaging data were obtained at 12 weeks and 24 weeks, and patients were stratified for it.

In some PCNSL patients over 60 years, standard high-dose methotrexate (HD-MTX) therapy may not be applicable due to comorbidities such as impaired renal function. This prompts the search for other chemotherapy agents that can be applied to these patients instead of HD-MTX. Progression-free survival, overall survival, and toxicity were retrospectively analyzed in patients with primary CNS lymphoma treated with temozolomide alone as primary therapy. We report 7 patients who had historically confirmed PCNSL (n = 6) or a steroid-responsive lesion highly suggestive of PCNSL by neuroimaging (n = 1). The patients (62–84 years old) received 1 to 8 four-week courses of temozolomide (200 mg/m2 for 5 days in 6 patients, reduced dose of 150 mg/m2 in 1 patient). The median number of courses applied was 3. Complete responses (CRs) were achieved in 4 patients and 1 patient had stable disease after 3 courses and therapy was then switched to WBRT due to myelosuppression, 2 patients had primary progressive disease and did not receive any further therapy. In the patients achieving CR, CR persisted for 5, 19, 21 and 48+ months. After a median follow-up time of 17 months (range, 1–48 months), median survival has

130. PRIMARY TEMOZOLOMIDE CHEMOTHERAPY IN ELDERLY PATIENTS WITH PRIMARY CNS LYMPHOMA (PCNSL)

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not been reached yet. One patient died after 1 month due to tumor progression, and 6 patients are alive. Acute toxicity consisted of high-grade thrombopenia and leukopenia with subsequent infection in one patient who had received 200 mg/m^2 temozolomide and high-grade leukopenia without further complications in another patient. Temozolomide appears to be an effective and tolerable therapy for elderly patients with PCNSL and comorbidity who cannot receive HD-MTX.

131. ADJUVANT CHEMOTHERAPY WITH TEMOZOLOMIDE AND LIPOSOMAL DOXORUBICINE IN THE FIRST-LINE THERAPY OF PATIENTS WITH GliOBLASTOMA: A PHASE II TRIAL

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Temozolomide (TMZ; Temodal/Temodar) recently showed promising results in the first-line therapy of glioblastoma (Stupp, 2004). Pegylated liposomal doxorubicin (PEG-Dox; Caelyx/Doxil) was successfully evaluated in patients with recurrent high-grade glioma (Fabel, 2001; Hau, 2002). Therefore, a combination of both agents seems promising. Here, we update data on a pilot phase 2 trial using this regimen. We initiated a combination regimen consisting of TMZ and PEG-Dox in the first-line therapy of patients with glioblastoma. TMZ is given using standard TMZ and 5 to 20 mg/m^2 of PEG-Dox.

132. SEVERE MYELOSUPPRESSION WITH THE FIRST COURSE OF STANDARD DOSE TEMOZOLOMIDE: THE "X" FACTOR

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Although suppression is a dose-limiting toxicity of most cytotoxic chemotherapies, a reported benefit of temozolomide is that myelosuppression is relatively uncommon. Most studies of temozolomide report an overall incidence of grade 3–4 myelosuppression of 5% to 8%. In the initial trial of 445 cancer patients treated with temozolomide, an 11% incidence of significant myelosuppression was reported in females, with older female patients who received higher doses having a greater chance of developing both neutropenia and thrombocytopenia. In our practice, we recognized a trend toward female brain tumor patients having a higher incidence of myelosuppression during the first course of chemotherapy with conventional dose temozolomide. Therefore, we analyzed the hematologic toxicity in 18 consecutive patients after their first course of treatment with standard dose temozolomide (200 mg/m^2, days 1–5 of a 28-day cycle). A marked difference in the incidence of clinically significant myelosuppression (grade 3 or 4) was noted between male 14% (1/7) and female 46% (5/11) patients. The solitary male patient with myelosuppression experienced both a grade 4 thrombocytopenia and neutropenia. Of the five female patients with myelosuppression, 4/5 experienced a grade 4 neutropenia and 1/5 a grade 3. Two out of five experienced a grade 4 thrombocytopenia and 2/5 a grade 3. Furthermore, four females required hospitalization for febrile neutropenia, the first time of the group (5 mg/m^2 of PEG-Dox), one out of 7 evaluable patients had a dose-limiting toxicity (DLT). In the second, third, and fourth treatment groups using 10, 15, and 20 mg/m^2 of PEG-Dox, evaluable patients had a dose-limiting toxicity (DLT). In the toxicity phase and time to progression in the efficacy phase of the trial, the regimen was tolerated without DLT. Concerning efficacy in the "treated-patients" analysis of 17 patients treated so far, 1 had a partial response in two courses of standard dose temozolomide and 5 to 20 mg/m^2 of PEG-Dox. Accrual started in November 2004. Considering the results of the dose escalation phase of this study, the regimen is feasible, tolerable, and able to induce objective responses and stabilizations in patients with glioblastoma using the standard dose of TMZ and 5 to 20 mg/m^2 of PEG-Dox.

133. DELAYS IN RADIATION THERAPY AFTER SURGERY FOR INTRINSIC BRAIN TUMOR ARE ASSOCIATED WITH CLINICAL DETERIORATION IN ELDERLY PATIENTS

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Beneficial effects of steroids and surgery are often counterbalanced by brain tumor progression in the interval between surgery and radiotherapy. We retrospectively studied patients examined pre- and post-radiotherapy to study the influence of the following: age (<60 vs. > 60 years), dexamethasone dose (mg), and time interval until start of radiation (>5 weeks vs. <30 weeks). Fifty-one consecutive patients with an intrinsic brain tumor consented to be prospectively, objectively assessed post-surgery and pre-radiotherapy using the Edinburgh Functional Impairment Test (EFIT). The EFIT consists of the nine-hole peg test (NHPT), timed-10m walk (TMW), Williams Delayed Recall Test (WDRT), and Boston Aphasia Severity Rating Scale (BASRS). Normal values and values for clinically significant change for these tests have been previously published. Patients were grouped as (a) no defects, (b) limb defects only, (c) memory defects only, (d) combination of limb and memory/speech. An initial dose of dexamethasone ≥12mg/day was arbitrarily considered "high dose". A waiting time of >5 weeks was considered to be "delayed". Of 51 patients, 49 (96%) completed EFIT at both time intervals. There were 28 males and 13 females, 34 patients were >60. Median age was 55; 81% of patients had high-grade glioma, 19% "other". Also, 96% were treated with steroids (39% high dose); 45% of patients had an abnormal NHPT, 76% had abnormal TMW, 43% had abnormal WDRT, and 41% had abnormal BASRS after surgery. Younger patients (91%) as opposed to older patients (81%) were more likely to have a degree of functional impairment post-surgery; 51% of patients improved and 22% deteriorated prior to radiotherapy. Patients <60 years were more likely to improve prior to radiotherapy (59% vs. 33%). Older patients were more likely to deteriorate (40% vs. 15%). There was no clear association between initial or mean daily dose of dexamethasone and type or degree on neurological impairment, although fewer patients who deteriorated with memory or speech impairment were given high doses of dexamethasone than those with limb impairments. Most started radiotherapy within 5 weeks. No patient treated within <5 weeks deteriorated compared with 36% >5 weeks. We conclude that most young patients with functional impairment post-surgery for intrinsic brain tumors improve in the period up to radiation therapy. Older patients and
patients waiting more than 5 weeks are more likely to have clinical deterioration. Initial high-dose demethasone seems to have no clear advantage over lower doses in the postoperative period. Delays in radiotherapy are associated with clinical (and probable radiological) tumor progression

135. FIRST-LINE CHEMOTHERAPY WITH TEMOZOLOMIDE IN RECURRENT/PROGRESSIVE OLIGODENDROGLIAL TUMORS: A PHASE 2 STUDY
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There are few data regarding the efficacy of first-line temozolomide in recurrent oligodendroglial tumors, and the responses range from 17% to 34%. The purpose of this phase 2 study was to investigate the efficacy and toxicity of temozolomide “standard schedule” in patients with oligodendroglial tumors at first relapse after surgery alone or surgery and radiotherapy. The inclusion criteria were as follows: age ≥ 18 yrs, Karnofsky performance status ≥ 60; histological diagnosis of oligodendroglioma or oligoastrocytoma (grade II or III according to WHO classification); tumor progression on MRI; chemotherapy naïve. Temozolomide was administered at 200 mg/m² for 5 days in cycles of 28 days up to a maximum of 24 cycles in responding or stable patients or to unacceptable toxicity. Primary end point of the study was overall response rate based on Macdonald’s criteria. Thirty-four patients are assessable, 24 males and 10 females, with a median age of 47 (range, 18–73). A median of 10 cycles (range, 1–24) were administered. Responses were as follows: CR, 2/34 (6%); PR, 10/34 (29%); SD, 21/34 (62%); PD, 1/34 (3%). Among patients with SD, 3 had a reduction of tumor volume of 20% to 40% (minor response). The overall response rate (CR + PR) was 35%. The maximum tumor response was observed after 3 cycles in 2/12, 6 cycles in 7/12, 9 cycles in 2/12, and 15 cycles in 1/12. Four of 12 responding (33%) had PR (33%) and 5 of 12 stable patients (42%) displayed a significant reduction of seizures. Nine of 23 (39%) pure oligodendrogliomas responded, including the 2 patients with CR (grade III tumors), compared to 3/13 (23%) oligoastrocytomas. Ten of 22 enhancing tumors responded (45%) compared to 2/12 nonenhancing tumors (17%), and 47% of grade III responded compared to 26% of grade II. Median TTP was 14 months (range, 2–29), with a PFS at 6 months of 88% and at 12 months of 59%. Grade III-IV myelotoxicity was observed in 26% of patients. Temozolomide shows activity as first-line treatment in oligodendroglial tumors at first relapse (CR + PR + minor response: 44%). Pure oligodendrogliomas and enhancing and high-grade tumors tend to respond better. A long-term treatment with temozolomide is well tolerated.

136. PHASE 1 CLINICAL TRIAL AND PHARMACOKINETIC STUDY OF KARENITECIN IN THE TREATMENT OF RECURRENT MALIGNANT GLIOMAS: A STUDY OF THE NABTT CNS CONSORTIUM

Camptothecins have clinical activity in colorectal, ovarian, lung and, to a lesser extent, brain cancers, but their use has been limited by gastrointestinal and hematopoetic side effects. Karenitecin is a highly lipophilic camptothecin derivative, with a 2-(trimethylsilyl)ethyl substituent at the 7-position, rendering the terminal lactone ring much more resistant to hydrolysis by hepatic esterases than the parent drug. Karenitecin is dramatically affected by the concomitant use of hepatic enzyme-inducing antiepileptic drugs (EIAEDs). This study was conducted to (1) determine the maximum tolerated dose (MTD) of karenitecin in adults with recurrent malignant glioma; (2) describe the effects of EIAEDs on pharmacokinetics, and (3) obtain preliminary evidence of activity. Karenitecin was administered intravenously over 60 min once a day for 5 consecutive days, every 3 weeks. The starting dose was 1.00 mg/m² per day. The dose was escalated by using the continual reassessment method independently in cohorts stratified by EIAED use. Three patients were treated at each dose level. Intrapatient dose escalation was not permitted. Treatment was continued until disease progression, treatment-related dose-limiting toxicity, or patient withdrawal. Pharmacokinetic samples were obtained to define the total karenitecin plasma profile for the first dose of drug. We accrued 32 pts (20 males) to this study. Their median age was 52 years (range, 34–72), median KPS was 90 (range, 60–100); 78% of the patients had glioblas- tomas multiforme, and the remainder had anaplastic gliomas. One prior chemother-apy regimen was permitted. Dose levels evaluated in the +EIAED arm were 1.0, 1.5, 1.7, 1.9, and 2.1 mg/m² and in the –EIAED cohort 1.0, 1.5, and 1.8 mg/m². Myelosuppression was the major toxicity observed (WBC > platelets > RBC), with a short-lived grade 3-4 neutropenia or thrombocytopenia occurring in 28% and 16% of patients, respectively. The MTD was determined to be 2.0 mg/m² in +EIAED patients and 1.5 mg/m² in the –EIAED patients, a difference of 25%. Statistical comparisons to assess the influence of EIAEDs were based upon data for patients treated with 1.5 mg/m² where there were maximal patient numbers (7 +EIAED, 6 –EIAED). In comparison to the –EIAED cohort, the mean (±SD) maximum concentration of drug in plasma was 36% lower (22.9 ± 12.1 vs. 35.2 ± 13.3 μg/ml), and the total mean total body clearance was 29% higher (13.5 ± 13.5 vs. 10.5 ± 3.3 l/h/m²) in the +EIAED group. The median survival after starting therapy was 6.3 months (95% CI, 4.0–9.7 months), and 23 of the 32 patients are deceased. No complete or partial responses were observed, although 12 patients were treated above the MTD. As observed with other camptothecin analogues, karenitecin elimination is enhanced by EIAED administration. Single agent karenitecin does not appear very active in recurrent high-grade gliomas.

137. LONG-TERM TREATMENT WITH TEMOZOLOMIDE IN HIGH-GRADE GLIOMAS IS FEASIBLE AND LEADS TO LONG-TERM SURVIVAL IN A SIGNIFICANT SUBSET OF PATIENTS
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Although survival in patients with high-grade gliomas is relatively poor, warranting intensified treatment approaches, postoperative radiochemotherapy is frequently limited by bone marrow toxicity, usually terminating chemotherapy after 6 cycles of chemotherapy. Clinical studies using temozolomide (TMZ) and/or EIAEDs have produced promising results concerning survival and long-term feasibility, which is in part due to the lacking cumulative toxicity of TMZ. Nevertheless, no systematic data concerning long-term feasibility of TMZ have been acquired yet. Within a retrospective field analysis, German neuro-oncologists were approached to report on their treatment strategies with TMZ in high-grade gliomas using a standardized questionnaire. Long-term application of TMZ (more than 12 cycles or more than 12 months of any dose) were analyzed and evaluated for feasibility, efficacy, and tolerability. Altogether, 128 patients with WHO Grade III or IV gliomas who fulfilled the study criteria were identified by 49 neuro-oncologists. Most of the patients were treated with the standard scheme using 150 to 200 mg/m², and some were treated according to protocols EORTC 26981/22981 or by modified schemes. In first-line treatment (n = 64), the median number of applied cycles was 13 (range, 12–40), with a mean progression-free duration of 36 weeks (range, 52–160 weeks). Recurrent patients (n = 55) received a median number of 14 cycles (range 12 to 44 cycles), with a median duration of 62 weeks (range, 52–176 weeks). Grade 3 or 4 (NCI-CTC) toxicity concerning the gastrointestinal system (n = 7), leukopenia (n = 13), thrombocytopenia (n = 7), or infection (n = 5) was observed only in a small amount of patients. Nevertheless, toxicity data may underestimate the true incidence of toxicity due to the retrospective character of the study. Although this was a retrospective analysis, we could identify a significant number of patients with long-term application of TMZ. Up to 44 cycles were given, and times of up to 160 weeks in the first-line and 176 weeks in the recurrent setting without signs of tumor progression could be reached, indicating that patients with active high-grade gliomas may have well-controlled disease over significant periods of time.

138. SUBCORTICAL MAPPING OF MOTOR TRACT PATHWAYS IMPROVES SURGICAL REMOVAL OF HUMAN GLIOMAS
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Surgery for lesions involving motor areas or pathways requires the identification of functional cortical areas to reduce tumor resection morbidity. Intraoperative identification of functional descending motor pathways has been recently advocated as a promising technique to further reduce postoperative motor morbidity. We have developed a system for identification of motor functional tracts during awake surgery for removal of gliomas involving motor tract pathways. There were 45 patients (25 males, 20 females, age ranging from 22 to 45 years) harboring a low-grade (39) or high-grade (6) glioma located in the frontal lobe and involving motor cortex and/or pathways. In 23 patients, the tumor was also involving language areas and/or pathways. Cortical and subcortical mapping was performed by the use of an Ojeann stimulator and using the largest current that did not produce afterdischarge. Cortical areas were found in all cases. Subcortical

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139. OUTCOME OF PATIENTS UNDERGOING SURGERY FOR BRAIN METASTASES

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Brain metastases (BM) represent one of the most severe complications of solid cancers. Surgical treatment of this debilitating condition, if feasible, seeks not only to prolong the survival but also to improve immediately the neurological condition and quality of life of the patients. Our objective is to report the results of surgical treatment with BM treated in Tel Aviv Sourasky Medical Center since March 2003. Eighty-two patients (47 females and 35 males) with a median age of 61 years (range, 44–82) underwent surgical removal of BM. The most common primary cancers were NSCLC (13 pts, 40%), breast cancer (13 pts, 16%), and melanoma (9 pts, 24%). In 20 patients (24%) BMs were first presentation of their malignancy. At diagnosis of BM, the majority of patients (58, 71%) had active systemic disease. Neurological symptoms (11%) already had in the past, and 7 of them (9%) received previously neurosurgical treatment. Sixty patients (73%) had single BM; in 7 patients (9%) there were 2 BM; 14 patients (17%) suffered from multiple (≥2) BM. In most cases of multiple BM only one (rarely two) symptomatic lesion(s) responsible for neurological deterioration were removed. The operation resulted in immediate neurological improvement in 72 patients (88%). The condition of 7 patients (9%) did not change. Three patients (3%) died within 2 weeks after the operation. Surgical removal of BM succeeds to achieve in most cases an immediate clinical improvement and should be considered in neurologically symptomatic patients, including patients with active systemic disease and multiple brain metastases.

140. PATTERN OF RECURRENCE IN PATIENTS WITH GlioBLASTOMA MULTIFORME TREATED WITH AN ANTIANGIOGENIC THERAPY

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Glioblastoma multiforme is the prototype of an angiogenic tumor and thus predestined for an antiangiogenic therapy. But a possible escape mechanism of the tumor cells is increased invasion. The aim of this study was to evaluate the progression free- and overall survival in patients with glioblastoma multiforme, treated with a continuous, low-dose chemotherapy with temozolomide and rofecoxib, with special respect to the localization of tumor recurrence. Twenty-two patients with glioblastoma multiforme received after operation and radiation therapy a continuous, low-dose chemotherapy with temozolomide and rofecoxib. Clinical and MRI follow-up examination was done every 8 weeks. Mean follow up time was 20 months. Tumor tissue was analyzed for microvessel density, COX-II expression, and VEGF expression in 13 patients. Mean progression-free survival of all patients was 9.7 months, and mean overall survival was 16.9 months. Treatment induced a tumor recurrence distant to the original tumor localization. These patients had a slightly shorter overall survival. Patients with a higher microvessel density responded significantly better to the therapy (P 5 0.03, vs. 6.7 months). There was no relationship of the immunohistochemical markers and the incidence of distant tumor recurrence. Despite the dramatic increase in distant tumor recurrences compared to historical controls, the continuous, low-dose chemotherapy seems to be a promising therapy option in highly vascularized glioblastoma, since the progression free-survival and the overall survival compare very well to actual studies.

141. PHASE II STUDY OF CLORETAZINE FOR THE TREATMENT OF ADULTS WITH RECURRENT MALIGNANT GLIOMA

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Alkylating agents are an important class of chemotherapeutic drugs used for the treatment of CNS neoplasms. A novel class, the 1,2 bis(sulfonyl)hydrazines (BCHs), has been synthesized. The BCH compounds produce chloroethylating species but do not generate vinylation or hydroxyethylating events unlike the nitrosoureas. The chloroethylating species preferentially alkylates the O6-position of guanine. Cloretazine is a novel BCH compound. In preclinical studies of xenograft models of malignant gliomas Cloretazine has demonstrated potent antitumor effect. In the HT29 tumor cell line that expresses O6-alkylguanine alkyltransferase (AGT), high concentrations of Cloretazine had similar cytotoxicity compared to a cell line that does not express AGT. We performed a phase 2 study of Cloretazine in adult patients with recurrent malignant glioma. Eligibility included adult patients with recurrent malignant glioma. Patients were divided into two strata: stratum 1, recurrent/progressive glioblastoma multiforme (GBM), and stratum 2, recurrent/progressive anaplastic astrocytoma (AA) or anaplastic oligodendroglioma (AO). Each patient was treated with a 15-min infusion of Cloretazine at 300 mg/m² every 6 weeks. Responses were assessed by functional and imaging criteria after one cycle (6 weeks). Patients were treated until disease progression or unacceptable toxicity. The primary end points were to determine the activity, the toxicity, the time to progression, and survival of recurrent and progressive malignant glioma treated with Cloretazine. To date, 38 patients have been enrolled, 32 in stratum 1 (planned accrual [PA] = 32), and six in stratum 2 (PA = 38). For a median age of 55.5 years (range, 31–68) and a 71% male population. Thirty-six patients are assessable for response. Twenty-two patients have presented disease progression after the first cycle. Thirteen patients remained stable for at least two cycles (12 weeks), two patients with minimal responses. Toxicities included thrombocytopenia (6 grade 3, 10 grade 4), neutropenia (2 grade 3, 3 grade 4), leukopenia (1 grade 3, 1 grade 4), AST elevation (1 grade 3), ALT elevation (1 grade 3, 1 grade 4), and infection (1 grade 3). Cloretazine shows very limited disease stabilization in recurrent GBM. However, it is too early to determine the responses in recurrent AA/AO. Toxicities are limited to hematologic events and transient transaminase elevation.

142. CELLULAR PATHWAYS MEDIATING APO2L/TRAIL-INDUCED APOPTOSIS IN GLIOMA CELLS

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Apo2L ligand/TNF-related apoptosis-inducing ligand (APO2L/TRAIL) is a member of the TNF family that has been shown to induce apoptosis in malignant cells but not in most normal cells. Moreover, APO2L/TRAIL exhibits synergistic effects with irradiation or chemotherapeutic drugs including lomustine (CCNU) and temozolomide. Previous work showed that most glialoma cell lines are resistant to APO2L/TRAIL-induced apoptosis unless co-sensitized by cotreatment with an inhibitor of protein synthesis, such as cycloheximide (CHX). Resistance is also observed in some cell lines expressing high levels of the agonistic receptors TRAIL-R1 and TRAIL-R2. However, even in the presence of CHX, some cell lines are still resistant to APO2L/TRAIL. Further, the mechanism by which CHX renders resistant cell lines sensitive to APO2L/TRAIL has remained largely unclear. Because PI3K and caspase kinase II signaling have been shown to modulate the sensitivity of glioma cell lines to APO2L/TRAIL, we investigated whether inhibitors of these signaling pathways would modulate the sensitivity of glioma cells to APO2L/TRAIL. We found that the inhibition of PI3K (LY294002) and casein kinase II (DRB), all resulting in sensitivity to APO2L/TRAIL-induced apoptosis in glioma cells.
143. MATRIX METALLOPROTEINASE INHIBITOR-INDUCED JOINT-RELATED TOXICITY PREDICTS PROLONGED PROGRESSION-FREE SURVIVAL IN RECURRENT HIGH-GRADE GLIOMA TRIALS M. Groves, Vinaykumar Puduvalli, Charles A. Conrad, Mark R. Gilbert, Kenneth R. Hess, W.K. Alfred Yung, and Victor A. Levin; Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

Recent reports have highlighted relationships between a drug’s toxicity and its anti-cancer effect. Variability in drug-induced toxicity can be due to differences in metabolism, drug-drug interactions, or pharmacogenomic factors. We reviewed outcomes for 93 patients treated on 2 prospectively conducted phase 2 studies evaluating temozolomide (TMZ) (150–200 mg/m²/day, days 1–5) plus the matrix metalloproteinase inhibitor marimastat (MMR) (24–160 mg/m² days 6–28) for recurrent glioblastoma multiforme or anaplastic glioma. Histologic subgroups were analyzed individually and together, looking for the effect of toxicity due to MMR (presence and grade of joint-related toxicity [JRT]) and its relationship to progression-free survival (PFS). We reviewed outcomes for all 93 patients, 44 with GBM, 23 with AA, 14 with AO, 12 with AOA. PFS was significantly longer in pts with JRT (median 36 weeks vs. 9 weeks, P < 0.0001, hazard ratio = 0.3). This difference remained highly significant after adjustment for clinical factors (age, KPS, extent resection, histology, prior pituitarys, enzyme-inducing anti-epileptic drug [EIAED] status). However, the difference was bigger in patients on EIAEDs (EIAED: 69 weeks vs. 9 weeks, No EIAED: 24 weeks vs. 9 weeks). Time to recurrence (TTR) is also 0.3, but not to prior adjustment (P = 0.20). The adjusted hazard ratio for JRT is 0.4 for pts without EIAED, and 0.1 in pts with EIAED. There was a graded difference in PFS with grade of JRT (0: Median PFS = 9 weeks, 1: 14 weeks, 2: 22 weeks, 3: 38 weeks). Only patients with grade 3 JRT were dramatically in patients taking MMR. After adjustment for clinical factors, this interaction between EIAED use and JRT grade was significant (P = 0.056). The adjusted hazard ratios by grade for patients not taking EIAEDs are 0: 1.0, 1: 0.7, 2: 0.5, and 3: 0.3, and for patients taking EIAEDs they are 0: 1.0, 1: 0.5, 2: 0.2, and 3: 0.1. The modifying effect of EIAEDs on JRT was also seen for CR/PR response: In patients not taking EIAEDs, CR/PR in 0% for patients with and without JRT, but in patients taking EIAEDs, 3% of the 29 patients without JRT had CR/PR, while 27% of the 33 patients with JRT had CR/PR (20% in grade 1, 14% in grade 2, and 33% grade 3). For those patients who developed JRT, median time to its onset was 7.7 weeks (1–109). Median time to the development of the most severe JRT was 15 weeks (1–109). This analysis demonstrates the association of MRM-induced JRT with significantly prolonged PFS in patients with recurrent malignant gloma. Patients with JRT on EIAEDs have further enhancement of PFS. Pharmacokinetic and pharmacogenomic interactions may be involved. Further trials assessing the efficacy of this combination and its relationship to EIAEDs, drug pharmacokinetics, and drug-induced JRT are warranted.

144. TIME TO TUMOR PROGRESSION (TTP) AND QUALITY OF LIFE (QOL) FOLLOWING PROPHYLACTIC INDUCTION OF CHEMICAL HYPOTHYROIDISM IN FAILED MALIGNANT GLIOMA E. Luntskey, A. Herchberger, S. Dotan, E. Shalom, and T. Siegfried; 1Leslie and Michael Gaffin Center for Neuro-Oncology, Hadassah Hebrew University Hospital, Jerusalem, Israel; 2Department of Radiation Oncology, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Under hypothyroid conditions, gene expression of several growth factors and their receptors is downregulated. A previous phase I/II clinical study using the high-dose tamoxifen and carbamazepine regimen in association with chemical induction of HT prolongs survival of pts with recurrent malignant glomas. However, a major drawback of induced HT may be a potential negative effect of HT on the QOL of these vulnerable pts. Our objective was to evaluate the toxicity and effect of induced hypothyroidism in pts with recurrent, failed malignant gliomas. Prophyphylactic HT was used to induce HT in 20 pts (median age 49 years) with failed malignant gliomas (8 GBM, 7 anaplastic astrocytomas, 5 anaplastic oligoastrocytomas). Temozolomine (240 mg/kg/d) was given only to pts who became hypothyroid. Clinical evaluation and QOL were assessed monthly by using a standard questionnaire. Thyroid function tests were done weekly until HT was induced (TSH > 8) and then repeated monthly and MRI every 8 weeks. Twelve pts (60%) achieved HT (median TSH 9.8) within a median time of 3 months (range, 1–36 months). KPS, age, gender, treatment with enzyme inducing anti-epileptic drugs, and baseline TSH levels did not differ between the 2 groups of pts retrospectively stratified as achieving or not achieving HT. Induction of HT often could be achieved in 3 months but no other clinical symptoms of HT were longer in the HT group (5 months vs. 2.7 months, P = 0.002), with 6 months PFS of 33% vs. 0%. Clinical improvement was noted in 8/12 pts (66%) with HT and led to dose reduction or withdrawal of steroid therapy. Marked decrease in seizure activity was noted, and 2 pts became seizure free. MRI showed objective response in 25% of the HT group and stable disease in 75%. At baseline evaluation, the QOL did not differ between pts who later achieved HT and those who did not. In the HT group, QOL improved after 3 months and remained significantly from QOL of pts without HT based on categories of weakness (P = 0.01), fatigue (P = 0.01), depression (P = 0.002), interference with family life (P = 0.001), global QOL (P = 0.02), and global health (P = 0.004) evaluations. Induction of HT was associated with significantly longer median TTP in pts with malignant gliomas. Clinical and objective responses are associated with significant improvement in QOL despite the induction of HT. Further studies are warranted to evaluate the benefit of early induction of HT shortly after diagnosis and prior to tumor recurrence.

145. SURGICAL TREATMENT OF LOW-GRADE GLIOMA RECURRENTS L. Rebbi,1 F. Acerbi,1 C. Giussani,1 J. Casagrande,2 P. Casagrande,2 D. Spagnoli,1 M. Caroli,1 R. Campanella,1 G. Tomesi,2 and S.M. Gaini;1 Neuro-Oncology, University of Milano, Milano;2NeuroOncology, University of Insubria, Varese, Italy

Treatment of low-grade gliomas recurrences is controversial and consists of a wait-and-see policy or treatment based on surgery, chemo, and/or radiotherapy. The role of surgery, particularly of repeated surgeries, should still be considered the cornerstone of therapy for the majority of patients with a recurrent low-grade glioma admitted at our Dpts during the decades 1990 to 2000. They were 68 males and 91 females, age ranging from 22 to 69 years. Follow-up ranged from 50 to 264 months (median 156 months). Surgical resection was performed in 106 patients (72%). The remaining 42 patients had neurological deficits and were not candidates for surgery. Among those, 34 had drug-induced joint toxicity (JRT) with a median time of 5 months (range, 0–72). Patients with JRT on EIAEDs had a median survival of 57 weeks vs. 90 weeks (P = 0.032), but not prior to adjustment (P = 0.20). The adjusted hazard ratio for JRT is 0.4 in pts without EIAED, and 0.1 in pts with EIAED. There was a graded difference in PFS with grade of JRT (0: Median PFS = 9 weeks, 1: 14 weeks, 2: 22 weeks, 3: 38 weeks). Only patients with grade 3 JRT were dramatic in patients taking EIAEDs. After adjustment for clinical factors, this interaction between EIAED use and JRT grade was significant (P = 0.056). The adjusted hazard ratios by grade for patients not taking EIAEDs and for patients taking EIAEDs (median age 49 years) with failed malignant gliomas (8 GBM, 7 anaplastic astrocytomas, 5 anaplastic oligodendrogliomas) were included. Time to first recurrence correlated with extent of surgery at first operation, tumor removal, and extension of the tumor toward deep structures. Seventy-eight percent of tumors recurred in the previous operation. At surgery, 42% of patients were symptomatic for seizures or neurological deficits, and in 58% an enlarging mass or an enhancing lesion on MR scan was documented. Global 50% survival was 126 months. Survival correlated with malignant transformation. Malignant transformation occurred in 57% of cases, at first or further surgeries, and correlated with extent of surgery at first operation and tumor extension and volume. Survival and malignant transformation correlated also with extent of recurrence at surgery before recurrence removal. Time to further recurrence correlated with extent of resection. A second surgery or further surgeries were performed in 39%, 22, and 4.5% of cases. Further surgeries did not result in an increase of morbidity (0.7% mortality, 11% of transient morbidity). Time to recurrence decreased with the increase in the number of surgeries (from 72 to 23 months). Percentage of total removal decreased as well (from 51 to 26%). In case of early recurrence, with no findings of malignant transformation, early surgery was associated with a longer survival than when surgery was postponed and a wait and see policy was adopted. When a recurrence appeared, the combination of surgery eventually followed by chemotheraphy was associated with a longer survival than when surgery followed chemotherapy. Awake surgery and cortical and subcortical stimulations reduced the morbidity and improved the surgical removal of lesions close to or involving functional motor or language areas of pathways. Surgery and repeated surgeries for low-grade glioma recurrences are safe, control or relieve symptoms, and may postpone the use of adjuvant therapies until the time of appearance of malignant transformation.
Evidence implicating cyclooxygenase-2 (COX-2) in tumor angiogenesis has generated interest in evaluating the role of inhibitors of this enzyme, such as celecoxib, in cancer treatment. Hepatic metabolism by cytochrome P450 2C9 (CYP2C9) is a major route of elimination for celecoxib. Anti-epileptic drugs that are frequently used in glioblastoma patients induce a number of CYP450 enzymes, including CYP2C9. This study was conducted to determine the effects of enzyme-inducing antiepileptic drugs (EIAEDs) on the pharmacokinetics of celecoxib. Secondary objectives were to determine the safety of celecoxib in this setting and estimate the duration of survival when celecoxib was administered concurrently with radiation therapy in patients with newly diagnosed glioblastoma multiforme. Patients were divided into 2 groups (+EIAED and −EIAED) based on anticonvulsant use. Celecoxib administration began one week before conventional radiation therapy was started. A single 400-mg dose of celecoxib was taken on the first day of treatment. Twice-daily dosing (400 mg every 12 h) was begun the following day and continued until tumor progression, dose-limiting toxicity, or study withdrawal. No adjuvant chemotherapy was permitted. Pharmacokinetic blood samples were obtained serially for 24 h after the first dose of celecoxib and prior to a morning dose once a week during weeks 2 to 6. A validated LC/MS assay was used to determine the concentration of celecoxib in plasma. A total of 35 patients (22 +EIAED and 13 −EIAED) were accrued from October 2003 to September 2004. Fourteen patients (40%) were African American. All patients in the +EIAED group were receiving phenytoin. The study was closed before reaching the stated goal of 22 patients in each cohort in light of the positive results of the EORTC adjuvant temozolomide study. Celecoxib therapy was very well tolerated, but not all patients continued until the final toxicity assessment was noted. Only one patient complained of grade 3–4 epigastric distress and he responded to this dose reduction. Pharmacokinetic data is currently available for 14 +EIAED patients and 10 −EIAED patients. There was a significant difference (P = 0.66) between the maximum concentration of drug in plasma following administration of the first dose in the +EIAED (1.85 ± 0.71 μg/mL) and −EIAED (1.98 ± 0.48 μg/mL) groups. Similarly, the area under the plasma concentration-time profile from time zero to 24 h was similar (P = 0.79) for the two groups (+EIAED, 14.9 ± 5.7 μg h/mL; −EIAED, 13.9 ± 9.1 μg h/mL). Survival figures remain too premature to report, as 31 of the 35 patients remain alive. These preliminary results strongly suggest that the plasma pharmacokinetics of celecoxib is not significantly affected by the concomitant administration of EIAEDs. Furthermore, plasma levels in this study are similar to data in published reports on patients with arthritis who were not receiving concomitant glucocorticoids. This drug was well tolerated in this clinical setting. Survival results will be presented as the data matures.
5 hypo-Na+, 2 intense skin reaction. We conclude that in the present study, LRTG, TPM and Ox-CBZ have shown the following, even in monotherapy: (1) a clinical efficacy in seizure control similar to the one obtained with “old-AEDs”; 30% of pts suffered from one or more Pts and/or GTCS in the whole follow-up, requiring polytherapy in about 10% (better for TPM and Ox-CBZ than LMT); (2) fewer side effects (especially for TPM); (3) no significant pharmacological interactions; and (4) no need for hematological or renal monitoring. Of general considerations are possible: (1) Rem-Tyr-AEC is more effective than high-grade glioma-TAE (75 vs. 64%); (2) plasma levels of the new AEDs do not correlate with clinical control; (3) maximum tolerated doses seem to be adequate and dose-increase often results in a seizure exacerbation after a severe-problem in neurooncological pts treated with 2nd-generation AEDs are (1) the necessity of a long period of titration, to avoid side effects and (2) the only possibility of an oral administration. In conclusion, new AEDs have proved to be useful and handy drugs in the management of a “difficult” epilepsy such as TAE.

151. TEMOZOLOMIDE (TMZ) IN GLIOMATOSIS CEREBRI
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Gliomatosis cerebri is a diffusely growing neuroepithelial tumor whose optimal treatment is unclear. Few data are available about response to radiotherapy and chemotherapy. The objective of this prospective study was to assess the efficacy of temozolomide in patients with gliomatosis cerebro. Since 1999, 28 patients with histologically confirmed (biopsy or resection) gliomatosis cerebri were treated with temozolomide either after progression after prior radiotherapy/chemotherapy or upfront. Tissue specimens were diagnostic for glioblastoma in 2 cases, malignant glioma in 3, anaplastic astrocytoma in 4, gemistocytic astrocytoma in 2, astrocytoma in 9, oligoastrocytoma in 1, oligodendroglioma in 2, glial proliferation typical of gliomatosis cerebri in 3. Patient characteristics were as follows: median age 56 years (range, 17–70 years); 15 males and 13 females; median KPS at diagnosis 70 (range, 50–90). Presenting symptoms were as follows: seizures (11 patients), intracranial hypertension (7), motor deficits (5), mental status changes (2), drowsiness and diplopia (2), dizziness and vomiting (1). Twelve out of 28 pretreatment MRI scans showed some contrast enhancement. Eleven of 28 patients had received radiation therapy, and 4 had received chemotherapy (BCNU) prior to temozolomide. All patients were treated with temozolomide, 200 mg/m² per day for 5 days every 4 weeks until progression or unacceptable toxicity. Response was evaluated, according to Macdonald criteria, on MRI using both T1-weighted with gadolinium and FLAIR images. The median number of cycles was 7 (range, 1–20). One patient (4%) showed a CR of the contrast-enhancing area, 2 patients (7%) a PR of the Flair hyperintense area, 14 (50%) an SD, and 11 (39%) a PD. Among patients with SD, 2 had a reduction of tumor volume of 20% to 40% (“minor response”). Overall response rate (CR + PR + “minor response”) was 18%. Median time to tumor progression (TTP) was 4 months (range, 1–127), with a median survival of 12 months (range, 3–119). A clinical benefit, consisting in a reduction of seizures or improvement of intracranial hypertension, was observed in 9/28 patients (32%). PFS at 6 months was 57%, at 12 months 25%. Three patients showed grade 3–4 toxicities within 2 weeks of the last dose of OSI-774 treatment first cycle. DLT was defined as grade 3 thrombocytopenia, grade 3 or 4 anemia or neutropenia. Nine patients were withdrawn from the study due to hematological toxicities. Twenty-two patients were evaluable for response. One patient continues to show a partial response after 10 cycles, 14 showed a stable disease for at least 4 cycles, and 23 patients progressed after either the first or second cycle. The MTD of this drug combination for the non-EIAC stratum has been defined at a dose of TMZ of 200 mg/m² and a dose of CPT-11 of 60 mg/m² with DLT limited to hematological events. The MTD has yet to be identified for the EIAC stratum. Partial response has been observed in one patient, which remains on treatment after 10 cycles.

152. TEMOZOLOMIDE (TMZ) WITH O6-BENZYLGUANINE (BG) PLUS CPT-11 IN THE TREATMENT OF RECURRENT MALIGNANT GLIOMA: A PHASE 1 TRIAL
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The combination of TMZ and CPT-11 reveals a marked increase in activity compared with either agent used alone. The addition of BG to this combination dramatically increased the growth delay of the O6-alkylguanine-DNA alkyltransferase (AGT)-positive malignant glioma D-456-MG6. Those results have prompted the initiation of a phase 1 study of TMZ with BG plus CPT-11 in patients with malignant glioma. Eligibility included adults with recurrent malignant glioma; all patients had undergone prior treatment with two strata: those receiving enzyme-inducing anticonvulsant (EIAEs) and those not receiving EIAE. Each patient was treated with a 1-h BG infusion at 120 mg/m², followed by a single dose of TMZ at 355 mg/m² and a 90–minute infusion of CPT-11 in dose escalation for TMZ was required from 335 to 267 and then to the present dose of 200 mg/m². Dose-limiting toxicities observed thus far have been limited to hematologic toxicities, including neutropenia (9 grade 4), leukopenia (1 grade 4), and thrombocytopenia (2 grade 4). Forty-one patients are assessable for response. One patient continues to show a partial response after 10 cycles, 14 showed a stable disease for at least 4 cycles, and 23 patients progressed after either the first or second cycle. The MTD of this drug combination for the non-EIAC stratum has been defined at a dose of TMZ of 200 mg/m² and a dose of CPT-11 of 60 mg/m² with DLT limited to hematological events. The MTD has yet to be identified for the EIAC stratum. Partial response has been observed in one patient, which remains on treatment after 10 cycles.

153. A COMPARISON OF RECOVERY FOLLOWING REMIFENTANIL-DESFLURANE AND REMIFENTANIL-SEVOFLURANE ANESTHESIA FOR PATIENTS UNDERGOING CRANIOTOMY FOR TUMOR
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Craniotomy for tumor carries the risk of postoperative complications such as bleeding, cerebral edema, and cerebral ischemia. If not diagnosed early and treated promptly, these can lead to neurologic deficit. Therefore, rapid recovery and early neurological assessment are useful goals in the anesthetic management of patients undergoing craniotomy for tumor. The anesthetic technique should enable a rapid and predictable recovery. The pharmacology of remifentanil and desflurane suggests that recovery will be faster if used in combination compared to remifentanil/sevoflurane anesthesia. We compared emergence from remifentanil/desflurane versus remifentanil/sevoflurane anesthesia in patients undergoing craniotomy for tumor. Forty patient undergoing anesthesia for elective craniotomy for tumor were randomly assigned to receive remifentanil/desflurane or remifentanil/sevoflurane anesthesia. Following induction with remifentanil, propofol and rocuronium, anesthesia was maintained with study vapor and remifentanil in oxygen and air. All treatment was standardized. Recovery staff blinded to the study recorded early recovery parameters. The times required for spontaneous ventilation, eye opening, extubation, stating name, stating date of birth and achieving post anesthesia recovery score (Aldrete) >9 were 50% shorter after remifentanil/desflurane compared to remifentanil/sevoflurane anesthesia. In patients undergoing craniotomy for tumor surgery, recovery is significantly faster and more predictable after remifentanil/desflurane compared to remifentanil/sevoflurane anesthesia allowing an earlier neurological examination.

154. A PHASE 1 TRIAL OF OSI-774 (TARCEVA) IN PATIENTS (PTS) WITH RECURRENT MALIGNANT GLIOMAS (MG) ON ENZYME INDUCING ANTI-CONVULSANTS: A NORTH AMERICAN BRAIN TUMOR CONSORTIUM TRIAL
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To determine the MTD of OSI-774, a small molecule tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR), in patients with recurrent malignant gliomas on enzyme inducing anti-convulsant drugs (EIAEs). Pts with recurrent MG or meningioma were treated in a standard phase 1 design with 3 pts per cohort until the DLT was reached. DLT was defined as grade 3 thrombocytopenia, grade 3 or 4 anemia or neutropenia, any grade 3 nonhematologic toxicity, or failure to recover from toxicities within 2 weeks of the last dose of OSI-774 treatment first cycle. The starting dose was 150 mg/day continuously and increased by 50 mg for cohort 2, 75 mg for cohort 3, and then in 125-mg increments for all
subsequent cohorts until the DLT was reached. Patients could not have had more than 3 prior relapses and 2 prior chemotherapies. Patients were evaluated for response with MRI every 56 days. PK studies and AGF levels were determined in most patients. Thirty-two patients were included in the study: 21 GBM (47%), 8 AA (4M:4F), 2 AO (Male), and 1 atypical meningioma (Female). Median age was 44 (19–76), and median KPS was 90 (60–100). Number of prior chemotherapies was as follows: 2 pts had none, 11 pts had 1, 12 pts had 2, and 3 pts had 3. The DLT occurred at 775 mg/day and consisted of one of the following: grade 3 rash, grade 3 hypophysopatia, and grade 3 thrombocytopenia. The MTD was determined to be 650 mg/day; 1 patient out of 6 patients had DLT at that level (grade 3 deep vein thrombosis/ pulmonary embolism). The maximal tolerated dose of OX177 for patients on EIAEDs is 650 mg/day. Pharmacokinetic data and outcome data will be presented.

155. CONTRIBUTION OF RADIOSURGERY IN THE SURGICAL TREATMENT OF SKULL BASE MENINGIOMA

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Management of skull base meningiomas has been challenging, even though they are benign tumors. Since tumors are located deep in the skull and can involve important neurovascular structures, surgical treatment has been associated with high morbidity and mortality. However, advances in surgical instrumentation and newly developed approaches to the cranial base have been introduced. Simultaneously, radiosurgery was introduced to treat skull base tumors. With this combination of modern techniques, we would like to discuss how radiosurgery contributes to the treatment of skull base meningiomas. During 1995 to 2004, 50 patients with skull base meningiomas underwent surgical treatment in our department. The mean patient age was 56 years (range, 22–72 years). Sixteen patients (32%) were men, and 34 (68%) were women. The tumors were located at anterior cranial base (n = 26: 3 olfactory groove, 2 planum sphenoidale, 10 tuberum sellae, and 10 anterior clinoid process), middle cranial base (n = 4: ethmoidal sinus), and posterior cranial base (n = 20: 12 petroclival, 7 petrous apex, 1 foramen magnum). Nineteen patients (38%) had progressive visual impairment. Cranial nerve palsy was found in 9 cases (18%). Nine patients (18%) showed conscious disturbance due to increased intracranial pressure. Our treatment strategies are (1) the tumor should be left undetached in case there is no CSF space between the tumor and the surrounding structures to avoid new deficit, (2) the residual volume should be reduced to less than 20 ml, which is small enough for radiosurgery (3) the distance between residual tumor and optic nerve should be less than 3 mm. Total removal was achieved in 33 cases (66%). In the remaining 17 (34%), a small amount of tumor was intentionally left because of invasion of the cavernous sinus (8 cases), brain stem (4 cases), the perforators from carotid artery or middle cerebral artery (3 cases), the lower cranial nerves (1 case), or the optic nerve (1 case). Postoperative mortality was none and morbidity was found in 6 cases (12%). Visual function was improved in 16 patients (32%). The radiosurgery was followed within three months after the open surgery in 14 patients. During the follow-up period (5–112 months; mean, 59 months), tumor regression was observed in 7 patients (50%), and the tumor was unchanged or decreased in 7 patients. The tumor growth control rate was 100%. No patients experienced deterioration of their clinical symptoms after radiosurgery. In the majority of the cases, we could totally remove the tumors safely. On the other hand, we left a small part of the tumors invading important neurovascular structures and treated residual tumors by radiosurgery. Radiosurgery was a safe and effective treatment for residual tumors. Our result indicated radiosurgery could change surgical strategy of skull base meningiomas invading important neurovascular structures.

156. PHASE 1/2 TRIAL OF A TWICE-DAILY REGIMEN OF TEMOZOLOMIDE AND CELECOXIB FOR TREATMENT OF RELAPSED/REFRACTORY GlioblAstoma MULTIFORME AND ANAPLASTIC ASTROCYtoma

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The COX-2 enzyme is overexpressed in anaplastic astrocytoma (AA) and glioblastoma (GBM), and COX-2 inhibitors have preclinical efficacy in glioma models. Since this drug class has nonoverlapping toxicity with chemotherapy, a phase 1/2 clinical trial was initiated for patients with recurrent or progressive AA or GBM with the combination of temozolomide (TMZ) and celecoxib (CEB). For phase 1, a modified Fibonacci design was used with 3 patients per cohort. Chemotherapy was fixed, with TMZ being given with an oral loading dose of 200 mg/m^2 followed by 9 doses of 90 mg/m^2 BID for 5 days. CEB was given in 5 escalating dose levels starting at 60 mg/m^2 BID up to 240 mg/m^2 BID (maximum of 400 mg BID) for 10 days. Cycles were repeated every 28 days. Forty-six patients (28 M, 18 F) received 235 cycles of therapy, with 37 of the patients carrying a diagnosis of GBM and 9 with AA. Prior treatment consisted of radiation (N = 46) and chemotherapy (N = 12). Median age was 54 years (range, 34–74). This BID regimen of TMZ plus CEB was welltolerated by most patients, and no dose-limiting toxicity was observed at any of the 3 CEB dose levels. Hematologic toxicity was mild with grade 4 neutrophenia occurring in 1/235 (0.4%) cycles, grade 3 neutrophenia in 2/235 (0.85%), and grade 3 thrombocytopenia in 3/235 (1.3%). Grade 3/4 toxicity did not recur following TMZ dose reduction. Grade 3/2 constipation was common, occurring in 1/235 (28%) patients. Tumor responses were evaluated every 8 weeks. In the 41 evaluable patients acceptable for response, after 2 cycles, 3/41 pts (7.4%) had a partial response (PR), 3/41 (7.8%) had stable disease (SD), and 6/41 (14.6%) had progressive disease (PD), resulting in an overall response rate (OR) of 85%. After 6 cycles, the responses were as follows: 1/18 (5.6%) CR, 5/18 (27.8%) PR, 5/18 (27.8%) SD, and 7/18 (38.9%) PD, for an OR of 61%. One patient had a PR in the brain for 13 cycles, but developed tumor in the cervicospinal cord. The average duration of response was 5.6 months (range, 2–15). The 6-month progression-free survival rate for the 41 evaluable patients was 13/41 (31.7%), with a 6-month overall survival rate of 31/41 (75.6%). A regimen of twice-daily TMZ and CEB is safe and potentially effective for the treatment of recurrent high-grade gliomas. Further study of TMZ plus CEB-based regimens is warranted.

157. LONG-TERM SURVIVAL WITH BLOOD-BRAIN BARRIER DISRUPTION THERAPY IN PATIENTS WITH GLIOBLASTOMA MULTIFORME: A REPORT OF THREE CASES

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Long-term survival is rare in patients with glioblastoma multiforme (GBM). The 5-year survival rate has remained at 2% for the last 30 years. One of the problems of glioblastomachemotherapy is the penetration of drugs through the blood-brain barrier (BBB). BBB disruption therapy protocols have been established and are mainly used in the therapy of primary central nervous system lymphoma. We present three cases of highly malignnant gliomas who became long-term survivors (LTGBMS) after bloodbrain barrier disruption therapy (BBBD). After debulking surgery, three patients with high-grade gliomas underwent a blood-brain barrier disruption therapy (BBBD) therapy consisting of a 30-s intracarotid infusion of mannitol followed by both intrarterial and intravenous chemotherapy. Cytotoxic regimens consisted of intraarterial methotrexate 1200 mg on days 1 and 2, intravenous cyclophosphamide 15 mg/kg on days 1 and 2, and intravenous etoposide 150 mg/m^2 on days 1 and 2. Treatment courses were repeated every 4 weeks for a total of 4 courses. Noteworthy, two of the patients had no radiotherapy after surgery. Cranial magnetic resonance imaging followup was performed every 3 to 6 months until progression occurred. 6 patients, 10 years, and 11 years and 11 months after initial diagnosis, respectively. At progression, patients received a second-line intravenous chemotherapy consisting of fotemustine in combination with dacarbazine. Despite the use of cytotoxic drugs that were of almost marginal benefit when used intravenously in patients with high-grade gliomas, after BBBD, patients survived for 8 years, 12.9 years, and 13.4 years, respectively. These patients had the benefit of periods of disease-free survival lasting 6 years, 10.9 years, and 11.9 years. Our data suggest that in selected cases of high-grade glioma patients, BBBD therapy is effective and leads to long periods of disease-free survival. Unfortunately, even more than ten years after therapy, patients relapsed near the initial tumor site, highlighting the need for further therapeutic progress in GBM therapy.
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158. TEN CASES OF SYMPTOMATIC SINGLE PARENCHYMAL CNS METATASES TO THE PINEAL GLAND FROM SYSTEMIC MALIGNANCIES

Metastases to the pineal gland are typically noted as incidental findings at autopsy. In contrast, we identified 10 cases of symptomatic single parenchymal metastases to the pineal gland. This is the largest antemortem series reported. Lung cancer was the primary tumor in 5 cases (2 non-small cell cases, 1 small cell case). Breast, renal, cervical, esophageal, gastric, and colon cancers were the primary tumor type for 1 case each, as was a cancer of unknown primary. In 4 cases, the neurologic symptoms antedated the discovery of a systemic malignancy; in the other 6 cases, the primary tumor was in remission when neurologic symptoms developed. There were 6 women and 4 men with an median age of 36 years (range, 36–70). The clinical and radiographic findings were fairly uniform. In all cases, a contrast-enhancing pineal lesion with obstructive hydrocephalus was discovered by cranial CT or MRI, and the pineal metastasis produced the first clinical manifestation of CNS disease. The metastasis or associated hydrocephalus induced mental status changes ranging from confusion to coma in 8 patients. Oculomotor or pupillary abnormalities were observed in 6 patients including features of Parinaud’s or Sylvian aqueduct syndromes. Documented leptomeningeal tumor spread was present at the time of diagnosis of the primary tumor in 4 patients. Treatment of hydrocephalus in 6 patients. Radiotherapy in 6 patients. Treatment of hydrocephalus by CSF diversion (with a third ventriculostomy in 1 case) led to transient clinical improvement in 7 patients and no change in 1. Radiotherapy was given to 6 patients with at least partial response in 4. However, there was no significant improvement in survival, with median survival of 4.5 months (range, 1–92+) from the time of first diagnosis of pineal metastases. Only 2 patients survived beyond 1 year (21 months and 92 months), neither of whom had leptomeningeal spread. Metastatic disease should be considered in the differential diagnosis of pineal tumors even in the absence of a history of systemic cancer. Leptomeningeal spread is an important feature that should be investigated in cases of metastases to the pineal gland.

159. PRODIGE: A PHASE 3 RANDOMIZED PLACEBO-CONTROLLED TRIAL OF THROMBOPHYLAXIS USING DALTEPARIN LOW-MOLECULAR-WEIGHT HEPARIN (LMWH) IN PATIENTS WITH NEWLY DIAGNOSED MALIGNANT GLIOMA

Venous thromboembolism (VTE) is common in patients with malignant glioma, occurring in 20% to 30% of patients per year of survival. Clinical risk factors may include tumor volume, the use of chemotherapy, and surgery. Recently it has been shown that Tissue Factor (TF), the principal initiator of coagulation, is overexpressed in malignant glioma cell lines and human tumor samples. TF may induce a thrombogenic state in patients and may be a therapeutic target. In addition to anticoagulant properties, LMWH has been associated with prolonged survival in patients with nonmetastatic solid tumors through an anticancer effect. We are conducting a large phase 3 RCT testing the efficacy and safety of chronic daily administration of dalteparin in patients with newly diagnosed malignant glioma. Patients are randomized 1:1 to receive dalteparin 5000 anti-Xa units s.c. daily versus s.c. placebo. The primary outcome is VTE-free survival at 6 months, and progression-free survival, overall survival, toxicity, and neurocognitive performance are secondary outcome measures. The expected cumulative risk of VTE in the control group is 13% at 6 months. At least 40 events will be needed in order to detect a 60% relative risk reduction in the dalteparin group. Allowing for dropouts, the projected sample size is 512 patients. Clinical risk factors, concurrent therapies, tumor response, and survival data are being collected. A companion molecular study examining the role of TF and other regulators of coagulation is underway. Patients must be adults with newly diagnosed glioblastoma multiforme or anaplastic glioma, with no history of prior VTE, and not on chronic anticoagulation. As of November 2004, 124 patients were randomized across 15 active study centers. Of 281 eligible patients, 157 (56%) were excluded for reasons including conflicting trials, needle aversion, comitant illness, and possible VTE. An independent DSMB is supervising this trial and an update on trial progress will be presented. A placebo-controlled trial testing the role of thromboprophylaxis in patients with malignant glioma is feasible and is accruing patients at an increasing number of clinical trial sites worldwide. Of particular interest to neuro-oncologists is not only the prevention of symptomatic VTE, but the potential surgical advantage conferred by LMWH in other solid tumors. This study is supported in part by a grant in aid from Pfizer Inc. and coordinated by the Ontario Clinical Oncology Group.

160. LONG SURVIVAL RESULTS WITH REPEATED BIOCHEMOTHERAPY (BUCLADESINE + FOTEMUSTINE) IN DE NOVO GBM PATIENTS
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This clinical study was designed to evaluate the impact of biochemotherapy (bucldesine-polymer locoregional therapy and systemic fotemustine chemotherapy) at the time of recurrence of the de novo glioblastoma multiforme (GBM) patients. In a randomized prospective manner, 50 patients who were diagnosed as de novo GBM were included in this study. Five different therapy protocols were used. The first group of 10 patients had tumor resection only. The second group assessed having only systemic chemotherapy as six i.v. infusions of fotemustine after tumor resection. The third group had implantation of bucldesine-loaded biodegradable polymer microspheres. The fourth group repeated fotemustine i.v. plus six i.v. infusions of systemic fotemustine as in the second group, in addition to local implantation of bcl-SR pellets. Finally, the fifth group assessed having stereotactic biopsy and implantation of bcl-SR rods under local anesthesia repeated in six-month intervals. In this trial of local interstitial biologic therapy with long acting (4 to 5 months of release time) bcl-SR did show a statistically significant delay of recurrence on the treatment of GBM patients. The best treatment results were obtained from the local bcl-SR + systemic fotemustine–treated group, in which the survival rate estimated by Kaplan-Meier method was 100% in de novo GBM at 12 months. Moreover, in the last group, the mean survival rate was 58 ± 25.9 weeks, and 1-year, 2-year and 3-year survival rates were 100%, 40%, and 10% respectively. Mean survival times of 28, 32, 37, 63, and 85 weeks for the control, fotemustine, bcl-SR, fotemustine+bcl-SR and repeated stereotactic bcl-SR + fotemustine groups were achieved, respectively. In this prospective clinical study conducted on primary GBM cases, although the numbers of patients are small, there is a significant benefit of repeated local bcl-SR when used in combination with systemic fotemustine therapy (biochemotherapy) with no adverse effects.

161. SYSTEMIC TEMOZOLOMIDE COMBINED WITH LOCOREGIONAL MITOXANTRONE IN TREATING RECURRENT GLIOBLASTOMA PATIENTS
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The purpose of this study was to evaluate in recurrent glioblastoma patients the feasibility and effectiveness of a combination treatment with systemic temozolomide and locoregional mitoxantrone. The first end point of the study was the 6-month PFS; the secondary end points were response rate and OS. Twenty-two recurrent GBM patients were enrolled for second tumor debulking, with local positioning of a Rickam reservoir in order to locally deliver chemotherapy into the tumor created resection cavity, with interstitial mitoxantrone, delivered through the reservoir (4 mg, days 1–5 every 28) in association with mitoxantrone, delivered through the reservoir (4 mg, days 1–5 every 28) positioned into the area of tumor excision. After reoperation, a residual tumor mass no larger than 2 cm was identified in 18/22 patients. The patients were treated with monthly cycles of chemotherapy until evolution of the tumor but in no case for more than 10 cycles. Responses were evaluated by MRI scans performed every two months and images assessed according to McDonald’s criteria. Response rates were as follows: no complete responses [CRs], 5 partial responses [PRs], 13 cases of stable disease [SD], and 4 cases of progressive disease [PD]. The median progression-free survival [PFS] and survival time [ST] of the whole group of treated patients was 7 and 11 months, respectively, and more than 1/5 of the patients survived over 18 months. During the study the patients’ compliance was complete, and no dropouts occurred. Hematological toxicity was mild, and after repeated local injections in the tumor regrowth group with 90% progression-free survival occurred. We conclude that although some bias in patient selection is not excluded in this pilot study, results are interesting: The PFS was as long as survival of recurrent GBM reported in the literature.

162. MANAGEMENT OF GLIOBLASTOMA MULTIFORME IN ELDERLY PATIENTS: SHOULD WE BE AGEIST?
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Past studies have suggested that aggressive treatment for glioblastoma multiforme (GBM) in the elderly is not appropriate because of apparent poor outcome. Global application of this view may deny some patients this potential survival advantage conferred by LMWH in other solid tumors. This ageist view still hold true? We conducted a retrospective audit of outcome in all elderly patients
165. EPHA2 RECEPTOR AND ITS LIGAND, EPHRIN A1, ARE DIFFERENTIALLY EXPRESSED IN MALIGNANT GLIOMAS AND NORMAL BRAIN
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EphA2 is a member of the largest family of tyrosine kinase receptors, the Eph receptors. These proteins play an important role in neural development and are involved in mediating contact-dependent processes between cells. EphA2 is expressed at low levels on the surface of adult epithelial cells, with EphA2 receptors and other ligands such as EphB4/ephrin-B4 and ephrinA1 as a useful means for diagnosis and therapeutic approaches to vascular endothelial cells in normal brain, but present at a uniformly low level throughout human GBM paraffin-embedded specimens. Thus, EphA2 is specifically overexpressed not only in malignancies of epithelial origin, but also in malignant gliomas. Furthermore, EphA2 is expressed differentially with respect to its ligand, ephrinA1, in both malignant gliomas and normal brain. Overall, EphA2 represents a novel target for the development of molecular therapeutics for the treatment of patients with high-grade gliomas. We are currently investigating the role of EphA2 and its interaction with ephrinA1 as a useful means for diagnosis and therapeutic approaches such as targeted drug delivery.

166. CHROMOSOME 10q LOSS IN MEDULLOBLASTOMA: ASSOCIATION WITH THE LARGE CELL/ANAPLASTIC VARIANT
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Medulloblastoma is by far the most common CNS embryonal tumor. Although patient stratification is based primarily on clinical variables, distinct histologic variants and molecular alterations have also been found to correlate with prognosis. In particular, large cell/anaplastic (LCA) medulloblastoma characteristically presents with CSF dissemination and follows a highly aggressive clinical course. Several studies have suggested that deletions involving chromosome 10q may play a role in medulloblastoma tumorigenesis, though no correlation has been established between these alterations and histologic subtype. Yellow fluorescence in situ hybridization (FISH) was performed on formalin-fixed paraffin-embedded tissue sections from tissue microarrays containing 79 medulloblastomas to determine c-myc and 10q status. Each case was designated as one of the following histologic variants: classic, nodular/desmoplastic (N/D), or large cell/anaplastic (L/A). Medulloblastomas had 10q loss while 21% had amplification. The cohort included 40 classic, 23 N/D, and 14 LCA medulloblastomas. Overall, detectable amplifications of c-myc and losses involving 10q were present in 6 cases (8%) and 9 cases (11%), respectively. In all cases with losses involving 10q, all three of the targeted loci were deleted indicating a large region of chromosomal loss, likely the entire long arm. Fifty percent of tumors with c-myc amplification and 67% with 10q loss were of LCA morphology. As a group, 43% of LCA medulloblastomas had 10q loss and 21% had amplification of c-myc; 14% harbored both abnormalities. Only 5% of classic and 4% of N/D cases
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had either alteration, and 2 of these cases had focal anaplasia. Our findings demonstrate that losses involving chromosome 10q are encountered in a subset of medulloblastomas and involve a large region of that chromosomal arm. Both c-myc amplification and 10q loss appear to correlate with the LCA/morphology, the later alteration encountered twice as often as the former in this series.

167. METHYLATION PROFILE OF PRIMARY CNS LYMPHOMAS
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Patients with systemic malignancies have been shown to have substantial quantities of tumor-specific DNA in their plasma. Studies in systemic malignancies are underway to determine if this can serve as a plasma marker of tumor burden. We recently used methylation-specific polymerase chain reaction (MSP) to identify the methylation status of four gene promoters (p16, p15, RARB, and MGMT) within resected brain tumor tissue and in the plasma of patients with low- and high-grade gliomas. (Proc. Am. Soc. Clin. Oncol. 2004). The presence of tumor-specific DNA in the plasma was defined as identification of the same methylated promoter (MP) in the primary brain tumor and the plasma. The gliomas contained methylation of at least one promoter in 9 of the 10 (90%) patients studied, and 6 (67%) had methylation of at least one of the same promoters. A plasma marker would be of particular importance in patients with primary CNS lymphomas (PCNSL), where genuine improvements in outcomes are being realized. In this study, we sought to characterize the methylation status of CNS lymphoma patients using a panel of 14 genes (p16(NKX4-1), p15(INK4c), p14(ARF), p73, Rb, hMLH1, GSTP1, MGMT, RARB, CRBP-1, TIMP2, TIMP1, and DAPK). With IRB approval, we isolated DNA from paraffin-embedded samples from 31 CNS lymphoma patients and studied the methylation status of gene promoters in the PCR product using a nested PCR approach. PCR product was diluted and amplified in the methylation-specific polymerase chain reaction (MS-PCR), which was performed with gene-specific or either methylated or unmethylated DNA using positive and negative controls. The results showed a high degree of aberrant methylation in PCNSL for DAPK (21/28 = 75%), p16(NKX4-1) (10/13 = 76%), p15(INK4c) (16/25 = 64%), THBS1 (7/17 = 61%), and p14(ARF) (13/24 = 54%). RARB (13/26 = 50%), MGMT (13/27 = 48%), TIMP2 (11/26 = 42%), TIMP3 (11/28 = 39%), and p15(INK4c) (12/31 = 39%). This methylation profile provides an important first step in developing a quantitative tumor-specific plasma biomarker that could be used to monitor tumor status in patients with PCNSL.

168. C-KIT, A POWERFUL TUMOR MARKER IN GERMINOMA, IS MUTATED, AND TACE REGULATES C-KIT SHEDDING IN GERMINOMAS
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The proto-oncogene c-kit encodes a transmembrane tyrosine kinase receptor for stem cell factor (SCF). C-kit is thought to have a crucial role for tumor formation such as testicular seminomas, gastrointestinal stromal tumors (GISTs), and intracranial germinomas. The soluble isoform of c-kit and generating a soluble isoform of c-kit (s-kit). Members of matrix-metalloproteinases, have been indicated to be a candidate for tumor formation such as testicular seminomas, gastrointestinal stromal tumors (GISTs), and intracranial germinomas. In this study we performed immunohistochemical staining of SHGC, staining the same sections for c-kit and placental alkaline phosphatase (PLAP). Moreover, immunohistochemical staining for TACE with STGC, was extracted from the germinomas and the mutation of c-kit gene was analyzed by using a semiquantitative PCR approach. PCR product was diluted and amplified in the methylation patterns in the CpG islands of the genes using a nested PCR approach. PCR product was diluted and amplified in the methylation-specific polymerase chain reaction (MS-PCR), which was performed with gene-specific or either methylated or unmethylated DNA using positive and negative controls. The results showed a high degree of aberrant methylation in PCNSL for DAPK (21/28 = 75%), p16(NKX4-1) (10/13 = 76%), p15(INK4c) (16/25 = 64%), THBS1 (7/17 = 61%), and p14(ARF) (13/24 = 54%). RARB (13/26 = 50%), MGMT (13/27 = 48%), TIMP2 (11/26 = 42%), TIMP3 (11/28 = 39%), and p15(INK4c) (12/31 = 39%). This methylation profile provides an important first step in developing a quantitative tumor-specific plasma biomarker that could be used to monitor tumor status in patients with PCNSL.

169. SURVIVAL OF ANAPLASTIC GLIOMAS CORRELATES WITH GENETIC PROFILE
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Based on WHO classification, anaplastic gliomas are divided into grade III oligodendrogliomas (OIII), oligoastrocytomas (OAIIN) and astrocytomas (AOII). Known prognostic factors include age, performance status, and genetic alterations such as 1p and 19q chromosome loss, 1q22 loss, and EGFR amplification. Our aim was to investigate the impact of the most common genetic alterations on survival, based on 739 anaplastic gliomas collected in our database, including 445 males, 294 females (sex ratio = 1.51) ranging from 10 to 98 years (median = 48 years). Genetic analysis included search for loss of heterozygosity (LOH) on chromosome 1p and 19q, 9p, 10q, EGFR and MDM2 amplification, P53 mutation and expression, PTEN mutation: Partial or complete screening was available for 261 anaplastic gliomas. Overall survival (OS) was 26.2 months and was correlated to age (15.5 months for patients > 48 years, vs. 47.9 months for patients < 48 years; P < 0.0001) but not to the histological subtype. Loss of 1p and 19q (both linked P < 0.10-11) were associated with OS (P < 0.001), 10q loss was related to EGFR amplification (P < 0.10-14), and to PTEN mutation (P < 0.05). On univariate analysis, survival correlated with 1p loss (66.5 vs. 26.2 months, P < 0.0001), 19q loss (65.3 vs. 26.2 months, P < 0.005), 10q loss (19.4 vs. 41.6 months, P < 0.001), PTEN mutation (11.1 vs. 36.1 months, P < 0.0004), EGFR amplification (16.6 vs. 37.1 months, P < 0.0001), MDM2 amplification (3.7 vs. 31.5 months, P < 0.0025), F16/C6K2 deletion (18.9 vs. 33.1 months, P < 0.013). The difference did not reach significance for LOH 9p (P = 0.064), P53 expression (P = 0.11), or mutation (P = 0.9). LOH 10q and EGFR amplification were the only genetic alterations correlated to age (P < 0.0001), being more frequent in older patients (51 vs. 43 years and 55.5 vs. 43 years, respectively). In conclusion, genetic analysis of anaplastic gliomas provides useful prognostic information for clinical practice and for stratifying clinical studies.

170. IMMUNOHISTOCHEMICAL EXPRESSION OF IMATINIB TARGETS IN GLIOBLASTOMA: AN APPROPRIATE METHOD TO SELECT PATIENTS FOR TARGTED THERAPY?
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The tyrosine kinase (TK) inhibitor imatinib (Gleevec) selectively targets PDGFRA-, β1, c-kit, c-abl, and arg and has proven successful in the treatment of chronic myeloid leukemia. In recurrent glioblastoma, phase 2 therapy trials using imatinib have been initiated. As only a fraction of patients seems to benefit from imatinib therapy, and because of potential side effects and high costs of imatinib therapy, selection of the right patients is important. The goal of our study was to assess systematically immunohistochemical expression of the major TKs targeted by imatinib in glioblastoma, as expression of these factors could be used to select patients for imatinib therapy. In a cohort of 101 glioblastoma patients, anti-PDGFR-a, -β1, c-kit, c-abl, and arg protein immunohistochemistry was performed, and expression of these proteins was assessed semiquantitatively. PDGFRA and arg expression in tumor cells was widespread in 1/101 cases, respectively. Focal PDGFRA-, -β1, c-kit, c-abl, and arg immunolabeling was detected in 25/101, 19/101, 4/101, 7/101 and 31/101 cases, respectively. We show here for the first time in a large series of glioblastomas that PDGFRA-, β1, c-kit, c-abl, and arg expression is immunohistochemically detectable in a fraction of cases. The value of anti-tyrosine kinase immunolabeling as predictive factor for patient selection remains to be clarified by comparative analysis of tumor tissue of therapy-responders versus nonresponders.
171. GAMMA-CATENIN EXPRESSION CORRELATES WITH GOOD PROGNOSIS IN MEDULLOBLASTOMAS
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In medulloblastoma, gene amplification and mRNA overexpression of the c-myc gene are reported to be adverse prognostic indicators. However, the frequency of mRNA overexpression (50%) cannot be explained by gene amplification (4%) alone, and therefore, other mechanisms independent of gene amplification may exist. Because c-myc is located downstream of the Wnt signal pathway, we examined associated molecules in primary tumors by immunohistochemical and cytogenetic analyses and have discussed their clinical relevance. Twenty-four medulloblastomas were studied. Immunohistochemistry for c-myc, beta- and gamma-catenins, and cyclin D1 was performed. Differential PCR was conducted for the gene amplification of c-myc, N-myc, and cyclin D1. Mutations of beta- and gamma-catenins were examined by PCR-SSCP analysis and direct DNA sequencing. Western blot analysis was available in 5 cases. The clinical significance of the results was statistically analyzed by the Kaplan-Meier method. Cytoplasmic/membranous staining of beta- and gamma-catenins was detected in 19 (79%) and 9 (37%) cases, and nuclear expression of cyclin D1 and c-myc was detected in 6 (25%) and 21 (83%) cases, respectively. The expression of gamma-catenin in immunohistochemistry was confirmed by Western blotting, and the expression levels were well correlated between the two. c-myc and N-myc amplification was detected separately in two cases. Mutations of beta- and gamma-catenins were not found. Statistically, patients without CSF dissemination (Chang M0) showed significantly better outcome than those with dissemination (Chang M1–3) (P = 0.0002), and only gamma-catenin expression correlated with good prognosis (P = 0.003) among the molecules analyzed. Furthermore, gamma-catenin expression was also significant in the M0 group (P = 0.022). Although insignificant (P = 0.057), cyclin D1 expression showed a trend of adverse outcome, and all patients with cyclin D1 expression expired. The expression of beta-catenin and c-myc was not associated with prognosis (P = 0.005 and 0.33, respectively). The immunohistochemistry of gamma-catenin is useful for further stratification or individualization in medulloblastoma treatment. It was also found that cyclin D1 expression has the potential to be an adverse prognostic indicator.

172. VH GENE ANALYSIS OF PRIMARY CNS LYMPHOMAS
H. Pels172. VH GENE ANALYSIS OF PRIMARY CNS LYMPHOMAS
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H. Pels,1 K. Misaki172. VH GENE ANALYSIS OF PRIMARY CNS LYMPHOMAS
K. Misaki,1 M. Montesinos-Rongen,4 C. Schaller,1 U. Schlegel,1 I. Schmidt-Wolf,3 O. Wiesler,1 and M. Deckert1; Departments of Neurology,1 Neurosurgery,3 and Internal Medicine, University of Bonn, Bonn;4 Department of Neuropathology, University of Cologne, Cologne;1 German Cancer Research Center, Heidelberg, Germany

Primary CNS lymphomas are highly malignant non-Hodgkin’s lymphomas of B-cell origin associated with a poor prognosis. These neoplasms show variable sensitivity to radio- and chemotherapy. A molecular basis for this differential treatment response has not yet been established. To date, the most frequent translocation in a large cohort of patients is the t(14;18) (Burkitt lymphoma) or the t(2;14) (MALT lymphoma). While t(14;18) is associated with a favorable prognosis, t(2;14) and the t(14;18) variant t(14;18)(q32;q21) are associated with a poorer outcome. It was recently shown that the VH4 gene family was observed in the majority of nonresponding patients. In medulloblastoma, gene amplification and mRNA overexpression of the c-myc gene are reported to be adverse prognostic indicators. However, the frequency of mRNA overexpression (50%) cannot be explained by gene amplification (4%) alone, and therefore, other mechanisms independent of gene amplification may exist. Because c-myc is located downstream of the Wnt signal pathway, we examined associated molecules in primary tumors by immunohistochemical and cytogenetic analyses and have discussed their clinical relevance. Twenty-four medulloblastomas were studied. Immunohistochemistry for c-myc, beta- and gamma-catenins, and cyclin D1 was performed. Differential PCR was conducted for the gene amplification of c-myc, N-myc, and cyclin D1. Mutations of beta- and gamma-catenins were examined by PCR-SSCP analysis and direct DNA sequencing. Western blot analysis was available in 5 cases. The clinical significance of the results was statistically analyzed by the Kaplan-Meier method. Cytoplasmic/membranous staining of beta- and gamma-catenin was detected in 19 (79%) and 9 (37%) cases, and nuclear expression of cyclin D1 and c-myc was detected in 6 (25%) and 21 (83%) cases, respectively. The expression of gamma-catenin in immunohistochemistry was confirmed by Western blotting, and the expression levels were well correlated between the two. c-myc and N-myc amplification was detected separately in two cases. Mutations of beta- and gamma-catenins were not found. Statistically, patients without CSF dissemination (Chang M0) showed significantly better outcome than those with dissemination (Chang M1–3) (P = 0.0002), and only gamma-catenin expression correlated with good prognosis (P = 0.003) among the molecules analyzed. Furthermore, gamma-catenin expression was also significant in the M0 group (P = 0.022). Although insignificant (P = 0.057), cyclin D1 expression showed a trend of adverse outcome, and all patients with cyclin D1 expression expired. The expression of beta-catenin and c-myc was not associated with prognosis (P = 0.005 and 0.33, respectively). The immunohistochemistry of gamma-catenin is useful for further stratification or individualization in medulloblastoma treatment. It was also found that cyclin D1 expression has the potential to be an adverse prognostic indicator.

173. PERINUCLEAR EXPRESSION OF LRIG PROTEINS IS A GOOD PROGNOSTIC INDICATOR IN ASTROCYTIC TUMORS
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The LRIG protein family has three members, LRIG1–3, which are expressed in glioma cell lines and control-matched tumor tissues, characterized the subcellular localization of a synthetic LRIG1-GFP fusion protein, and analyzed the immunohistochemical staining patterns of LRIG1–3 in 404 astrocytic tumors. LRIG1–3 mRNA was detected in all human glioma cell lines and matched tumor samples. Ectopically expressed LRIG1-GFP localized to nuclear, perinuclear, and cytoplasmic compartments in a cell line-specific manner. Immunoreactivity of LRIG1–3 was seen in different patterns in the astrocytic tumors. Perinuclear staining of LRIG1–3 inversely correlated with WHO grade and survival of the patients. Positive LRIG3 perinuclear and cytoplasmic expression correlated with a lower proliferation index. LRIG3 perinuclear staining was in addition to tumor grade an independent prognostic factor. Thus, expression of LRIG1–3 might be of importance in the pathogenesis and prognosis of astrocytic tumors.

174. DEVELOPMENT AND APPLICATION RT-PCR SYSTEMS TO DETERMINE HERV EXPRESSION IN ASTROCYTOMA CELL LINES
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Human endogenous retroviruses (HERVs) belong to the family of transposable elements that make up 8% of the human genome. Unlike exogenous retrovirus (e.g., HIV and HTLV), HERVs are inherited in a Mendelian manner. More than 22 families of HERVs have been identified over the past two decades. Importantly, some HERVs have been found to possess large open reading frames and produce virus-like particles. More lately, it has been found that HERVs have been linked with certain autoimmune diseases and cancers. Indeed, HERVs may contribute toward carcinogenesis through retrotransposition, promoter insertion, immunomodulation, disruption of normal HERV-related functions, or by the production of fusion proteins. Of importance, HERK-V, HERV-W, and HERV-H have the potential to be transcriptionally active in the brain. We have developed robust RT-PCR systems using primers/probes specific to HERK-V and HERV-W to assess mRNA expression in control and glioma cell lines. In our study, we evaluated the mRNA expression level of HERV-K in the cell line U251-MG (derived from a glioblastoma multiforme; WHO grade IV astrocytoma) as compared to a control cell line SW480 (colon adenocarcinoma): RT-PCR values; 1.0 and 0.42, respectively. This observation raises an intriguing possibility that HERV-K expression can impact tumor growth. In addition, this approach provides a useful approach to optimize primers and probes prior to using real-time quantitative PCR.

175. MOLECULAR GENETICS AND RESPONSE TO PCV CHEMOTHERAPY IN OLIGODENDROGIAL NEOPLASMS: A SINGLE CENTRE PROSPECTIVE STUDY
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Recent research suggests that the -1p/-19q genotype is associated in oligodendroglial neoplasms with prolonged survival and chemosensitivity. However, the role of genetic analyses to guide patient management remains controversial. In this prospective study, the impact of genotype on the response of oligodendroglial neoplasms to procarbazpine, lomustine, and vincristine (PCV) chemotherapy was investigated in a routine clinical setting. Twenty-six patients treated with PCV chemotherapy at a single center between 2000 and 2003 were investigated. PCV was given as first therapy (50 cases) or at recurrence following radiotherapy (26 cases). Response was assessed by using Macdonald criteria and T1-weighted MR or CT images taken with contrast agents for enhancing tumors or T2-weighted MR images for nonenhancing tumors. Alteleic imbalance in 1p36, 1q13, 17p13, 10p12-15 and 10q22-26 and p53 mutation in the series as at Vrije Universiteit - Library on July 27, 2011 neuro-oncology.oxfordjournals.org Downloaded from
trend toward longer PFS and significantly greater time from first to second therapy. In the study overall, 26% of cases with intact 1p/19q responded to PCV; 5 had p53 mutation, 3 had no detectable genetic alterations, and 1 had a BRAF V600E mutation and chromosome 10, monosomy plus enhancing and nonenhancing grade II tumors with the 1p/-19q genotype responded to PCV chemotherapy. This prospective study supports previous retrospective observations that the 1p/-19q genotype is highly predictive of chemosensitivity in oligodendroglial neoplasms, but some tumors with intact 1p/19q also benefit from PCV.

The precise mechanisms governing the direct effect of IFN-b, including apoptosis induction, are not yet fully understood. To gain a better insight into these mechanisms, we investigated the signaling pathways focusing particularly on IFN regulatory factor 1 (IRF-1) and IFN regulatory factor 2 (IRF-2) in glioblastoma cell lines. Furthermore, we attempted to determine whether or not IRF-1 and IRF-2 act as additional prognostic indicators in diffusely infiltrating astrocytomas (DIA). We first assessed the cytotoxic effects of IFN-b based on a cell growth study and modified MTT assay, and then quantified the expression of caspase-3 and caspase-7, a sandwich enzyme immunoassay following IFN-b treatment in the cell lines, U-87MG, T98G, and A-172. Subsequently, we carried out an analysis of apoptosis-related molecules as evaluated by densitometric scan of type I IFN receptor, IRF-1, and IRF-2 using immunohistochemical techniques in 63 DIA (15 of WHO grade II, 18 of grade III, and 30 of grade IV), and analyzed their impact on prognosis. An increase in apoptosis was observed after 48 h of IFN-b treatment (1 × 10⁶ IU/ml) in T98G but not in U-87MG or A-172. IFN-b treatment for 6 h significantly enhanced the expression of IRF-1 in all cell lines. However, an enhanced expression of IRF-2 was observed only in the most-sensitive, apoptosis-induced U-87MG and A-172. While minimal processing of caspase-8 was noted in the 3 cell lines throughout the experiment, caspase-9 activation was observed in the apoptosis-detected T98G after 48 h of treatment, as indicated by a 1.33-fold increase (P = 0.037). On the other hand, the IRF-1 LI and IRF-1/IRF-2 LI ratios were greater in low-grade DIA and were negatively correlated with the histopathological grade in DIA (P = 0.017 and P = 0.001, respectively). Furthermore, the IRF-1/IRF-2 LI ratio was negatively correlated with the MIB-1 LI in DIA (P = 0.004) and represented an independent and most powerful determinant of overall survival compared to other conventional prognostic factors (P = 0.018). However, the relation was not statistically significant when only patients with high-grade DIA were assessed. Our findings suggest that upregulation of IRF-1 and IRF-2 might be an important determinant of susceptibility to IFN-b-mediated cytotoxicity including apoptosis. Furthermore, the IRF-1/IRF-2 LI ratio may reflect the proliferative state of DIA and constitute an important prognostic marker in DIA. Thus, IRF-1 and IRF-2 could represent one of the therapeutic target sites for the regulation of cell growth in DIA.

The prognostic implication of patients’ epidemiological data (including age and gender), MRI scan characteristics (diffuse, ring, and no enhancement), extent of resection, histopathology, postoperative KPS, proliferation index (Ki-67 expression), and chromosome 10, monosomy plus enhancing and nonenhancing status of the promoter. Ninety-three patients with anaplastic glioma (WHO grade III) were analyzed for MGMT protein expression by immunohistochemistry. Overexpression of type I IFN receptor, IRF-1, and IRF-2 using immunohistochemical staining in the cell lines, U-87MG, T98G, and A-172. Subsequently, we carried out an analysis of apoptosis-related molecules as evaluated by densitometric scan of type I IFN receptor, IRF-1, and IRF-2 using immunohistochemical techniques in 63 DIA (15 of WHO grade II, 18 of grade III, and 30 of grade IV), and analyzed their impact on prognosis. An increase in apoptosis was observed after 48 h of IFN-b treatment (1 × 10⁶ IU/ml) in T98G but not in U-87MG or A-172. IFN-b treatment for 6 h significantly enhanced the expression of IRF-1 in all cell lines. However, an enhanced expression of IRF-2 was observed only in the most-sensitive, apoptosis-induced U-87MG and A-172. While minimal processing of caspase-8 was noted in the 3 cell lines throughout the experiment, caspase-9 activation was observed in the apoptosis-detected T98G after 48 h of treatment, as indicated by a 1.33-fold increase (P = 0.037). On the other hand, the IRF-1 LI and IRF-1/IRF-2 LI ratios were greater in low-grade DIA and were negatively correlated with the histopathological grade in DIA (P = 0.017 and P = 0.001, respectively). Furthermore, the IRF-1/IRF-2 LI ratio was negatively correlated with the MIB-1 LI in DIA (P = 0.004) and represented an independent and most powerful determinant of overall survival compared to other conventional prognostic factors (P = 0.018). However, the relation was not statistically significant when only patients with high-grade DIA were assessed. Our findings suggest that upregulation of IRF-1 and IRF-2 might be an important determinant of susceptibility to IFN-b-mediated cytotoxicity including apoptosis. Furthermore, the IRF-1/IRF-2 LI ratio may reflect the proliferative state of DIA and constitute an important prognostic marker in DIA. Thus, IRF-1 and IRF-2 could represent one of the therapeutic target sites for the regulation of cell growth in DIA.

178. ESTHESIONEUROBLASTOMA: A STUDY OF PROGNOSIS

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Esthesioneuroblastoma is a rare tumor, for which experience at individual institutions has been limited. At present, there is no consensus regarding its systemic treatment. In order to determine prognostic factors for outcome in the management of this tumor, we retrospectively studied the records of 78 patients treated for esthesioneuroblastoma at the Mayo Clinic between 1976 and 2003. Histological slides from original biopsies/surgery were reviewed in 60 (of 78) patients, and Hyams pathologic grade (1–2, low; 3–4, high) was determined. Statistical analysis included Kaplan-Meier overall and disease-free survival and Cox proportional hazards modeling, uni- and multivariate. We assess the strength of association of the following variables with survival: age and gender, Hyams grade, modified Kadish stage (A to D), extent of surgery, and adjuvant treatment. There were 35 females (45%). The average patient age at diagnosis was 51 years and average age follow-up was 6.4 years (Hyams grade, n = low, 31; high, 29; modified Kadish stage, n = A, 4; B, 15; C, 42; D, 15). Fifty-four patients underwent gross total resection, 16 subtotal, and 8 biopsy only, while 51 patients received radiotherapy and 31 chemotherapy. The 3-year survival (68% overall) was: 84% for low and 50% for high grade tumors (P = 0.004); 88% for stages A and B, 72% for stage C, and 34% for stage D patients (P < 0.005). Controlling for age and extent of resection, stage D exhibited significantly higher mortality than either stage A + B (P = 0.0005) or stage C. P = 0.006, while high-grade tumors had significantly higher mortality than low grade (P = 0.007). In total, 53 patients were at risk for recurrent disease, and 32 (60%) of these went on to develop recurrence: 9% at 1 year, 36% at 3 years, and 77% at 10 years. Our data suggest that both modified Kadish stage and Hyams grade at the time of diagnosis have a significant impact on prognosis.

179. GENETIC ANALYSIS OF MENINGIOMAS REVEALS CHROMOSOME-SPECIFIC CORRELATIONS WITH GRADE AND RECURRENCE

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Meningiomas are common central nervous system neoplasms that exhibit a remarkably diverse histopathology and biological behavior. Apart from the 22q gene, the molecular biology of meningioma development and progression is poorly understood. We examined 25 benign (WHO grade I; M1), 10 atypical (WHO grade II; MII) and 6 anaplastic, malignant (WHO grade III; MIII) meningiomas, including 14 tumors of 5 patients showing recurrence (n = 3) and progression (n = 2), for DNA copy number gains and losses by comparative genome hybridization (CGH) and for chromosome 1, 7, and 22 alterations by fluorescence in situ hybridization (FISH) using centromere- and NF2 gene–specific probes. In addition, 44 tumors of 12 patients showing malignant progression were
studied by microsatellite analysis using 7 markers for loci on chromosomes 1p and 22q. Results were statistically correlated with available clinical data. CGH analysis identified (1) genomic alterations in all but 6 MI tumors; (2) a marked accumulation of the average number of alterations per tumor with increasing WHO grade, i.e., 5.2 for MI, 10.0 for MII, and 11.8 for MIII (P = 0.001; MI vs. MII + MIII); (3) besides 22q losses most frequently 17p and 20q gains (36%–40%) in MI, losses of 1p, 14q (both 50%), 6q (40%), and gains of 17q (70%), 20q (60%), 17p (50%), and 11q (40%) in MII, and a further increase of most of these alterations (1p losses and 17q gains; 83%), as well as amplifications on 5p15, 5q35, 8q24, and 20q13 in MII tumors; (4) a significant association of 1p and 6q losses and 1q gains with higher WHO grade (MI vs. MII/MIII; P = 0.05); and (5) a strong correlation of tumor recurrence with 1p and 10p losses (P = 0.057 and 0.006, respectively) and 1q gains (P = 0.032), as well as with an increase in total number of alterations and losses per tumor, i.e., 5.7 vs. 11.5 (P = 0.007) and 2.4 vs. 6.0 (P = 0.003), respectively. FISH analysis in these tumors showed chromosomes 1 and 7 copy numbers indicating a diploid DNA content, while 1 copy of the NFI locus was lost in 18/23 (78%) MI, 8/9 (89%) MII and 5/5 (100%) MIII tumors, predominantly together with a chromosome 22 centromere. Microsatellite analysis revealed LOH for at least 1 marker at 22q in 8/10 and at 1p in 7/11 additional patient cases showing progression, which was already detected in the primary tumor. Our study indicates that meningiomas are mainly diploid tumors showing high frequencies of chromosome 22q loss including the NFI gene as well as 17p and 20q gains already early in tumorigenesis. Tumor recurrence proved to be associated with 1p and 10p losses and 1q gains, as did 1p and 6q losses and 15q gains with tumor progression. The identified regions of amplification and the location of the discovered novel genes that play a role in meningioma tumorigenesis and may help to predict their biological behavior. This study was supported by the Royal Netherlands Academy of Sciences (KNAW), Van Leeuwen Foundation, and the Foundation Neurosurgery Heerlen.

180. PROSPECTIVE ANALYSIS OF MATRIX METALLOPROTEINASE-9 (MMP-9) IN THE SERUM OF PATIENTS WITH HIGH-GRADIE GLIOMAS


The objective of the study is to determine if matrix metalloproteinase-9 (MMP-9) can be used as a serum marker in patients with malignant gliomas. Our previous work has shown that the level of YKL-40 protein can predict survival of patients with high-grade gliomas. However, the YKL-40 does not significantly correlate with response to therapy. We have now identified MMP-9 as a potential marker for response to therapy in gliomas. MMPs are proteases that degrade extracellular matrix proteins, and MMP-9 is expressed by human gliomas and associated with tumor invasion and progression. Serum MMP-9 levels were determined by ELISA assay prospectively and correlated with magnetic resonance imaging (MRI) scans in a prospective longitudinal study. The tumor response was determined by standard criteria: complete response (CR), partial response (PR), stable disease (SD) and progression of disease (P). For each MRI, we analyzed serum samples from 57 patients with anaplastic gliomas (AG) and 48 patients with glioblastoma multiforme (GBM) (range, 1–12 samples; median of 4 and 3 per patient, respectively). For AG the mean value of MMP-9 was 232 ng/ml for patients who had a radiographic CR, 196 ng/ml for PR, 372 ng/ml for SD, and 519 ng/ml for POD. For GBM, the mean value of MMP-9 was 190 ng/ml for CR, 356 ng/ml for PR, 425 ng/ml for SD, and 562 ng/ml for POD. To determine if MMP-9 levels predict the response categories, we used a cumulative logit model that corrected for multiple measurements per patient. This analysis showed a significant difference of MMP-9 values per response type for AG (P = 0.002) and a marginally significant difference for GBM (P = 0.08). Similar to what is seen with YKL-40, the MMP-9 levels transiently increase immediately following surgery reaching the maximum level at 48 h after tumor resection (mean 174 ng/ml and 1070 ng/ml, respectively) and decreased over the next two weeks. Therefore, reliable levels must be obtained at least two weeks post-surgery. In conclusion, serum levels of MMP-9 correlate with response type at least for AG. Ongoing longitudinal studies are designed to (1) assess correlation with survival and (2) determine whether MMP-9 can complement YKL-40 as a reliable serum marker for disease status in patients with high-grade gliomas and be used to guide clinical decisions during a patient’s disease course.

181. CLINICAL SIGNIFICANCE OF EGFR AMPLIFICATION AND THE EGFRVIII TRANSCRIPT IN CONVENTIONALLY TREATED ASTROCYTIC GLIOMAS

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The aim of this study was to evaluate the clinical value of assessing amplification and the common 5’ rearrangement of the EGFR gene (EGFRvIII) in 223 astrocytic tumors by correlating the data with patient survival. Previous studies have evaluated amplification alone and provided contradictory results. Amplification was analyzed by densitometry of Southern blots or quantitative PCR, and the EGFR transcripts were examined by RT-PCR and sequenced to identify alterations. In addition, RNase protection assay was carried out on a subgroup of the tumors to confirm the PCR results. We found no significant association between EGFR amplification or rearrangement and survival in a series of 160 glioblastoma patients. There was no association between EGFR status and age in this patient group. We noted a tendency toward decreased survival in the 42 patients with anaplastic astrocytomas with any EGFR abnormality. This was most marked for the EGFRvIII rearrangement (P = 0.061). The latter anaplastic astrocytoma patients were significantly older than those without 5’ rearrangements (P = 0.020). No EGFR abnormalities were identified in the astrocytoma patients. We conclude from this large set of astrocytic tumors that neither EGFR amplification nor the presence of the EGFRvIII transcript predicts patient outcome in conventionally treated glioblastomas. In anaplastic astrocytomas, however, although uncommon, EGFR aberrations are associated with shorter survival.

182. MGMT PROMOTER HYPERMETHYLATION IS MORE FREQUENT IN SECONDARY Glioblastomas AND IS INDEPENDENT FROM OTHER PROGNOSTIC FACTORS

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O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that specifically removes mutagenic, carcinogenic, and cytotoxic O6-alkylguanine DNA adducts induced by alkylating agents like nitrosoureas. Repair of cytotoxic DNA damage by MGMT is a potentially important factor of resistance to alkylating agents, commonly used in the treatment of glioblastoma multiforme (GBM). Using methylation-specific PCR (MSP) we investigated the inactivation of the DNA-repair gene MGMT by promoter hypermethylation in 67 GBMs obtained from patients subsequently treated by conventional radiotherapy and CDDP + BCNU. We observed that the MGMT gene was methylated in 24 patients (36%). This finding was associated with prolonged overall survival (24 vs. 14 months; log-rank P = 0.002) and with a longer progression-free survival (PFS) (11.3 vs. 7.9 months; log-rank P = 0.03). Among these 67 GBMs, secondary GBMs had prolonged overall survival (26 vs. 14 months; log-rank P = 0.01) than de novo tumors, whereas other prognostic factors were not statistically associated with ST or PFS. Moreover, the frequency of MGMT methylation was higher in secondary than in primary GBMs (78% vs. 22%, P = 0.01), but was not associated with age, KPS, and RTOG class risk. Other genetic markers (LOH on chromosome 19q and chromosome 17p, EGFR amplification and p53 mutation) were used to assess their influence on the treatment response and overall survival of patients with GBM. These findings indicate the relevance of epigenetic and genetic factors in the prognosis of glioblastomas.

183. HYPOXIA INDUCIBLE FACTOR-1 ALPHA EXPRESSION CORRELATES WITH HISTOLOGICAL GRADING IN HUMAN GLIOMAS

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Hyoxia inducible factor (HIF-1) is considered to play an important role in the adaptation of cells to hypoxia. HIF-1α as a hypoxia-inducible factor (HIF) regulates the expression of several genes that encode proteins involved in the regulation of angiogenesis. The aim of this study was to evaluate the expression of HIF-1α in various astrocytic tumors. HIF-1α expression was assessed by immunohistochemistry in 35 specimens of supratentorial astrocytomas (15 glioblastomas, 11 anaplastic astrocytomas, and 9 low-grade astrocytomas). Histological
184. MICROGLIA IN GEMISTOCYTIC ASTROCYTOMAS
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Gemistocytic astrocytomas behave more aggressively than other diffuse gliomas. Gemistocytic tumor cells express MHC class II molecules, and a high fraction of gemistocytes is present. The mean Ki-67 labeling index was 20.4% in the strongly and moderately HIF-1 alpha expression group and 8.5% in the no HIF-1 alpha expression group. There was correlation of HIF-1 alpha expression with VEGF expression, Ki-67 labeling index, and histological grading. Malignant astrocytomas with high degree of HIF-1 alpha, which contributes to angiogenesis via VEGF. Furthermore, HIF-1 alpha would correlate with tumor cell proliferation. These results suggest that neovascularization due to upregulation of HIF-1 prevents hypoxic damage and permits tumor cell progress.

185. PROGNOSTIC VALUE OF MGMT METHYLATION, TP53 MUTATION, AND MDM2, EGFR, OR CDK4 AMPLIFICATION IN MALIGNANT GLIOMA PATIENTS TREATED WITH ADJUVANT TEMOZOLOMIDE CHEMOTHERAPY
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The response of malignant gliomas to chemotherapy with alkylating drugs such as temozolomide (T) varies from patient to patient. The present study investigates the significance of tumor-associated genetic aberrations in the TP53, MDM2, EGFR, and CDK4 genes, as well as the methylation of the MGMT promoter for response to T and overall survival (OS) of patients with malignant gliomas. Fifty-one patients with malignant gliomas (anaplastic WHO grade II [AG], n = 14, WHO grade IV [GBM], n = 37) were treated with T as first-line chemotherapy after tumor resection and radiation therapy. From each patient, clinical data were systematically recorded, including index of resection, KPS, performance score (KPS), extent of resection, tumor volume (determined before and after resection, before chemotherapy, and every 3 months), OS, and response to treatment. The tumor tissue was investigated for mutations in the TP53 gene (exons 5–8), amplification of the EGFR, CDK4 and MDM2 genes; and hypermethylation of the MGMT promoter. Molecular findings were correlated to OS and response to T. Fifty-nine percent of the patients were men, and median age at surgery was 54.3 years. The mean preoperative KPS was 80%. OS were 277.4 weeks (AG) and 87 weeks (GBM). A median number of 8 cycles of T was administered. Grade 3 or 4 toxicity occurred in 6 patients. T was terminated because of tumor progression in 31 patients (4 AG, 29%; 27 GBM, 73%). Thirty-three percent of tumors demonstrated EGFR amplification (17% AG, while MDM2 amplification was detected in 7% (8 AG, 6% GBM), CDK4 amplification in 16% (8 AG, 18% GBM), and TP53 mutation in 20% (12.5% AG, 22% GBM). MGMT hypermethylation could be demonstrated by a 78% (66.6% AG, 46% GBM). Statistical results revealed that MGMT hypermethylation was significantly associated with longer OS (P = 0.0013, log-rank test) and better response to T (P = 0.034, log-rank test). Neither EGFR, MDM2, and CDK4 amplification nor TP53 mutation, alpha expression were correlated to OS or response to T. In line with recent publications from other groups, our study clearly indicates MGMT hypermethylation as a clinically important molecular marker that is significantly associated with longer overall survival and better response to temozolomide treatment with malignant gliomas. Genetic alterations in the TP53, MDM2, EGFR, and CDK4 genes appear to be less important as prognostic factors. This study was supported by grants (70-3088-Sa 1) from Deutsche Krebshilfe.

186. SECRETORY MENINGIOMAS AS MORPHOLOGICALLY DISTINCT MENINGIOMA ENTITY
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The unique features, both concerning clinical and pathological aspects, of secretory meningiomas have not been completely understood so far. We present a hitherto not noticed pathological finding that could shed new light upon this rare entity. Fourteen cases of secretory meningiomas could be identified out of more than 1,300 meningiomas from our institutions during the last 25 years. Tumors were retrospectively analyzed regarding their clinical and pathological features with special emphasis upon mechanisms that might explain the clinically observed common edema. The main microscopic finding was the occurrence of a significantly higher proportion of mast cells in secretory meningiomas compared to other unselected meningioma subtypes: mean 2.43 (range, 0.020–5.60) versus 0.27 (range, 0.00–2.00; P = 0.0001, Mann-Whitney-U-test). These mast cells can be visualized and counted with the CD-117 immunoreaction, an antibody directed against the product of the c-kit oncogene. Mast cells were identified in close connection to vessel wall (pericytic) proliferations. Electron microscopy of such proliferated vessels revealed increasing vacuolization in the peripheral layers of proliferated vessels, constantly adjacent to small edematous areas, in which the mast cells often were freely floating. Yet, we could not establish a positive correlation between microscopic mast cell density and macroscopic edema as found by CT scans in respective cases. Furthermore, the pseudopsammoma bodies that characterize these tumors are surrounded by cells that react vividly with cytokeratins. Cytokeratin subgroups such as CK 7, CK 8, and CK 5/6 are equally found at random and in combinations; only CK 20 was regularly absent from these cells. Further attempts to characterize mast cells and secretory products by a panel of tissue hormones (serotonin and others) failed to give clues as to the action of mast cells and to the pathogenesis of pseudopsammoma bodies (secretory products) within these tumors. Pericytic vessel proliferation, edema formation of secretory meningiomas, and the newly described occurrence of mast cells may well be correlated. The further analysis of these features and of their connection to the formation of pseudopsammoma bodies is difficult because these tumors are as rare, only approximately 1% of all meningiomas.
troscopy data was performed, and the following ratios were calculated: (i) choline/creatine, (ii) choline/nAA, (iii) choline/creatine+NA, (iv) lactate/creatine, (v) choline/creatine+pH, (vi) choline/creatine+NA/histidine. Five Cox proportional hazards models were constructed, (i) WHO grade, (ii) histopathological features of the tumor, (iii) MR spectroscopy, (iv) MRI characteristics of the tumor, and (v) combined MRI/MR spectroscopy. Each model also included covariates for age at diagnosis and information on whether surgical debulking and/or further oncological treatment occurred. Because some of the patients remained alive at the time of analysis, the Kaplan-Meier algorithm was used to calculate the survival function. The survival predicted by each model was compared to the actual survival at three months, one year, and two years.

Oligodendrogliomas frequently show allelic loss of chromosomal arms 1p and 19q. These deletions are associated with an improved response to chemotherapy and longer overall survival when compared to tumors with no such allelic loss. The assessment of the genetic status of 1p/19q is routinely performed by analyzing the primary neoplasm, while the implications of the re-evaluation of the recurrent tumor is unclear. Our objective was to evaluate the status of 1p and 19q in both the primary and the recurrent oligodendrogliomas. The status of 1p/19q was evaluated from paired tumor blood DNA samples by using PCR-based microsatellite analysis. Ten tumors of 5 patients were evaluated and included the primary and recurrent neoplasm. For 3 patients, initial diagnosis was oligodendroglioma (WHO II, 2 pts; WHO III, 1 pt). Two of these tumors had codeletions at 1p and 19q chromosomes, and one had no loss. At recurrence, the two low-grade tumors transformed to a higher grade and were now diagnosed as anaplastic oligodendroglioma (WHO III). The repeat evaluation of 1p/19q status showed again, a combined allelic loss. The anaplastic tumor with no initial loss remained +1p/+19q. Two other patients had an initial diagnosis of an oligoastrocytoma, one low grade and the other anaplastic (WHO III). The low-grade tumor had codeletions at 1p/19q, and the anaplastic tumor had an intact 1p with a deletion at 19q. At recurrence, both tumors were reevaluated by anaplastic biopsy, obtained from a part of the tumors that demonstrated radiographic changes but were located at sites of the residual tumors that were not resected on initial surgery. The pathology of the low-grade tumor has not changed, but no deletions were found in the recurrent neoplasm. The anaplastic tumor transformed to WHO IV grade, and this recurrent tumor presented no deletions. Our findings may indicate that pure oligodendrogliomas are of monoclonal origin, while mixed oligoastrocytomas probably contain a biallelic population. It is possible that different parts of the heterogeneous tumors represent different clonal expansion. Alternatively, it can not be excluded that over time and following treatment, the more resistant clones that do not manifest deletions may overtake and become dominant.

189. ANAPLASTIC ASTROCYTOMA PATIENTS WITH GLOBLASTOMA-LIKE TUMOR GENOTYPE HAVE A POOR OUTCOME

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Anaplastic astrocytoma (AA, WHO grade III) is, second to glioblastoma, the most common and most malignant type of adult CNS tumor. Since survival for patients with AA varies markedly and there are no known useful prognostic or therapy response indicators, the primary purpose of this study was to examine whether the knowledge of genetic abnormalities in AA had any value in this regard. The survival data on 37 carefully sampled AA was correlated with the results of a detailed analysis of the status of 9 genes involved in the development of astrocytic tumors. These included 3 genes coding for proteins in the p53 pathway (i.e., TP53, p14ARF, and MDM2), 4 in the Rb1 pathway (i.e. CDKN2A, CDKN2B, RB1 and CDK4) and PTEN and EGFR. We found that abnormalities in at least one of the four genes (CDKN2A, CDKN2B, RB1 and CDK4) for coding imbalances of the Rb1 pathway were associated with shorter survival (P = 0.009). This finding was consistent in multivariate analysis, including adjustment for age (P = 0.015). The findings suggest that analysis of the genes coding for Rb1 pathway components provides additional prognostic information in AA patients.

190. PRIMITIVE NEUROECTODERMAL TUMOR ARISING WITHIN LOW-GRADE ASTROCYTOMA: A REPORT OF THREE CASES

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We present three cases of primitive neuroectodermal tumor (PNET) arising in low-grade astrocytomas treated with radiotherapy. One case was identified at clinical presentation, two from a review of our histopathology database. All three patients had histologically proven low grade astrocytomas and had received radiotherapy following biopsy. Two patients had partial resection for recurrence, one at five years post-surgery and the other at one year following histological confirmation of low-grade astrocytomas. The third patient had a recurrence at the same site nine and 29 years after initial diagnosis and further surgery. Histology now showed PNET (WHO grade 4). Two patients died with one year of final surgery, one with PDGF-a activational analysis of B-RAF, a member of RAF kinase family, are frequently altered in gliosarcoma, a member of RAF kinase family, are frequently altered in gliosarcoma. B-RAF mutations. Activating analysis of B-RAF, a member of RAF kinase family, are frequently altered in gliosarcoma, a member of RAF kinase family, are frequently altered in gliosarcoma. B-RAF mutations. Activating mutations of B-RAF, a member of RAF kinase family, are frequently found in several tumors, mainly melanomas. The evaluation of this pathway is of great importance, owing to the fact that signal transduction mediated by c-KIT and PDGF receptors can be efficiently blocked by specific tyrosine kinase inhibitors such as Imatinib (Gleevec, Novartis). Gliosarcoma is a rare and poorly characterized malignant brain tumor that exhibits a biphasic tissue pattern with areas of glial and mesenchymal differentiation. Molecular studies showed that gliosarcomas display a genetic profile similar to glioblastomas and support the concept of monoclonal origin of both components. The aim of this study was to characterize the molecular alterations of PDGF subfamily of receptor tyrosine kinases pathway in gliosarcomas. Immunohistochimistry for PDGF-A, PDGF-B, PDGF-C, and c-KIT were analyzed in both components of six gliosarcomas. Activating mutations in PDGF-A (exons 12 and 18) and c-KIT (exons 9, 11, 13, and 17) genes were studied by PCR followed by direct sequencing. B-RAF gene (exons 11 and 15) was screened by using PCR-SSCP followed by direct sequencing. Expression of PDGF-A was found in all cases: three with similar moderate to intense immunoeexpression in both glial and mesenchymal components, two with predominant intense expression in the glial component, and one case with predominant moderate mesenchymal expression. Co-expression of PDGF-A was observed in three cases, mainly in glial component. c-KIT immunopositivity was observed in both components of one case. The muta- tional analysis of PDGF-A was performed on 11 cases. Five cases showed an intrinsic insertion in two cases, and a 2472C->T silent mutation in two cases. No mutations were detected in c-KIT and B-RAF genes. PDGF-A was overexpressed in gliosarcomas. PDGF-A was overexpressed in 50% of cases, predominantly in the glial component supporting an autocrine loop pattern.
192. EXPRESSION OF CD15 ON NEOPLASTIC CELLS OF INTRINSIC BRAIN TUMORS: A MODULATORY ROLE IN METASTASIS

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CD15, the adhesivity sialyl LewisX oligo saccharide, acts as the cellular ligand for E-, L- and P-selectins as well as binding homophilically. It is widely distributed in epithelial cells and granulocytes. Within the developing normal brain neurones, astrocytoma, oligodendroglioma, and Bergmann glia have also been reported to express this epitope. CD15 is also strongly expressed in many highly metastatic somatic cancers and is thought to mediate tumor cell adhesion to vascular endothelium prior to extravasation, enabling entry to, and colonization of, new sites for metastatic spread. We have previously shown that CD15 is expressed by experimental rat and mouse gliomas but that human glioma cells in vitro are invariably negative (Martin et al., Anticancer Res. 15, 1159, 1995). The ability of such cells to adhere to non-brain vascular endothelial cells was also seen to be poor. In the present study we examined archival histological sections from 19 cases of intrinsic brain tumor (including astrocytoma, anaplastic astrocytoma, oligodendroglioma, and glioblastoma multiforme) from which extraneural metastatic spread was apparent. In each of these cases, taken from five different hospitals, sections of the original tumor plus the secondary deposit (in chest wall, lymph nodes, scalp, bone marrow and lung) were examined by immunohistochemical staining using the DAKO anti-CD15 antibody (MO733). While CD15 expression was seen in infiltrating heterogenous cells, and discounted, expression on neoplastic cells within both primary and secondary sites was seen. Corresponding primary site “control” cases for the various histological types of tumor that had not been seen to metastasize showed CD15 positivity for neoplastic cells in only one case (glioblastoma multiforme). We conclude and postulate that expression of CD15 in primary brain tumors may indicate a possible propensity to spread outside the nervous system by virtue of facilitating adhesion of neoplastic glia to non-brain vascular endothelium. The Sama thra Dickson Research Trust is gratefully acknowledged for their support of this research.

193. YKL-40 EXPRESSION IS ASSOCIATED WITH ACTIVATED PI3K AND MAP-KINASE PATHWAYS IN Glioblastoma: IMPLICATIONS ON AGE AND SURVIVAL

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We have previously shown that YKL-40 is overexpressed in glioblastoma (GBM) and its overexpression is associated with poorer response to radiation and reduced survival time. YKL-40 has been shown to activate the PI3K/Akt and MAPK pathways in vitro. Therefore, in this study, we test for an association of YKL-40 with activated intermediates of these pathways in GBM tumor specimens. The interaction between age and molecular marker status, with respect to overall survival (OS), is analyzed as well. The study set comprised 294 patients with newly diagnosed GBM who underwent resection from 1994 to 2003. Immunohistochemical staining was performed on patient tissue for YKL-40, activated (phospho-) species of the AKT pathway (p-AKT, p-mTOR, and p-70S6K) and MAPK. Statistical analysis revealed a significant association between decreased OS and increased YKL-40 expression in patients with newly diagnosed GBM (p = 0.003, Kaplan-Meier). Expression of YKL-40, p-mTOR, p-70S6K and p-MAPK were highly correlated with each other (p = 0.009, Spearman). There was a trend in correlation between YKL-40 and p-AKT. Expression of YKL-40, p-AKT, and p-MAPK correlated with advanced age (all p < 0.01). YKL-40, p-mTOR, p-70S6K, and p-MAPK expression levels were associated with decreased overall survival within the entire patient set (all p < 0.02, Kaplan-Meier). However, nearly all of the survival variation was explained by the markers was observed within the young patient population. Specifically, among patients younger than the median age (39 years), univariate analyses showed YKL-40, p-mTOR, p-70S6K, and p-MAPK to be prognostic factors (all p < 0.01). Multivariate analysis (Cox regression) revealed that lower KPS (HR 3.8, P = 0.03) and YKL-40 positivity (HR 1.8, P = 0.03) were independent prognostic factors after adjusting for extent of surgery, and p-mTOR, p-70S6K, and p-MAPK staining scores. None of these factors were significant in univariate analysis within the older patient group. We have previously shown that YKL-40 activates the AKT and MAPK pathways is supported by the demonstrated correlation with these activated species in patient tumor specimens. While these markers are in general more prevalent in older patients, the survival differences of these markers is far greater than on older patients. These findings may explain the survival differences between older and younger patients with GBM. The presence of these markers in patients younger than the median age for GBM (55–60) should be considered in risk stratification.

194. INVESTIGATION OF THE EGFR-GENE AMPLIFICATION STATUS DETERMINED BY FISH AS A PREDICTIVE OR PROGNOSTIC MARKER FOR PATIENTS WITH RECURRENT HIGH-GRADE GLIOMA TREATED WITH TEMOZOLOMIDE


The epidermal growth factor receptor (EGFR) gene is frequently amplified and over-expressed in glioblastoma (HGG). Immunohistochemical staining for the EGFRVIII mutant is a negative prognostic factor for patients with anaplastic glioma (Buckner et al., J. Clin. Oncol. 22 (14S), 1508, 2004). We investigated the prognostic and predictive value of EGFR- gene amplification determined with fluorescence in situ hybridization (FISH) in HGG treated with temozolomide (Temozol, TMZ) at recurrence following surgery and radiotherapy. Clinical data and tumor material were collected from a retrospective cohort of patients treated at 4 Belgian hospitals. All patients were treated with TMZ at recurrence. In addition to conventional diagnostic histopathology, HGG was characterized by FISH for the amplification status of the EGFR gene (Vysis, LSI EGFR/C EP7 Dual Color Probe). Our patient population consisted of 36 men and 22 women (N = 58) with a median age of 58 years (range, 16–80 years). At the initiation of TMZ treatment, tumors consisted of 14 AA/AOA and 44 GBM. Eighteen tumors were found to have an amplification of the EGFR gene (4/14 AA/AOA, 14/44 GBM). No significant association was found between EGFR-gene amplification and sex, age (at diagnosis or at initiation of TMZ), glioma localization, type of surgery (resection versus biopsy), histology (at diagnosis or at initiation of TMZ), the interval between diagnosis of HGG and the initiation of TMZ, or response to TMZ (objective response or disease control). We found no significant correlation between EGFR-gene amplification and time to progression or overall survival following the initiation of TMZ treatment (Kaplan-Meier survival statistics, log-rank test, P > 0.3). In a subgroup analysis of the 17 patients with an AA/AOA or secondary GBM, EGFR-gene amplification was associated with an inferior overall survival (log-rank test, P = 0.04). We could not demonstrate a predictive or prognostic value of EGFR-gene amplification determined with TMZ. Our observations support the concept that EGFR- amplification is an independent prognostic factor for OS in patients with an AA/AOA or secondary GBM.

195. LOW SERUM S100ß LEVELS IN PATIENTS WITH NEWLY DIAGNOSED LUNG CANCER CORRELATE WITH AN ABSENCE OF BRAIN METASTASES ON MRI

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Early detection of metastatic brain tumors in patients with systemic cancer may provide these patients with less invasive treatment options. We have previously observed that disruption of the blood-brain-barrier (BBB), which occurs in the setting of metastatic brain tumors, is accompanied by a release of the astrocytic protein S100ß into the serum where it can be detected by use of a simple, relatively low cost assay. We hypothesized that measurement of S100ß in the serum may serve as a screening test for cerebral metastases in individuals with lung cancer. Individuals with newly diagnosed non-small cell lung cancer were enrolled in the study if there were no symptoms or signs of neurologic dysfunction suggestive of the presence of metastatic brain tumors. Each participant underwent MRI of the brain and had serum drawn with measurement of the S100ß level. The level of S100ß was compared to the results from MRI imaging.

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Thirty-eight individuals agreed to participate in the trial. Seven of the 38 had evidence of metastatic disease on MRI of the brain, and 22 had normal MRI scans. The remaining 9 patients had no brain metastases but had 12 classic clinical signs of cerebral vascular disease in addition to chronic microvascular disease were lower in those with normal MRIs than those with chronic microvascular disease or metastatic disease (0.07, 0.49, and 0.28 μg/liter respectively; P < 0.002 for patients with normal MRIs compared to those with chronic microvascular disease, and P < 0.03 for normal MRIs compared to those with metastatic disease). Utilizing a cutoff of 0.12 μg/liter the sensitivity of S100β for cerebral metastatic disease was 100%, specificity was ~13%, negative predictive value was 1, and positive predictive value (PPV) was 0.47. If patients with chronic microvascular disease were excluded, the specificity was 86% and the PPV was 0.88. Low serum levels of S100β (< 0.12 μg/liter) accurately identified lung cancer patients who do not have metastatic brain tumors. However, an elevated serum S100β in this group of patients does not specifically indicate the presence of brain metastases. Nevertheless, the results of this study suggest that assessment of serum S100β can be used to narrow the population of lung cancer patients who may benefit from prospective surveillance imaging.

196. LOSS OF HETEROZYGOSITY (LOH) OF 1p IN OLIGODENDROGLIAL TUMORS IS RELATED TO REDUCED EXPRESSION OF NUCLEAR FACTOR KAPPA B (NFKB)

In oligodendrogliomas, LOH on chromosome 1p is associated with tumor chemosensitivity. The molecular mechanism for this association is unknown. Nuclear factor kappa B (NFκB) is a transcription factor found in the cytoplasm as hetero- or homodimer that exerts its effects through binding to nuclear DNA and activating transcription of target genes. While the ultimate gene targets of NFκB are diverse, its activation can block apoptosis in numerous tumor cell lines. Moreover, NFκB inactivation is associated with chemotherapy resistance (3). To the best of our knowledge, this is the first study to address NFκB’s role in glioma chemosensitivity. We postulated that the chemosensitivity of oligodendrogliomas may be related to reduced NFκB activation. Our objective was to evaluate NFκB activation in oligodendrogliomas and its relationship to 1p status. We evaluated 41 oligodendrogliomal tumors (WHO II, 26; WHO III, 15) for their LOH status by PCR. Activated NFκB which undergoes nuclear translocation was assessed by immunohistochemistry (IHC) of the p65 subunit. The level of NFκB activation was defined as high (50%–100% nuclear staining), intermediate (10%–50%) or low (< 10% nuclear staining). All tumors (100%) with an intact 1p (n = 17) had high activation of NFκB as opposed to 43% (10/23) of tumors with 1p LOH. NFκB level of activation differed significantly (P < 0.02) between tumors with or without 1p LOH. This difference was more pronounced in the WHO II group, where high activation was found in all 11 tumors with intact 1p, but in only 30% (5/17) of tumors with 1p loss (P = 0.0004). We suggest that low NFκB activation plays a role in the chemosensitivity of oligodendrogliomas with 1p LOH. Recent publications demonstrated that TNFa has a central role in NFκB activation and in protection against apoptosis. The deleted region < 0.1p (1p36) contains some of the tumor necrosis factor receptor superfamily (TNFRSF) such as TNFR2 that was shown to mediate NFκB activation. Further investigations are needed in order to conclude whether a deletion of TNFRSF at 1p site can induce chemosensitivity as a result of reduced NFκB activation.

197. DETERMINATION AND PROGNOSTIC SIGNIFICANCE OF MITOTIC INDEX IN GRADE II AND III INFILTRATING ASTROCYTOMAS USING THE MITOSIS MARKER PHOSPHO-HISTONE H3

Histologic classification of infiltrating astrocytomas into malignancy grade III astrocytomas confirmed that mitotic activity, as determined by the presence of mitotic activity, is a significant independent predictor of survival in grade II and grade III astrocytomas. An index of 2 mitoses or less per 1000 cells in the most mitotically active area appears to define a group of tumors with survival time typical of grade II tumors, while an index of 3 or greater portends a prognosis typical of grade III anaplastic astrocytoma.

198. NESTIN, CXCL12/SDF1, PDGFR-BETA AND VEGF EXPRESSION IN ENDOTHELIAL PROLIFERATION ON 80 GLIOMAS: CLINICOPATHOLOGICAL CORRELATIONS AND SUGGESTIONS FOR ANGIOGENESIS

Nestin is a class VI intermediate filament (IF), highly expressed in embryonic progenitor cells and is a marker for multipotential neural- and endothelial stem cells, involved in early stages of lineage commitment, in proliferation and in differentiation. Nestin is detected in neuroepithelial tumors and in endothelial cells in active proliferation. The VEGF expression upregulates CXCR4 on endothelial cells, binding the proangiogenic chemokine SDF1/CXCL12 (Stromal Derived Factor). CXCL12 have a role on angiogenesis and chemotaxis of endothelial cells throughout the activation of its receptor. PDGFR in healthy tumor tissue is expressed in endothelial cells, and is overexpressed in gliomas in angiogenic endotelial cells. To investigate the amount and the meaning of endothelial proliferating cell–expressed nestin related to the presence of VEGF, SDF1/CXCL12, and PDGFR-beta in gliomas, we performed an immunohistochemical study, with their specific antibodies, on 80 patients with gliomas of different phenotype and malignancy grade. The patients were categorized by disease: 20 glioblastomas, 12 anaplastic astrocytomas, 12 anaplastic oligoastrocytomas, 4 anaplastic oligodendrogliomas, 10 oligoastrocytomas, 12 oligodendrogliomas. Moreover, all the cases were immunostained for GFAP, vimentin, Ki67/MIB1, and CD34. Nestin was variably expressed in neoplastic cells and in endothelial cells in low-grade gliomas, with shorter time to tumor progression suggests a role in early angiogenesis. A correlation was found also to expression of VEGF and PDGFR-beta. These results suggest that some cells expressing nestin, a marker of neural progenitor cells, stimulated by growth factors and chemokines, may be involved in angiogenesis as proliferating endothelial cells. Our data, especially in low-grade gliomas, might provide useful information related to angiogenesis and might add prognostic information in patients with variable life expectancy.

199. LOSS OF HETEROZYGOSITY ON CHROMOSOME 1p 1q 1q9 IS A MAJOR PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL IN LOW-GRADE GLIOMAS

The morphologic classification of low-grade gliomas remains controversial and insufficient. To investigate whether commonly described genetic alterations have prognostic significance, search for loss of heterozygosity (LOH) on chromosome 1p, 9p, 10q, 19q, EGFR amplification, and p53 expression was performed in a series of L1 low-grade gliomas. The profile of molecular alterations was then correlated with clinical parameters and
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200. A GENETIC POLYMORPHISM OF THE CHEK2 GENE AS A PROGNOSTIC FACTOR IN GLOBLASTOMA MULTIFORME

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CHEK2 gene germline mutations have been identified in some families with Li-Fraumeni syndrome. Li-Fraumeni patients suffer from various malignancies including glioblastoma multiforme (GBM). The CHEK2 gene is located on chromosome 22q11.2. Up to 30% of sporadic GBMs may harbor LOH (loss of heterozygosity) for markers from chromosome 22q. The CHEK2 gene product is a negative cell cycle regulator and important effector of the DNA damage control pathways. We tested the hypothesis that a genetic polymorphism of the CHEK2 gene might modify the incidence and the clinical course of sporadic human GBM. DNA was isolated from peripheral blood of 213 patients with primary GBMs and 192 control subjects. Subjects with glioblastoma were compared with respect to CHEK2 IVS1-711A/T (single nucleotide polymorphism), CHEK2 IVS1-711A/T, using PCR and restriction digestions. For all patients the following clinical parameters were recorded: age, sex, histology (GBM NOG, giant cell GBM, gliosarcoma), localization, degree of resection (biopsy, subtotal, gross total resection), postoperative radiotherapy, postoperative chemotherapy, postoperative Karnofsky performance score. No association between the CHEK2 SNP and GBM of resection (biopsy, partial, subtotal and gross total resection), postoperative histology (GBM NOS, giant cell GBM, gliosarcoma), localization, degree of resection (biopsy, subtotal, gross total resection), postoperative radiotherapy, postoperative chemotherapy, postoperative Karnofsky performance score, and postoperative radiotherapy and postoperative chemotherapy and postoperative Karnofsky score (all P < 0.0001, log–rank test). This study confirms the important role of well-known clinical prognostic factors such as age, Karnofsky performance index, and postoperative radiotherapy. Our data support the use of cytodestructive surgery and chemotherapy for GBMs. Most interestingly, this investigation suggests a genetic polymorphism as a prognostic marker for GBMs. Since CHEK2 is involved in the detection and repair of DNA damage (e.g. after chemotherapy), possible explanations include differential chemosensitivity mediated by different CHEK2 genotype. This information may be important when considering the (high) probability of sampling error and treatment of these tumors, and glioma classification in general. For this study a selected group of 14 gliomas, consisting of 6 anaplastic oligodendrogliomas, 6 mixed anaplastic oligoastrocytomas, and 2 glioblastomas, all with partially classical oligodendroglial and other histology, were used. Within each tumor, up to 8 regions (total number of regions studied was 60) were characterized morphologically and genotyped by fluorescent in situ hybridization (FISH) for 1p and 19q. Loss of 1p was found in 8/14 tumors and loss of 19q in 7/12 tumors. Fifty percent of the losses of 1p were detected in all tumor areas, while the other 50% were only seen in part of the tumors. In 57% of tumors, loss of 19q was found in all tumor areas, and in the other 43% only in parts of the tumors. Loss of 1p or 19q was detected in tumor areas other than those with classical oligodendroglial histology in 47% of cases, and loss of 19q in 45% of cases. The results illustrate that, besides phenotypical heterogeneity, there is also genotypical heterogeneity and that genotyping of gliomas without classical oligodendroglial histology may result in positive identification of 1p/19q losses. This may be relevant for small (stereotactic) biopsies. Alternatively, these findings may reflect limitations of the tests used; therefore, further research is indicated.

201. MORPHOLOGICAL AND MOLECULAR HETEROGENEITY WITHIN TUMORS WITH OLGODENDROGLIAL CHARACTERISTICS

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Gliomas are known for their morphologic heterogeneity. Generally, gliomas are typed according to areas with classic histological parts, but within morphologically lesional tissue often less typical histology exist. The classic morphology of oligodendrogliomas is the honeycomb architecture with chicken wire-like vasculature, the tumor cells having a perinuclear shrinkage artifact. However, tumor parts showing astrocytoid differentiation, mixed histology, pseudoparenchymal changes, rhabdoid changes, or mucoid degeneration are variably encountered in oligodendroglial tumors. This protein remains controversial, it does appear to be involved in cell division and signal transduction through both G-protein and Ca1 binding proteins. A-PROTEIN expression in glioblastoma is normally localized to the intracellular plasma membrane. It is located in both normal and neoplastic tissue. Abnormal expression of A-PROTEIN has been identified in a number of tumors including small cell carcinoma of the lung, carcinoma of the breast, colon, prostate, and cervix. Brain tumors have been reported to express exceedingly high plasma levels of A-PROTEIN and thus be of significant diagnostic and prognostic value. Pediatric neuro-oncology patients were prospectively identified from the neuro-oncology program at Children’s Hospital Boston and the Dana Farber Cancer Institute. Patients included those with newly diagnosed disease pre- and post-surgery, during treatment, and during routine follow-up while maintaining remission (based on conventional MRI scans) as well as at the time of diagnosis, progression, or recurrence. A-PROTEIN levels were evaluated. Of these samples, 46% were positive, while 29% were negative and 25% were equivocal. For patients with stable disease, 73 samples were obtained, and 51% demonstrated a negative A-PROTEIN level, 19% of these samples were positive and 30% were equivocal. By contrast, 20 samples from patients with progressive disease were evaluated. Of these, 35% had normal A-PROTEIN levels, 35% were positive, and 10% were equivocal. Based on these results, the sensitivity of the assay was 39% and the specificity was 82%. Further research is needed to clarify these findings. These findings may reflect limitations of the tests used; therefore, further research is indicated.

202. A PROSPECTIVE, BLINDED ANALYSIS OF A-PROTEIN LEVELS IN PEDIATRIC PATIENTS WITH CENTRAL NERVOUS SYSTEM TUMORS

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The purpose of this prospective blinded study was to evaluate the sensitivity and specificity of A-PROTEIN levels in pediatric patients with brain tumors. A-PROTEIN is a 23 kD molecule that is highly conserved among mammals and non-mammalian species and was initially named for its presumed role in adenosine nucleotide exchange. While the exact function of this protein remains controversial, it does appear to be involved in cell division and signal transduction through both G-protein and Ca1 binding proteins. A-PROTEIN expression in glioblastoma is normally localized to the intracellular plasma membrane. It is located in both normal and neoplastic tissue. Abnormal expression of A-PROTEIN has been identified in a number of tumors including small cell carcinoma of the lung, carcinoma of the breast, colon, prostate, and cervix. Brain tumors have been reported to express exceedingly high plasma levels of A-PROTEIN and thus be of significant diagnostic and prognostic value. Pediatric neuro-oncology patients were prospectively identified from the neuro-oncology program at Children’s Hospital Boston and the Dana Farber Cancer Institute. Patients included those with newly diagnosed disease pre- and post-surgery, during treatment, and during routine follow-up while maintaining remission (based on conventional MRI scans) as well as at the time of diagnosis, progression, or recurrence. A-PROTEIN levels were evaluated. Of these samples, 46% were positive, while 29% were negative and 25% were equivocal. For patients with stable disease, 73 samples were obtained, and 51% demonstrated a negative A-PROTEIN level, 19% of these samples were positive and 30% were equivocal. By contrast, 20 samples from patients with progressive disease were evaluated. Of these, 35% had normal A-PROTEIN levels, 35% were positive, and 10% were equivocal. Based on these results, the sensitivity of the assay was 39% and the specificity was 82%. Further research is needed to clarify these findings. These findings may reflect limitations of the tests used; therefore, further research is indicated.

203. RESPONSE TO RADIOThERAPY IS NOT ASSOCIATED WITH LOSS OF HETEROZYGOsITY (LOH) FOR CHROMOSOMES 1p36 AND 19q13 IN WHO GRADE II ASTROCYTIC GLIOMA

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Loss of heterozygosity (LOH) of 1p36 and 19q13 is probably both prognostic for survival and a predictive marker of chemosensitivity in patients with WHO grade II gliomas. A high proportion of patients with WHO grade II oligoastrocytomas (OA) and astrocytomas (A) respond to radiotherapy, whereas not the present study we investigated whether or not the presence of LOH of 1p36 and 19q13 might be related to response to radiotherapy in those tumors. From our database of patients with gliomas WHO grade II (OA, A) treated between 1991 and 2000, we selected patients who received radiotherapy either at primary diagnosis or after tumor progression. The tumor volume was measured and monitored after radiation therapy. Tumor response was assessed according to standard criteria and graded as complete response (CR), partial response (PR), >50%, minor response (MR), >25%, and progressive disease (PD), >25%. The presence of LOH for chromosome 1p (4 loci) and 19q (3 loci) was assessed on archival, paraffin-embedded tumor specimens
by using a PCR-technique. Thirty-eight patients received radiotherapy. There were 31A and 7 OA. The tumor volume was available for 27 patients (71%), 12 having received radiotherapy at primary diagnosis and 15 at time of progression. There was no CR, (18.3%), 8 patients MR (29.6%), 12 patients a SD (44.4%), and 2 had PD. The LOH status could be assessed in 17 and 16 patients for 1p and 19q, respectively. Of 12 patients with intact chromosome 1p36, 6 had either PR or MR, and 6 had either SD or PD. Approximately 50% of patients with astrocytic WHO grade II tumors show objective tumor response after radiotherapy. Tumor response is not associated with LOH for 1p36 and 19q13.

204. PROGNOSTIC VALUE OF 1p/19q REARRANGEMENT IN OLIGODENDROGLIOMAS: RETROSPECTIVE STUDY WITH 86 PATIENTS

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Oligodendrogliomas represent up to 25% of diffuse gliomas, the most frequent primary tumors of the central nervous system. The tumors, which present a 1p/19q co-deletion, have a better prognosis, with a higher chemoradiosensitivity and longer survival. A retrospective study of 1p/19q rearrangements in primary oligodendrogliomas treated between 1990 and 2001 at the Rennes Hospital was done. A confrontation between clinical data and factors influencing the prognosis was made. The tumors were graded by two neuropathologists. FISH (fluorescent in situ hybridization) was performed on 1.5µm thick sections from archival formalin-fixed paraffin-embedded tumors. The probes used, located in 1p36 and 19q13 regions, were developed from BAC and were directly labeled by nick translation. For each probe, hybridization signals were scored from a minimum of 200 nuclei. A univariate and a multivariate statistical analysis was performed. The prognosis was correlated with the age of the patients at diagnosis (odds ratio = 1.1), with the presence of necrosis (odds ratio = 13.7), and with and the chromosomal status (odds ratio = 0.026). We found 58% (n = 59) of tumors with a deleted status, 22% (n = 19) with a disomic status, and 20% (n = 17) with a polysomic status. Tumors with a deleted status had a better prognosis and a better response to chemotherapy than the 1p/19q disomic tumors. A third population of tumors had a polysomic status, which cannot be detected by LOH search and is hardly identified by CGH (comparative genomic hybridization). This population of patients had a poor prognosis whatever the treatment was. This study reveals the coexistence of three independent prognostic factors: age, necrosis presence, and mainly chromosomal status. Even though the deleted population is known, we reveal for the first time a new population, the polysomic status tumors population, which has a very poor prognosis.

205. IMMUNOHISTOCHEMICAL SCREENING BASED ON GENE EXPRESSION PROFILES PREDICTIVE OF MENINGIOMA RECURRENT

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Current clinicopathological models used in the diagnosis, prognostica- tion, and treatment of meningiomas do not always reliably predict tumor behavior and patient survival. A pathological tool that better distinguishes the more aggressive subset of these tumors will allow a more accurate prediction of their behavior, leading to earlier therapeutic intervention and improved patient outcome. Differential gene expression in 34 meningio- mas was analyzed by using Affymetrix Human Genome microarrays. Expression profiles based on histologic grade, primary/recurrent identity, invasion, and whether the tumor recurred within 18 years old were evaluated for correlation with survival. Pathologic data and total survival were evaluated by multivariate analysis with age, tumor site, and total survival. Morphometric analysis of nuclear size, malignant nuclear pleomorphism, and MIB-1 labeling index (LI) value have been evaluated by multivariate analysis with age, tumor size, and total survival. Morphometric analysis of nuclear size was performed by using the Eclipse 90i microscope. Tumors treated with standard postoperative radiation therapy (35 Gy to the craniospinal axis and 50 Gy to the posterior fossa) were considered for correlation with survival. Pathologic data and total survival were compared by Kaplan-Meier and log-rank analysis. Nodular/desmoplastic features were present in 50% of cases; however, none of them qualified for diagnosis and prognostication. Searches of publicly available antibody databases based on genes whose expression can predict aggressive behavior reveal candidates for further screening with antibodies on tissue microarrays. Current candidates include CD40, CD44, and SPRY1. These findings are being further correlated with histologic grade, subtype, invasion, and recurrence. The use of microarray-based expression profiling to identify prognostically significant genes that can then be analyzed by immunohistochemical screening provides a new approach to improve clinical management of meningiomas and improve overall patient outcome.

206. TEMOZOLOMIDE (TMZ) AS INITIAL TREATMENT FOR PROGRESSIVE LOW-GRADE OLIGODENDROGLIOMAS: TREATMENT RESULTS AND CORRELATION BETWEEN GENETIC PROFILE AND MGMT PROTEIN EXPRESSION

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Loss of heterozygosity (LOH) on chromosomes 1p and 19q has been associated with favorable response to chemotherapy and good prognosis in oligodendrogliomas. The DNA repair enzyme O6-methylguanine DNA methyltransferase (MGMT) may induce resistance to DNA-alkylating drugs. Recent studies show that TMZ, an oral alkylating agent, has efficacy in progressive low-grade oligodendrogliomas (LGO). Yet, limited data is available regarding the association between 1p/19q profile and MGMT protein expression in these tumors. The objective of this study was to evaluate the response of progressive LGO to TMZ and to assess the association between 1p/19q profile and MGMT protein expression. Adult patients whose MRI findings and/or clinical deterioration were compatible with progressive LGO were eligible for the study. Clinical and pathologic data was available for 204 patients. MGMT dose was 200 mg/m2/d for 5 days repeated every 28 days. Clinical and MRI data served for evaluation of outcome, and Kaplan-Meier estimates were used to assess median time to tumor progression (TTP) and progression-free survival (PFS). 1p/19q status was evaluated from paired tumor-blood DNA samples by using PCR-based microsatellite analysis. MGMT protein expression was studied in paraffin-embedded tumor sections by immunohistochemistry. Twenty-eight patients (median age, 38; range, 17–77) were enrolled. Median time between tumor diagnosis and TMZ treatment was 33.5 months (range, 1–133). Median number of TMZ cycles/patient was 12 (range, 2–24). Marked clinical improvement was recorded in 15 pts (54%), an objective response in 17 (61%) with 7 minor responses, stable disease in 10 (36%), and progressive disease in one patient. Median TTP is 11 months with PFS of 70% at 24 months. LOH of 1p and/or 19q was found in 14/16 tumors of whom, 11 improved on TMZ, and 3 had stable disease. Of the 2 tumors with no LOH, one had stable disease and the other progressed. MGMT protein expression was evaluated in 16 LGO, whose LOH status showed 13 codeleons at 1p/19q and 19q of an intact 1p chromosome. LGO with an intact 1p demonstrated high expression of MGMT (>50% nuclear staining) as opposed to tumors with 1p/19q losses, that exhibited a relatively low MGMT (0–50% nuclear staining). MGMT expression was significantly associated with 1p loss (P < 0.0004). TMZ is active in progressive LGO. 1p/19q deletions are associated with response to TMZ, and MGMT protein expression significantly correlates with the allelic loss on 1p chromosome.
for medulloblastoma with extensive nodularity. Small isomorphic nuclei characterized 70% of tumors, while others had a moderate nuclear pleomorphism without significantly enlarged nuclei. Isolated large cells with pleomorphism were found in two cases from 9.6% to 53.5% (median 30.2%) and were not significantly different in nodular versus classic medulloblastomas. No correlation was found between total survival duration and the evaluated pathologic features. Assessment of anaplasia grade based either on coexistence or increasing degree of histopathological features did not predict outcome. Severe degrees of anaplasia were not found. Based on our data, the histologic evaluation of medulloblastomas from adults with respect to features of anaplasia does not give prognostic information for a satisfactory stratification of adult patients with medulloblastoma, we have to turn to biological and molecular factors and analyze their prognostic role.

208. RANDOMIZED CONTROLLED TRIAL OF A PATIENT SPECIFIC REMINDER OF ROYAL COLLEGE OF PHYSICIANS GUIDELINES FOR COMMUNICATION IN PATIENTS WITH MALIGNANT GLIOMA

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Some studies have shown that Patient Specific Reminders (PSR) can improve the standard communication between treating doctors and patients or their family. We developed PSR cards to remind treating doctors of the Royal College of Physician guidelines on communication, for example, breaking the news of diagnosis of brain tumor, diagnosis, and prognosis. We were interested to see if a PSR attached to the patient notes improved communication. This was a randomized controlled trial involving 163 patients with intrinsic brain tumors treated in three centers (Edinburgh, Aberdeen, and Dundee), randomized to PSR card to inform the neurosurgeon about RCP guidelines on communication with patient and collected the PSR card (standard care). Quality of communication with (a) patient, (b) GP, and (c) radiation oncologist was assessed after discharge by confidential questionnaire. Completed questionnaires returned by patients were scored by 3 blinded independent assessors of a brain tumor charity. The GP questionnaires returned by GPs were sent to 3 independent GPs for scoring; the Radiation Oncology questionnaires were sent to 3 independent hospital doctors for scoring. The assessors rated the individual questionnaires for overall communication as very good, good, poor, very poor. Assessors only knew the age and the presumed diagnosis. The median score of the 3 assessors was taken as the overall grade for each questionnaire. Statistical analysis was performed using the chi squared test. Quality of communication in patient questionnaires was considered good or very good in 55% to 74% and very poor in 9%. There was no statistical difference between those allocated a PSR and those with “standard care” (DF = 6, P = 0.365). GPs considered communication good or very good in 36% to 48% of cases and very poor in 24% (PSR 13%; standard care 24%), no statistical difference (DF = 6, P = 0.53). Hospital doctors considered rated communication with radiation oncologists as good or very good in 55% to 62% of cases and very poor in 2.5% to 5%, no statistical differences (DF = 6, P = 0.963). The only exception to the effect of a patient-specific reminder regarding a guidelines on communication for neurosurgeons dealing with brain tumor patients. The PSR alone did not dramatically or statistically significantly improve the quality of information given to patients, GPs, or radiotherapists by the neurosurgical teams in 3 centers in this study.

209. THE COLLECTIVE CARE PATHWAY OF THE NEURO-ONCOLOGY NURSE PRACTITIONER AND THE ADVANCED PRACTITIONER IN RADIOTHERAPY

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Primary brain tumors (gliomas) represent a small proportion of all cancers, yet the impact of the disease is catastrophic, as the illness causes both physical and cognitive disabilities. For the client and family, this presents many challenges, as the journey is unpredictable. It is this uniqueness that requires specialist input, ranging from advanced technical treatments to palliative care. The concept of the above roles evolved from The Calman-Hine Report (DOH 1995) and The NHS Plan (DOH 2000 A), which subsequently initiated The NHS Cancer Plan (2000 B). It was this report that explicitly identified recommendations for staffing investment and patient care and acknowledged the need for site-specific multidisciplinary teams. It proposed that core members of the team should include a specialist nurse and a therapy radiographer. Since their implementation, both roles have evolved, and the current designations replace the previous titles of Specialist Radiog-
211. THE PRESENTATION OF CHILDHOOD BRAIN TUMORS: A META-ANALYSIS
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Brain tumors can be difficult to diagnose. Many of the initial symptoms and signs are also seen in other more common and less serious childhood illnesses. The challenge for health care professionals is to identify children whose presenting symptoms suggest a CNS tumor from the majority that do not. A meta-analysis of the presenting symptoms and signs of childhood brain tumors was conducted as the initial stage in a project to devise guidelines for the referral and imaging of children who may have a CNS tumor. The objective of this study was to conduct a meta-analysis of the presenting symptoms and signs of childhood brain tumors. The English-language literature was searched from 1996 to 2004 by using Medline, Embase, and the Cochrane library. Case-series and cohort studies detailing the presenting symptoms and/or signs of children with brain tumors and including at least 10 children were included in a meta-analysis of presenting symptoms and signs. Thirty-two papers describing 2200 patients were identified as satisfying the inclusion criteria. Sixty-eight symptoms and signs were identified as occurring in children with brain tumors, although not every paper contained data on every symptom and sign. Meta-analyses of the data revealed that the most frequent presenting symptoms and signs were headache (43%; 41%–45%); precocious puberty and vomiting (41%; 39%–43%); abnormalities of gait and co-ordination (29%; 27%–31%); papilloedema (17%; 16%–19%); changes in behavior and school performance (13%; 12%–15%); seizures (13%; 12%–15%); signs of raised intracranial pressure (11%; 10%–13%); somnolence (11%; 9%–13%); ataxia (10%; 9%–11%); and anorexia (8%; 7%–9%). Thirty-one additional symptoms and signs occurred at lower frequencies. This analysis emphasizes the diverse ways in which childhood brain tumors present. While the classical symptoms of raised intracranial pressure and headache, and vomiting are identified as the commonest presenting symptoms of childhood brain tumors, they occurred in fewer than 50%. Childhood CNS tumors may present in multiple ways and thus to many branches of medicine. Brain tumors have a long symptom interval in comparison to other childhood tumors. Families and patients find this distressing, and it may adversely affect outcome. Awareness among health care professionals that the presentation of brain tumors may mimic that of more common childhood illnesses may facilitate diagnosis and reduce their symptom interval.

212. THE IMPACT OF THE NURSE PRACTITIONER ON LENGTH OF STAY OF NEURO-ONCOLOGY PATIENTS IN AN ACUTE CARE SETTING IN A LARGE TEACHING HOSPITAL
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The value of the nurse practitioner (NP) as part of a multidisciplinary team has been well documented in both nursing and medical literature. The Massachusetts General Hospital (MGH) established a full-time nurse practitioner position to decrease the length of stay (LOS) of neuro-oncology patients, with the additional goals of improving patient education and outpatient transition. The neuro-oncology NP program was initiated in 1999. A retrospective analysis was performed of the average LOS of 627 patients admitted to the neuro-oncology service for three periods: 8 months prior to the addition of the NP (period 1) and the two 8 month periods following addition of the NP (periods 2 and 3). We also analyzed the number of LOS outliers between the first two periods. Data were obtained from the hospital's Clinical Performance Management (CPM) database. The neuro-oncology team consisted of a staff neuro-oncologist, neuro-oncology fellow, and NP. The NP assisted in the medical management and discharge planning of general neuro-oncology patients hospitalized due to complications of their disease or side effects of treatment. The NP also managed all elective chemotherapy patients by coordinating pre-admission laboratory evaluation, writing chemotherapy and admission orders, performing daily medical care, and planning patient discharges. Average LOS was 10.7 days in period 1, prior to the addition of the NP. There was a decrease in average LOS of 3.3 days (from 10.7 to 7.4 days) at the end of period 2, and 4.5 days (from 10.7 to 6.2 days) at the end of period 3 (P < 0.005). There were 10 non-chemotherapy patients who had LOS greater than 2 SD of average LOS. Of the 10 patients, 7 were admitted in period 1, prior to the addition of the NP, whereas 3 were admitted in period 2. Informal interviews with staff neuro-oncologists, nursing administrators, and NP revealed an improvement in patient education, smoother transitions to the outpatient setting, and better coordination of patient care in general. This subsequently resulted in an overall improvement in patient satisfaction. Nurse practitioners are effective in the acute care setting in decreasing LOS of neuro-oncology patients. The addition of the nurse practitioner improved the care of neuro-oncology patients both by decreasing average LOS and increasing patient satisfaction.

213. QUALITY OF LIFE IN BRAIN CANCER PATIENTS AND COPING STRATEGIES
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The aim of our study was to measure the quality of life (Qol) in brain cancer patients using objective and subjective measures, in order to assess which factors fit patients’ needs and the contextual setting. We studied 111 consecutive subjects with high-grade gliomas between 23 and 79 years old (mean age, 48.9) during their treatment at the National Neurological Institute C. Besta of Milan (Italy). Each patient completed the following scales: the Fact-G scale 4.0 (Italian version) and the Fact-Br brain specific module for the evaluation of Qol in brain cancer patients, the HAD scale for depression and anxiety, and the half-structured interview SeiQol-Dw for subjective evaluation of Qol, and patient’s experience. For each patient we first measured the functional status by the Karnofsky index (KPS) and the cognitive status through the Mini Mental State Examination. The mean (± SD) score of the Fact-G was 73.4 ± 13.7 and the Fact-Br was 123.1 ± 23.7. The HAD depression sub-scale showed that 19% of patients had a light to moderate depression and 3% a severe depression state. Concerning the SeiQol-Dw interview, we performed a content analysis of the SeiQol descriptions, and we categorized answers in eight main classes of coping behavior. Most patients found in familial or social support and in positive actions and thoughts good strategies to cope with the illness. Further, women were observed to use spiritual and positive thoughts more frequently. At the opposite extreme, men preferred more concrete strategies directed to improve their functional role in family and society. Males also reported negative thoughts more frequently than women. The psychological adjustment to the illness by males seemed to be more difficult. Interaction between measures showed that global Fact-Br score was better predicted by Kps and depression, while the SeiQol general score was mainly predicted by depression scores. Depression did not appear to change in correlation with age, sex, and time from first diagnosis. Our data confirm that patients with high-grade gliomas may cope with the illness without developing severe depression and anxiety. Though Qol is strongly affected by physical symptoms, patients are able to use different strategies to fit the situation, during aggressive therapy, radiotherapy, and chemotherapy. We think that the coping strategies used by patients help them to adjust to the illness and continue their treatment in a hopeful and protective context. This indicates the necessity to sustain patients’ positive context with an adequate assessment and comprehension of the potential coping strategy. This could be done by adopting specific instruments that help to develop an understanding of the patient’s subjective experience.

214. PRE-OPERATIVE AND FOLLOW-UP FMRI EVALUATION OF CONDITIONAL VISUO-MOTOR ASSOCIATIVE FUNCTION IN PATIENTS WITH LOW-GRADE GLIOMAS
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The goal of this study is to develop a functional Magnetic Resonance Imaging (fMRI) protocol that assesses higher cognitive functions, other than language and somatosensory functions, in patients with low-grade gliomas. These patients have a life expectancy that ranges between 2 and 10 years, and therefore it is crucial that great care be taken to preserve higher cognitive functions in order to preserve their autonomy and quality of life post-operatively. In this study, we developed an fMRI protocol for the pre-operative and follow-up (8 months after the surgery) evaluation of higher cognitive functions in patients exhibiting gliomas near the premotor region. In order to assess the function of the premotor region in these patients, we developed a conditional visuo-motor associative protocol that was tested in 8 healthy control subjects. In this protocol, the subjects had to associate each one of four different colors with a specific key press on a mouse with four buttons. The fMRI results revealed that the premotor region and the supplementary motor area are specifically involved in conditional visuo-motor associative function. These data demonstrate that the protocol we developed provides robust and reliable measures of conditional visuo-motor associative function within the premotor region. We then conducted a pre-operative fMRI study using this protocol in three patients with tumors near the premotor...
region. As in healthy control subjects, pre-operative fMRI data obtained in these patients showed activity increases within the premotor region. Coupled with the structural MRI, the clinical pre-operative fMRI data were transferred to an integrated image guided system in the operating room. The neurosurgeon was able to use the data in the planning of the surgery in order to spare functional tissue in the premotor region. As a result, the neurosurgeon was able to optimize the extension of the tumor resection in the three patients without any deficits in the pre-operative fMRI; only one patient demonstrated a low level of tumor-like activity in the premotor area. The follow-up fMRI revealed activity differences similar to the ones observed in the pre-operative fMRI scan. These results suggested that functional reorganizations occurred following the tumor resection. The results provided by such preoperative, postoperative and follow-up fMRI studies are a useful clinical tool for the patient’s long-term prognosis.

215. QUALITY OF LIFE IN PEDIATRIC BRAIN TUMOR SURVIVORS
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Studies suggest that survivors of pediatric brain tumors may be at risk for developing symptoms consistent with a normative learning disability (NLD) as a consequence of their disease and its treatment. Rourke (1988) described NLD as affecting children’s functioning across multiple domains including cognitive, academic, and social areas. As a result, individuals with NLD may experience learning difficulties in reading and mathematics, comprehension, math calculation, and written language. In addition, those with NLD may exhibit deficits in social perception, judgment, and interaction skills that place them at higher risk for social withdrawal or isolation. In contrast to these weaknesses, individuals with NLD often manifest relative strengths in rote skills and memorization, verbal processing, reading decoding and dictation skills. Preliminary data are now available that provide evidence of impairment in cognitive and academic deficits associated with NLD; however, the social functioning of survivors is not well investigated. The primary objectives of the current study are to demonstrate that a sample of survivors of pediatric brain tumors have greater impairments in social functioning than do healthy controls, using both questionnaire and skill assessment data, and to determine what risk factors are associated with social impairment. To date, 24 patients (14 females and 10 males) ranging in age from 7 to 16 (M = 13.1, SD = 2.77) have been enrolled in the study. Diagnoses were varied, with 8 patients (33.3%) with medulloblastoma, 5 (20.8%) with ependymoma, 5 (20.8%) with pilocytic astrocytoma, and 6 (25%) with other tumor types. The majority of patients had been treated with surgery (n = 20, 83.3%), 17 (70.9%) had received hemotherapy, and 34 (58.3%) had been treated with radiation. Age at diagnosis ranged from 2 to 14 (M = 6.58, SD = 3.20). Participants had been off treatment an average of 2.52 years (SD = 2.56; range = 1−11 years) at the time of study. Cognitive functioning of the sample was variable, with estimated IQs averaging 87.61 (SD = 16.18). Broadly speaking, parents rated their children’s social functioning as somewhat lower than would be expected by age-based norms. Subjects were also rated as having somewhat more social problems, including difficulties with nonverbal communication, than would be expected. Contrary to expectations, parent-reported social problems were not significantly associated with age at diagnosis, treatment type, functional or cosmetic impairment, or estimated IQ scores. However, social problems correlated significantly and negatively with scores of performance on a measure of ability to decode facial emotions. Findings from this study contribute to a more complete phenotype of the neuropsychological late effects experienced by survivors and to the development of interventions aimed at ameliorating disease and treatment-related deficits in the growing population of brain tumor survivors.

216. COGNITIVE FUNCTIONS AND APOE GENOTYPE IN LOW-GRADING GLIOMA PATIENTS
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The purpose of this study was to assess cognitive functions in patients with low-grade gliomas (LGG) who received radiation, chemotherapy, or no treatment. We also compared the cognitive performance of patients who carried the Epsilon-4 allele of the Apolipoprotein E (APOE) gene to those who carried other APOE alleles. Forty adult patients with LGG and no evidence of disease progression participated in the study; 16 patients who received conformal radiotherapy + chemotherapy, and 24 patients had no adjuvant treatment. All patients underwent a neuropsychological evaluation, and test scores were compared to normative reference groups; 7 composite cognitive domain scores were calculated. APOE genotype was obtained in 33 patients who were classified in two groups based on the presence or absence of at least one APOE Epsilon-4 allele. Patients who received radiation + chemotherapy had significantly lower scores than untreated patients on the Psychomotor (P = 0.03) domain. Analysis of covariance, adjusting for anticonvulsant regimen (i.e., monotherapy vs. polytherapy), suggested no significant adjuvant treatment effect on Psychomotor performance; the results showed that patients on anticonvulsant polytherapy had lower scores on the Psychomotor domain, regardless of tumor treatment status. Treated patients obtained significantly lower scores than untreated patients on the Non-Verbal Memory (P = 0.02) domain. Analysis of covariance, adjusting for age, showed that patients who completed treatment at intervals longer than 36 months had significantly lower scores on the Non-Verbal Memory domain than untreated patients. Over 60% of treated patients showed mild to moderate white matter confluence on MRI, whereas 90% of the untreated patients had either no or only minimal white matter changes (P = 0.002). Comparisons between APOE Epsilon-4 carriers (n = 8) and noncarriers (n = 25) on cognitive domain scores revealed no significant differences, but Epsilon-4 carriers had lower scores on the Verbal Memory domain than did noncarriers. The findings suggest that LGG patients treated with radiation + chemotherapy had more difficulties in nonverbal memory and were more likely to show white matter abnormalities on MRI than untreated patients. Psychomotor slowing was most prominent among patients who were on anticonvulsant polytherapy, regardless of adjuvant treatment status.

217. THE COURSE OF NEUROCOGNITIVE STATUS IN HIGH-GRADING GLIOMA PATIENTS
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This work was conducted to study the course of neurocognitive functioning in newly diagnosed high-grade glioma patients and to determine the tumor, treatment, and patient-related factors affecting neurocognitive functioning in the course of the disease. Following baseline assessment (i.e., before surgery, prior to the start of radiotherapy), follow-up evaluations on neurocognitive functioning were performed at 8 and 16 months in newly diagnosed high-grade glioma (HGG) patients and in patients with non-small-cell lung cancer (NSCLC). HGG patients’ level of functioning was compared to the level of functioning in NSCLC patients who received different chemotherapy and sex-matched healthy controls. A battery of standardized tests was used to assess neurocognitive functioning. In order to accomplish data reduction, summary measures were calculated to detect possible deficits in the neurocognitive domains of (1) information processing speed, (2) psychomotor function, (3) attentional functioning, (4) verbal memory, (5) working memory, and (6) executive functioning. Follow-up data could be obtained in 35 of the 68 HGG patients initially included in the study. Of these, 20 patients had only one follow-up at 8 months, whereas 15 patients also had a 16-month follow-up. The patients who also had a follow-up at 16 months had a better neurocognitive status, were younger, had a significantly lower tumor grade, and received lower fraction doses than patients with only an 8-month follow-up. HGG patients with a shorter follow-up performed worse when compared to NSCLC patients with the same follow-up. Compared to the performance of NSCLC patients during the follow-up, no statistically significant deterioration was found in neurocognitive function in the course of the disease in the HGG patients. However, evaluation of HGG patients with versus NSCLC patients without tumor recurrence indicated that neurocognitive decline in these patients often precedes clinical signs of tumor progression. Neurocognitive functioning in HGG patients is mostly affected by tumor effects and not unequivocally by treatment effects. In the course of their disease and in the absence of tumor progression, no clear trend toward further worsening in neurocognitive functioning is to be expected in these patients. Additionally, neurocognitive function appears to be a prognostic factor in the course of the disease in these patients.
Despite aggressive treatment, outcome of patients with malignant gliomas is poor. In the terminal course of the disease, due to social, economical, individual, and cultural reasons, some patients are admitted to hospital care. For the terminal phase, there is a lack of investigations in the neurological literature. The purpose of this study was to evaluate the end of life phase in a hospital setting for patients with malignant glioma. Twenty consecutive patients with malignant gliomas were included in this analysis regarding symptoms, medication, diagnostic, and interventional procedures. The last ten weeks before death were divided into three periods: Period I: from ten weeks to six weeks before death, Period II: six weeks to two weeks before death; and period III: last two weeks before death. The patients were comparable regarding age, sex, and overall survival. The Karnofsky performance scale decreased continuously from period I (average age absolute 70%) to period III (average absolute 10%). Relevant clinical complications, medications, and diagnostics as well as interventional procedures increased from period I to period III. For the period I symptoms of headache and seizures, treatment with antiepileptic drugs as well as steroids and analgesics was prescribed. Diagnostic procedures such as MRI, blood tests, or X ray of the chest, as well as interventional procedures (e.g., urinary catheter, intravenous drug administration), were less frequent compared to period II or III. In the last period, confusion, encephalopathy, fever, somnolence, and pneumonia were the most prominent clinical features. Almost all patients in period III received transdermal or subcutaneous opioids, fluid replacement, antiemetics, intermittent oxygen, antimycotics, gastric protection, and urinary catheters, as well as a pressure relief mattress. The majority of patients died due to infection or respiratory distress. Providing adequate palliative care to dying patients with malignant gliomas is an important aspect of treatment. Our study demonstrates that the end-of-life phase of patients with brain tumors has severe therapeutic implications, the number of therapeutic and diagnostic interventions increases toward the terminal phase. Future patient management needs to be more symptom-oriented and palliative.
224. NEUROPSYCHOLOGICAL IMPACT OF BONE MARROW OR HEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROSPECTIVE STUDY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Bone marrow or hematopoietic stem cell transplantation (SCT) is a current cancer treatment for patients with malignant hematological disorders. Better patient selection and development of reduced-intensity preparatory regimens and new transplant techniques have expanded the use of SCT. SCT is preceded by the use of high-dose chemotherapy and total body irradiation to eradicate the malignant disease and suppress the immune system to allow engraftment of the donor (or autologous) stem cells or bone marrow. The complications associated with SCT treatment are significant due to severe toxicity associated with myeloablative therapy (including central nervous system toxicity), the period of profound immunodeficiency, and the risk at graft failure or graft-versus-host reaction. The neurotoxic side-effects on cognitive functioning and the consequences on patients’ quality of life are major concerns. Cognitive deficits following SCT have been documented in subgroups of patients. Unfortunately, no attempt has been made to evaluate a progressive decline or stability in cognitive functioning prospectively. The purpose of this study was to address the extent of cognitive changes associated with SCT in adult patients with hematological malignancies. A standardized neuropsychological test battery assessing multiple cognitive domains was administered to a longitudinal group of patients before undergoing SCT (18 patients, 12 females, 6 males, ages 16–58 years) and 20 months (T2) and 20 months (T3) after baseline. To control for SCT treatment, a reference group of 82 hematological patients treated with conventional systemic chemotherapy and/or involved-field radiotherapy was included. Effects of subjective cognitive functioning, quality of life (QOL), fatigue, and psychosocial functioning were measured with several self-report questionnaires. Results were compared to normative data. Analysis employed random regression modelling (RRM). No between-group differences were found in cognitive functioning at baseline. Changes over time were observed in attention (P = 0.01) and psychomotor functions (P = 0.03) with poorer functioning in SCT patients. Performance on verbal memory, visual memory, and visuospatial functions remained stable at follow-up. Negatively correlated with gender and age were found, suggesting poorer performance in, respectively, females and older patients. Positive effects of education were observed in all cognitive domains, suggesting that patients with higher educational had better test results. Impaired cognitive functioning in SCT patients was weakly correlated to mental fatigue, reduced motivation, and anxiety at follow-up. More cognitive deficits were observed in patients treated with TBI, in patients who received prednisone, and in patients who experienced long-term infections. Intensive myeloablative cancer therapy had an adverse impact on cognitive functioning over time, in particular, on psychomotor functions and attention.

225. LATE EFFECTS IN YOUNG ADULTS SURVIVING A CHILDHOOD PRIMARY TUMOR OF THE CNS

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Central Nervous System (CNS) tumors account for about 20% of all childhood tumors. They are second in incidence only to leukemia. As treatment results have improved significantly, leading to an increased number of patients surviving over the last decades, attention is drawn toward the long-term effects and quality of life of these patients. In order to monitor the late effects of childhood cancer treatment, a long-term follow-up clinic was opened at the Academic Medical Centre in Amsterdam; it has successfully completed their treatment 5 years before they were transferred to the long-term follow-up clinic. Participants in this study had to be aged 16 years or older and had to be treated for a primary malignancy of the CNS at childhood. The study included a questionnaire and interview for psychosocial and educational functioning, a physical examination, and a laboratory screening of endocrine-axis. Of 61 patients, the late effects were divided into 11 different groups: endocrinological, fertility, neurological, sexual, cancer, cognitive, emotional, occupational, social, and psychosocial functioning. Further, a self-report measure on health-related quality of life (FHS) was used. The majority of patients (n = 3 [5%]) had late effects associated with cognitive functioning in 15 (24%) patients. In four patients (15%), a secondary malignancy occurred. In total, 42 patients had a mild (70%), severe (22%), or total (8%) alopecia, of which 23 patients received a combination of radiation and chemotherapy. The psychological...
problems consisted of a broad variety of social and cognitive disabilities. Twenty patients reported a learning disability, in fifteen cases leading to a decreased level of education compared with the pre-treatment level. Ten patients attended special schools for learning disabilities. Since patients (15%) were treated by a psychiatrist or psychologist. Four out of seven patients (57%) who received carboplatine experienced hearing loss. The total number and severity of the late effects exceeded our expectations, and they should play a role in the assessment of future treatment strategies.

226. DEVELOPMENT OF NEW FMRI AND INTRA-OPERATIVE TOOLS FOR NEUROSURGERY GUIDANCE IN PATIENTS WITH BRAIN TUMORS INVADING THE PARIETAL LOBES

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The objective of this study is to develop new pre-operative neuroimaging and intra-operative behavioral tools for the assessment of high-order parietal functions in patients with brain tumors. Pre-operatively, we used a Functional Magnetic Resonance Imaging (FMRI) protocol to assess mental rotation function in three patients with brain tumors invading the parietal lobes. We aimed to localize functional regions surrounding the tumor that were involved in the task. The pre-operative FMRI showed that these functional regions were critically involved in this task, that is, the superior and inferior parietal lobules, as well as the cortex surrounding the intraparietal sulcus. These FMRI data were then transferred to a neuronavigation system to provide direct intra-operative guidance to the neurosurgeon. Using some of these methods, the neurosurgeon is able to identify the critical areas in the operating room not only the location of the tumor but also the critical areas involved in the mental rotation task. The neurosurgeon used the functional data to plan the neurosurgery and to determine the safest surgical route for the tumor resection of each patient. It was therefore possible, with the use of this technology, to remove as much of the tumor as possible while preserving critical areas in each patient. A standardized intra-operative protocol was also developed. During the operation, patients were tested on the mental rotation protocol at regular intervals and at specific stages of the surgery. The online assessment of performance during surgery allowed the neurosurgeon to verify whether the cognitive and behavioral processes for mental rotation were preserved throughout the surgery and to adapt the surgical approach in order to minimize as much as possible the potential deficits post-operatively. Using this procedure, the neurosurgeon spared the areas that were critical for mental rotation in each patient. This was reflected in stable performance of the patients on the protocol during surgery. The results suggest that this procedure is of great clinical value. It provides the neurosurgeon with the possibility to balance the benefits of a complete tumor resection with the possible post-operative functional deficits. Ultimately, it allows an optimal tumor resection with the minimal post-operative neurological deficits. The use of this new procedure could therefore become crucial for the patients’ post-operative quality of life and autonomy.

227. PALLIATIVE CARE IN BRAIN TUMOR PATIENTS: COMPLICATIONS AND SUPPORTIVE THERAPY IN 215 PATIENTS ASSISTED AT HOME

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Although the poor prognosis of malignant brain tumors has not been substantially modified in the last years by antitumor treatment, palliative care in neuro-oncology received very little attention. Since October 2000 at the Regina Elena National Cancer Institute of Rome, we started a palliative home-care program for patients affected by malignant brain tumor after hospital discharge, with financial support of Regional Health System. The aims of this model of assistance are to meet the patient’s need of care during the evolution of the disease, to provide rehabilitation at home, to improve the patient’s quality of life with palliative care, and to facilitate the patient’s death at home. Neuro-oncologic home staff includes 1 neurologist, 5 nurses, 2 rehabilitation therapists, and 1 psychologist. In the first three years of our program 213 patients have been assisted at home and 131 died. The complications in the last phase of disease were pulmonary infections (10.6%), deep venous thrombosis (9.7%) with embolic complications in 6 cases, diabetes due to chronic steroid treatment (8.4%), and psychiatric syndromes (5.7%). Seventy-nine patients (37%) presented epilepsy despite anticonvulsant treatment; 24% presented adverse effects to medication (chemotherapy, antiepileptics, and steroids). Sixty-nine percent (90/131) of the patients were able to die at home. Among the 131 patients who died, the most frequent symptoms in the terminal phase were lethargy (35.5%), dysphagia (31.8%), pain, and neurocognitive decline (23.5%). A team of palliative nurses was therefore involved in the care of patients receiving continuing home care, the hospital readmission rate and the median time spent in hospital in the last four months of life are significantly lower than in a control group (P < 0.01). Future research should include new models of care for brain tumor patients, with special attention to palliative home-care models.

228. EXTENDED ABSTRACT: PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: RECENT ADVANCES IN DIAGNOSIS AND TREATMENT

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Primary central nervous system lymphoma (PCNSL) has been considered to be a rare tumor, but most literature published since the 1980s has reported that PCNSL has been increasing in frequency. It now affects approximately 300 people in Japan and 1000 in the United States each year.

The pathogenesis of PCNSL remains obscure. It arises in the central nervous system where no lymphocytes are present in the normal state. Most PCNSL is B-cell derived (non-Hodgkin lymphoma (NHL)). Patients with NHL frequently have multiple lesions in the brain. Compared to non-central nervous system (CNS) NHL, PCNSL is refractory to any treatment, and prognosis is dismal. Primary central nervous system lymphoma often relapses within the radiation field in which a dose sufficient to control CNS NHL is given. Primary central nervous system lymphoma arises in both immunocompetent and immunocompromised populations. These two groups present different clinical features. In this report, PCNSL in an immunocompetent patient population is described.

Primary central nervous system lymphoma affects any age, with a peak incidence in the fifth to seventh decade. Recently, PCNSL arising in the elderly seems to be increasing. Eighty-two percent of cases has been reported at all ages. Primary central nervous system lymphoma has no specific diagnostic features. It may exhibit various clinical presentations, including increased intracranial pressure, focal neurological symptoms, and demential symptoms. Primary central nervous system lymphoma anywhere in the brain. Most common sites are the periventricular and cortical regions. The tumor is multifocal in 20% to 30% of cases. Unlike glioblastoma, which typically shows ringlike enhancement in CT and MRI, PCNSL exhibits circumscribed homogeneous enhancement.

Peritumoral edema is common. Calcification, cyst formation, hemorrhage, and necrosis are not accompanied.

Histopathological confirmation is essential to establish diagnosis and determine treatment strategy. Stereotactic biopsy is currently the diagnostic procedure of choice. Total resection does not correlate with prolongation of survival in PCNSL. Therefore, extended resection of the tumor, enhancing the risk of new neurological deficits, is not warranted in most patients. Stereotactic biopsy should be withheld before resection because the biopsy may appear completely. T-cell-derived PCNSL has been rarely reported. But, the vast majority of PCNSLs correspond to B-cell NHL and are categorized into the subtype of diffuse large cell lymphomas. Immunostaining using various antibodies is required for the final diagnosis. Primary central nervous system lymphoma differs from systemic lymphomas in several aspects. However, until now, no evidence supporting that PCNSL is biologically or genetically different from systemic lymphomas has been proposed. From cytological and genetic observations, CNS B-cell-derived diffuse large cell lymphomas are related to lymphocytes in the germinal center in the different stage.

Primary central nervous system lymphoma had been an incurable disease. If untreated, median survival time (MST) of patients was reported to be two to three months. Surgical resection alone did not provide any benefit to patients with MST of one to four months. The object of the surgery is to improve the patient’s quality of life with palliative care, and to facilitate the patient’s death at home. Neuro-oncologic home staff includes 1 neurologist, 5 nurses, 2 rehabilitation therapists, and 1 psychologist. In the first three years of our program 213 patients have been assisted at home and 131 died. The complications in the last phase of disease were pulmonary infections (10.6%), deep venous thrombosis (9.7%) with embolic complications in 6 cases, diabetes due to chronic steroid treatment (8.4%), and psychiatric syndromes (5.7%). Seventy-nine patients (37%) presented epilepsy despite anticonvulsant treatment; 24% presented adverse effects to medication (chemotherapy, antiepileptics, and steroids). Sixty-nine percent (90/131) of the patients were able to die at home. Among the 131 patients who died, the most frequent symptoms in the terminal phase were lethargy (35.5%), dysphagia (31.8%), pain, and neurocognitive decline (23.5%). A team of palliative nurses was therefore involved in the care of patients receiving continuing home care, the hospital readmission rate and the median time spent in hospital in the last four months of life are significantly lower than in a control group (P < 0.01). Future research should include new models of care for brain tumor patients, with special attention to palliative home-care models.

Abstracts from the World Federation of Neuro-Oncology Meeting
suggestion the superiority of preradiation HD-MTX over radiotherapy alone. High-dose methotrexate followed by radiotherapy brings a response rate of 80% to 90% and MST of 30 to 40 months. Long-term survival or even cure can be achieved in some patients. Clinical results from a limited number of patients, that HD-MTX followed by irradiation produces better treatment results than irradiation followed by chemotherapy. Any combined chemotherapy without HD-MTX did not surpass HD-MTX. Now, systemic HD-MTX followed by whole cranial irradiation is recommended as a standard treatment. These reports were phase 2 trials, and efficacy of HD-MTX has not been validated by randomized prospective phase 2 studies. Because of the small number of patients and large difference of survival between these two treatments, phase 2 study has not been accepted to perform by most physicians.

The molecular weight of MTX is 454, and MTX is not lipid soluble. When given in a low dose, MTX cannot cross the blood-brain barrier. When a high dose of 5mg/kg or 1g/m² is administered, the cerebrospinal fluid (CSF) level of MTX was demonstrated to elevate over 11M, which has an antitumor effect against lymphoma cells. In most clinical trials, ‘high-dose’ is defined as greater than 1g/m² of intravenous MTX. Optimal dose and number of cycles of HD-MTX has not been established. We reported a single institutional trial of HD-MTX followed by whole brain irradiation. Median survival time and median relapse-free survival were 39.3 and 35.2 months, respectively, for 28 assessable patients. Response rate was 93.8% in rapid (three-hour) infusion and 58.3% in regular (six-hour) infusion. Rapid infusion produced a higher level of MTX in the CSF and significant tumor volume reduction. Thus, HD-MTX improved the prognosis of the patients with PCNSL. However, the five-year survival rate still is less than 25%. High-dose methotrexate is not effective in all cases. A few negative trials were also reported. Why some tumors are resistant to MTX has not been clarified. For relapsed or refractory tumors, there is no established standard treatment.

For further improvement, new trials are being tested. Combined chemotherapy trials based on HD-MTX have been reported with MST of up to 60 months. High-dose combined chemotherapy with bone marrow or stem cell transplantation has also reported. The role of high-dose chemotherapy to PCNSL is not clear. New therapeutic modalities have been tested. In systemic NHL, response rate to the humanized anti-CD20 antibody rituximab is 50%. Most PCNSL expresses the cell surface molecule CD20. If rituximab (MabCamp) is administered intravenously, its CSF level are very low. Intrathecal rituximab may be of advantage. Currently, there is limited evidence for effectiveness of rituximab against PCNSL. Temozolomide, an alkylating agent recently used to treat glomas, has a modest activity against PCNSL. These agents have been recently used in relapsed or refractory tumors after HD-MTX as a salvage therapy. Delayed neurologic toxicity due to a combination of HD-MTX and whole brain irradiation has been recognized. As survival is prolonged by chemotherapy, this complication overshadows quality of life, especially in the elderly. Patients with this complication present cognitive dysfunction, dementia, and ataxia. In MRI, diffuse brain atrophy and leukoencephalopathy are observed. Delayed neurotoxicity is more frequent in patients older than 60 years. To reduce this complication, several trials have been reported. Radiotherapy is excluded in aged patients or deferred in patients with complete response with chemotherapy.

In immunocompetent persons, PCNSL will be expected to be more common in the country where the aged population further increases. Basic research to understand the pathogenesis of PCNSL is mandatory. Multi-institutional prospective trials will ensure the establishment of evidence-based standard treatment strategies.

**New Drugs**: The reduced number of available active drugs limits further improvements in chemotherapeutic efficacy, which remains the most pressing issue in PCNSL. Preliminary results from small phase 2 studies in relapsed patients have been reported with temozolomide, topotecan, and rituximab, and some retrospective evidence suggests that the addition of high-dose cytarabine to HD-MTX could be associated with survival improvement (Ferreri et al., 2002).

Topotecan, a camptothecin derivative that inhibits enzyme topoisomerase I, has been tested in 16 patients with refractory or relapsed PCNSL, obtaining four complete remissions and two partial remissions (overall response rate: 38%) and a one-year progression-free survival rate of 13% (Fischer et al., 2004). Promising results but on small groups of patients with relapsed PCNSL have been reported with ifosfamide and trofosfamide (Jahnke et al., 2005), while infusional 5 bromo-2′-deoxyuridine given as radiomimetic with whole brain radiotherapy has been associated with modest disease control and unacceptable neurotoxicity (Dabaja et al., 2003). Temozolomide is an oral second-generation alkylating agent that spontaneously undergoes chemical conversion to MTIC (5-[3-methyl-1-triazene]-imidazole-4-carboxamide), resulting in O-6 methylguanine-DNA methyltransferase depletion. This drug has been associated with excellent tolerability and a 26% overall response rate, mostly complete and partial. A multicenter phase 2 trial on 23 patients with PCNSL relapsed or refractory to HD-MTX (Reni et al., 2004). Considering it permeates the BBB, it is well tolerated even in elderly patients, and exhibits additive cytotoxic activity with whole brain radiotherapy, temozolomide could be used as induction, maintenance, or radiomimetic treatment against PCNSL.

**New Strategies**: Chemoradiotherapy is associated with a higher risk of neurotoxicity in PCNSL patients. Thus, some authorities focused their efforts on new strategies, that is, BBBD and high-dose chemotherapy supported with autologous peripheral-blood stem-cell transplantation (APBSCT), to dose intensify chemotherapy and eliminate the need for consolidation radiotherapy. BBBD by intra-arterial infusion of hypertonic mannitol followed by intra-arterial cytarabine delivery is a strategy leading to increased drug concentrations in the lymphoma-infiltrated brain to enhance survival. BBBD plus HD-MTX has been associated with five-year survival of 42%, and a 14% cognitive loss rate at one year (Kraemer et al., 2002). In immunocompetent persons, PCNSL was associated with a 36% response rate, with a median duration of 6.8 months (Tyson et al., 2003). Given its good efficacy and acceptable complication rates, the role of BBBD deserves further investigation in PCNSL.

Preliminary results indicate that high dose chemotherapy supported by APBSCT is feasible in PCNSL patients. In a study on 28 patients with newly diagnosed PCNSL (Abrey et al., 2001), HD-MTX and HD-cytarabine, followed by BEAM consolidation chemotherapy and APBSCT was well tolerated, but only five remained in remission at a median of 26 months after transplantation. In an ongoing study, 19 of 24 enrolled patients have achieved a complete remission, without relevant toxicity, after a combination of MTX, thiotepa, and cytarabine, followed by high-dose BCNU and thiotepa and hyperfractionated radiotherapy (Illerhaus et al., 2001). In a study on 22 patients with recurrent or refractory primary CNS or intracranial lymphoma, induction cytarabine and etoposide followed by high-dose cyclophosphamide and BBBD achieved a 36% response rate, with a three-year overall survival of 64%, but with a significant treatment-related morbidity/mortality in elderly individuals and risk of neurotoxicity in pre-irradiated patients (Soussain et al., 2001). The role of high-dose chemotherapy and APBSCT in PCNSL remains to be defined, considering that worldwide experience is still limited, and further studies will need to be done to identify the optimal induction and high-dose chemotherapy regimen.

**229. ENHANCED ABSTRACT: NOVEL THERAPIES AGAINST PRIMARY CNS LYMPHOMAS**

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Despite recent progress, primary central nervous system lymphomas (PCNSL) still exhibit one of the worst prognoses among non-Hodgkin lymphomas (NHL). Chemotherapy followed by radiotherapy is the most commonly used strategy. The most effective drug is high-dose methotrexate (HD-MTX) (response rate: 52%–100%; two-year overall survival: 58%–72%), while chemotherapy regimens without this drug comprehensively do not perform any better than radiotherapy alone (Ferreri et al., 2003a). Several attempts to improve outcome by adding other drugs, empirically chosen on the bases of extracerebral NHL experience or of the capability to penetrate the blood-brain barrier (BBB), to HD-MTX have been performed. However, only a few drugs had been previously evaluated as single agents in phase 1/2 trials, in patients with relapsed or refractory PCNSL. New strategies aimed to intensify chemotherapy as well as to replace consolidation radiotherapy, by a matter of investigations focusing on improving drug bioavailability in different areas of the CNS, such as eyes and meninges. This paper summarizes new drugs and strategies against PCNSL and discusses their current role and future developments.
a sustained release formulation of cytarabine (liposomal cytarabine) for intraarticular injection is available and allows dosing once every 14 days (Jacek et al., 2003). Indications and efficacy of intraarticular chemotherapy are, however, limited, and in fact, this strategy is associated with increased risks of neurotoxicity and chemical meningitis (Bessell et al., 2002; Ferreri et al., 2002), and its efficacy in PCNSL patients has not been prospectively assessed. Moreover, leptomeningeal relapse is almost always associated with brain recurrence (Ferreri et al., 2002), which constitutes the cardinal prognostic event in PCNSL, obscuring the effect of concurrent leptomeningeal relapse on survival and, consequently, the potential benefit of intraarticular chemotherapy.

Chemotherapy efficacy against intraocular lymphoma is dependent on intracocular pharmacokinetics, which is not well understood. One case series suggests that micromolar concentrations of MTX are achieved in the aqueous and vitreous humor when the drug is given at a dose of 8 g/m² (Batchelor et al., 2003; Ferreri et al., 2003). However, intravitreal drug concentration is erratic, it is not predictive of response, and it is lower in the aqueous and vitreous humor when the drug is given at a dose of 8 g/m². One case assessed. Moreover, leptomeningeal relapse is almost always associated with brain recurrence (Ferreri et al., 2002), which constitutes the cardinal prognostic event in PCNSL, obscuring the effect of concurrent leptomeningeal relapse on survival and, consequently, the potential benefit of intraarticular chemotherapy.

Perspectives: The optimal treatment of PCNSL remains a relevant challenge for international cooperation (Ferreri et al., 2003b). Collaborative efforts should be focused on the identification of new active drugs and combinations and on the role of emerging strategies against NHL. Different combination strategies may be needed because of the capability of lymphomatous cells to infiltrate more than one compartment of the CNS. To improve our knowledge of the molecular mechanisms underlying genesis and dissemination of malignant lymphocytes constitutes an essential step to improve therapy efficacy, and the establishment of animal models will allow us to investigate a variety of novel molecules to be included in the armamentarium against PCNSL.

References

232. EXTENDED ABSTRACT: STROKES IN CANCER PATIENTS: CEREBRAL INFARCTION, CEREBRAL HEMORRHAGE, DISSEMINATED INTRAVASCULAR COAGULATION, NEOPLASMS

Stroke in the cancer patient is rarely associated with the common causes of stroke in patients without cancer. The type of stroke is tumor-specific and is often associated with the stage of cancer and type of antineoplastic therapy (Cestari et al., 2004; Graus et al., 1985).

Nonmetastatic

1. Coagulopathy

Infarction/Thrombosis: A hypercoagulable state frequently accompanies carcinomas due to a complex interplay of immature cancer blood vessels, inflammation, and interaction of the host with procoagulant substances secreted by the tumor. In nonbacterial thrombotic endocarditis (NBTE), sterile platelet-fibrin vegetations develop on heart valves. This material embolizes to the brain and is accompanied by systemic small-vessel thrombosis due to the underlying hypercoagulable state. Other cancer patients have disseminated intravascular coagulation (DIC) and diffuse small-vessel cerebral thrombosis without NBTE. Hypercoagulability, sometimes from chemotherapy administration, underlies venous sinus thrombosis, typically in patients with leukemia and lymphoma (Raizer and DeAngelis, 2000). Nonbacterial thrombotic endocarditis is the most common multifocal cerebral signs from TIA (transient ischemic attack) or infarction (Rogers et al., 1987). Confusion alone or with focal signs or partial seizures may result from disseminated thrombosis in NBTE or DIC. Venous occlusion usually results in a headache that is accompanied by focal neurologic signs if infarction or hemorrhage develops. Signs of systemic thrombosis may be observed in NBTE and DIC, but laboratory tests of coagulation function are often not diagnostic. Cardiac vegetations visualized on transesophageal echocardiography and evidence of systemic or cerebral microvascular thrombosis are clues to NBTE. MRI in NBTE will typically reveal varying sizes of infarctions in multiple territories (Singhal et al., 2002). MRI or magnetic resonance venography (MRV) is diagnostic of venous occlusion. Oral anticoagulation therapy for cancer-related thrombosis may also be inadequate. However, intravenous heparin should be individualized. Heparin should be considered for NBTE and DIC. Venous occlusion may require anticoagulation, thrombolysis, or thrombectomy but more often can be observed.

Hemorrhage: Acute DIC is most common in leukemia, especially myelogenous leukemias. In acute promyelocytic leukemia, coagulopathies released from the tumor activate the clotting pathway and deplete clotting factors. In other cancer patients, cerebral hemorrhage results from thrombocytopenia that is due to marrow metastasis, marrow suppression from radiation or chemotherapy, or microangiopathic hemolytic anemia or liver dysfunction. Acute or subacute headache, focal signs, vomiting, and/or encephalopathy are signs of periventricular or subdural hemorrhage from coagulopathy. There may also be systemic bleeding. In acute DIC there may also be systemic thrombosis. Microangiopathic hemolytic anemia also causes pulmonary edema, hypertension, and renal insufficiency. CT or MRI will show single or multiple parenchymal or subdural hemorrhages. Low platelets and fibrinogen, elevated prothrombin time, activated partial thromboplastin time, and D-dimer are signs of acute DIC. Treatment for DIC is directed to the tumor and replacement of clotting factors. Sometimes anticoagulation is indicated. Subdural hemorrhages thrombocytopenia can usually be managed conservatively (Graus et al., 1996).

2. Treatment-Related

Infarction/Thrombosis: Arterial or venous thrombosis is an uncommon complication of chemotherapy, possibly related to vasospasm, vasculitis, or effects on the coagulation system. It is most common in children with leukemia who develop venous sinus thrombosis after induction therapy with L-asparaginase. Arterial infarction is also reported with cisplatin administration and in breast cancer patients receiving tamoxifen and multiagent chemotherapy (Bushnell and Goldberg, 2004). Therapeutic radiation to treat head and neck cancer is associated with accelerated carotid atherosclerosis (Dorrestein et al., 2002). Venous thrombosis typically causes headache. There are focal signs if infarction or hemorrhage ensues. Chemotherapy and radiation-related thrombosis may result in TIA or infarction. Long segments of carotid stenosis confined to the area of radiation are visualized on angiography in radiation-induced atherosclerosis. Radiation-induced carotid disease is effectively treated surgically.

Hemorrhage: Radiation or chemotherapy with marrow suppression may result in thrombocytopenia and brain, subdural, or subarachnoid hemorrhage. The hemolytic-uremic syndrome is a complication of some chemotherapies, especially mitomycin (Gordon and Kwaan, 1999).

3. Other

Infarction: Fungal septic embolus is a rare cause of stroke, resulting in symptomatic bland or hemorrhagic infarctions, most often in leukemia patients after bone marrow transplantation. Granulomatous angiitis is a rare complication of lymphoma or leukemia.

Metastatic

1. Vessel Compression/Infiltration

Infarction/Thrombosis: Metastatic tumor in the skull or meninges can produce thrombosis in an underlying venous sinus due to compression or infiltration. Parenchymal arterial compression or spasm is a rare complication of leptomeningeal metastasis. Intravascular and intraparenchymal tumor infarction from proliferation of lymphoma cells within cerebral vessels. Metastatic venous occlusion causes gradual signs of increased intracranial pressure. Papilloedema is often present. Subacute and progressive focal signs or encephalopathy result from lymphomatosis. MRI or MRV shows venous occlusion and skull or dural enhancement in metastatic venous occlusion. Infarction, hemorrhage, and nonspecific white matter changes are seen on MRI in lymphomatosis (Williams et al., 1998). Treatment for neoplastic infarction/ thrombosis includes brain radiation therapy and/or chemotherapy. 

Hemorrhage: Brain or dural metastasis can result in acute or subacute hemorrhage. The most common parenchymal tumors are lung cancer, melanoma, breast, and hematopoietic tumors. In breast cancer, subdural metastases are most common in breast and prostate carcinoma, less frequent in leukemia and lymphoma. Hyperleukocytosis in acute leukemia with leukostasis and brain hemorrhage is now rare. Signs of systemic thrombosis are typically a sudden headache and focal signs or encephalopathy. Subdural hemorrhages present subacutely with headache and focal signs or encephalopathy.
A clue to neoplastic parenchymal hemorrhage on MRI is early edema and enhancement, heterogeneous signal, and other brain areas of enhancement. Metastatic subdural hemorrhages are accompanied by dural and/or skull enhancement. A dural biopsy may be required for diagnosis. Steroids are often indicated to treat tumor-associated edema. Removal of the parenchymal hematoma or drainage of subdural fluid, followed by brain radiation, is indicated if the patient is symptomatic.

2. Embolism

Infarction: Cerebral TIA or infarction results from mucin or large tumor emboli, typically from the lung (O’Neill et al., 1987), less often from cardiac or aortic arch tumors. Embolization may occur from surgical manipulation of the lung to remove cancer. Brain CT or MRI shows focal or multifocal infarctions and may show enhancement from growing tumor. Echocardiography is diagnostic of cardiac tumor. The treatment for tumor emboli is brain radiation and treating the systemic tumor in order to prevent further episodes.


References


233. EXTENDED ABSTRACT: PARANEOPLASTIC NEUROLOGICAL SYNDROMES

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Paraneoplastic neurological syndromes (PNS) are important in clinical practice because they are associated with specific types of tumors and usually antedate the diagnosis of the cancer that usually is in a localized stage where the chances to cure the tumor are highest. The clinical evaluation of patients with suspected PNS is difficult because similar syndromes may occur in the absence of cancer and the tumor is not evident at the onset of the neurological disorder in the majority of patients. Some neurological syndromes defined as classical must suggest a paraneoplastic etiology as one of the leading diagnoses (Table 1). Most PNS have in common the subacute onset, severe neurological deterioration, and, in those involving the central nervous system, frequent evidence of mild cerebrospinal fluid pleocytosis or IgG oligoclonal bands. The most helpful test that suggests the paraneoplastic etiology of the syndrome is the detection of onconeural antibodies (Table 2). Recently, the term “well-characterized” onconeural antibody has been introduced to designate those onconeural antibodies (Hu, Yo, Ri, Ma2, CV2, and amphiphysin) for which (1) there are recognizable patterns on routine immunohistochemistry and for which immunoblotting on recombinant proteins is available to confirm their specificities, (2) the number of cases reported is associated with tumors, (3) the description of well-characterized neurological syndromes is associated with the antibodies, (4) the identification of the antibodies occurs among different studies, and (5) the frequency of these antibodies is less in patients without cancer. Most onconeural antibodies are tightly associated with particular PNS and tumor types. However, the predictive value depends on the onconeural antibody and the PNS.

Recently, a panel of neurologists interested in PNS suggested that there should be two levels of diagnostic evidence to define a neurological syndrome as paraneoplastic: definite and possible. Each level can be reached by combining a set of criteria (Fig. 1). The panel recognized that the term “possible” may include true PNS but also the coincidental association of two unrelated disorders (the neurological syndrome and cancer). However, this level of evidence may be useful to identify disorders that in the future may be aggregated to define PNS and to recognize PNS based on the identification of specific trends such as a higher than expected association with a specific type of cancer. The panel emphasized that definite and possible PNS have in common the need to exclude other known causes that could explain the neurological syndrome under study even if onconeural antibodies are positive.

Early diagnosis of the underlying tumor affords the best chance to cure the neoplasm. In addition, effective treatment of the neoplasm contributes to improving or stabilizing the PNS. Early tumor diagnosis requires a high index of suspicion of the radiologist who performs the radiological examination. Recently, positron emission tomography showed a better sensitivity than the usual CT to demonstrate the underlying neoplasm. Sometimes the tumor discovered is not the one usually associated with the PNS or the onconeural antibody. In this situation, the tumor may be responsible for the PNS, or the patient may harbor another tumor that is responsible for the PNS. A way to solve this dilemma is to determine if the tumor expresses the antigen recognized by the onconeural antibody.

The clinical course of PNS is not always uniform. Spontaneous improvement is reported in a few patients with several PNS. Furthermore, some patients with radionecrosis present with a slowly indolent clinical course over years in absence of any treatment. Several immunosuppressor therapies including corticosteroids, plasmapheresis, and intravenous high-dose immunoglobulins have been used in the treatment of PNS. These therapies are useful in the opsoclonus-myoclonus syndrome associated with neuroblastoma, with LEMS, with multineuritis with vasculitis, with dermatomyositis, and in a few patients, with limbic encephalitis, particularly those with anti-Ma2 antibodies. In most of these disorders, the damage to the nervous system is functional more than structural, so a clinical improvement may be expected after treatment.

In PNS with neuronal degeneration such as paraneoplastic cerebellar degeneration, immunosuppressor therapies have not been successful. However, we favor a trial of immunosuppressor or immunomodulating drugs based on the evidence that these PNS probably are immune mediated and on occasional case reports that they improved with intravenous immunoglobulins or other immunotherapies. Although theoretically, immunosuppression could exacerbate tumor growth, we did not find that these treatments were an adverse prognostic factor for survival.

References


234. TREATMENT OF HUMAN GLIOMA CELLS WITH HUMAN ANTI-HUMAN TNF-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL/APO2L) RECEPTOR MONOCLONAL ANTIBODIES


TNF-related apoptosis-inducing ligand (TRAIL/APO2L), a member of TNF family, induces apoptosis preferentially in human tumor cells but not in normal cells, suggesting TRAIL, through its cognate death receptors DR4 or DR5, may serve in a potential therapeutic role for intractable malignant gliomas. We applied complete human anti-human TRAIL receptor monoclonal antibodies (mAbs) to specifically target one of TRAIL’s death receptors in human glioma cells, which could reduce potential TRAIL-induced toxicity in human. All mAbs were provided by the Kirin Brewing Co. Ltd., Tokyo. Fourteen human glioma cell lines were treated with either a human anti-DR4 mAb (clone B12), or anti-DR5 mAbs (clones E11 and H48), and their cytotoxic effects were determined by using MTT assays. Anti-TRAIL receptor mAb-induced cytotoxicity was compared with that induced by soluble TRAIL-R1 and TRAIL-R2. Caspase activation studies were performed using whole cell lysates prepared after mAb treatments. Anti-DR5 mAb treatments induced significant cytotoxicity in a majority of human glioma cell lines tested, which was blocked in the presence of DR5-Fc, a TRAIL-neutralizing fusion protein composed of the extracellular domain of DR5. Sensitivity to anti-DR5 mAb correlated with that to soluble TRAIL in these cells, suggesting that the apoptosis signals triggered by ligation of DR5 with human mAbs may be transduced similarly to that by soluble TRAIL. In contrast, anti-DR4 mAb treatment was ineffective in most human glioma cell lines except two, and only one cell line exhibited cross sensitivity to both mAbs. Established TRAIL-resistant sublines, 198G TR, and LNZ308 TR, showed lower response rates to the mAb treatments. Anti-DR5 mAb treatment resulted in cleavage and activation of initiator and executioner caspases, as well as cleavage of poly(ADP-ribose) polymerase, an intrinsic substrate of caspase 3. Furthermore, treatment with anti-DR5 mAbs suppressed growth of subcutaneous xenografts derived from LNZ108 cells in athymic mice. These results suggest that DR5 may represent the major functional TRAIL receptor mediating receptor-induced apoptosis in human glioma cells, and targeting DR5 with human mAb agonistic to DR5 could provide a potential therapeutic strategy against intractable malignant gliomas.

235. INHIBITION OF C-JUN N-TERMINAL KINASE ENHANCES TEMOZOLOMIDE-INDUCED CYTOTOXICITY IN HUMAN GLIOMA CELLS

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Previous studies revealed that the p38, a member of stress-activated protein kinases (SAPKs), cooperates with the Chk1-pathway to bring about TMZ-induced G2 arrest, and the inhibition of either pathway alone is sufficient to sensitize U87MG glioma cells to TMZ-induced cytotoxicity. We hypothesized that other SAPKs might be involved in cellular responses to DNA damage and that blocking of such protein might sensitize glioma cells to chemotherapeutic agents. In the present study, we analyzed alteration of c-Jun N-terminal kinase (JNK), another SAPK, in U87MG cells treated with DNA-methylating agent temozolomide (TMZ). Immunoblot analysis showed that JNK was phosphorylated 1 to 2 days after TMZ treatment. Since a previous study suggested that TMZ induces severe DNA damage 1 to 2 days after drug treatment in primary glioma cells, it is plausible that repair system, we speculate that activation of JNK is triggered in response to the creation of severe DNA damage, probably DNA double strand breaks which are potentially lethal to the cells. To analyze the role of JNK phosphorylation in survival of glioma cells with DNA damage, we pre- (for 24 h) and post- (for 72 h) treated U87MG cells with JNK inhibitor (Calbiochem, USA) in combination with TMZ treatment. Colony formation efficiency assay revealed that the clonogenicity of TMZ-treated U87MG cells was remarkably reduced by JNK inhibitor at 200 nM or higher concentra- tion. Immunoblot for phosphorylated cdk2 revealed that this potentia- tion of TMZ-induced cytotoxicity was not associated with abrogation of G2 checkpoint pathway. Phosphorylation of JNK target protein c-Jun was inhibited with 200 nM JNK inhibitor. However, phosphorylation of ATF-2, another JNK target, was not affected by this concentration of JNK inhibitor, and it was suggested that c-Jun-related responses were more important in JNK-mediated survival of glioma cells with DNA damage. Finally, we performed similar experiments using another human glioma cell line, U251, and confirmed that the events mentioned above were not cell line-specific. The mechanism of the JNK inhibitor-induced enhancement of the cyto- toxicity of TMZ is still unclear, and JNK inhibitors are not available for clinical use. Nonetheless, we performed additional investigation based on the result of this study which may provide a viable approach for the sensitization of human gliomas to TMZ-induced cytotoxicity.

236. BLOCKADE OF THE PI3-KINASE P110A CATALYTIC SUBUNIT INDUCES G2M ARREST AND APOPTOSIS IN HUMAN GLIOMA CELL LINES

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Amplification of EGFR occurs in 40% of astrocytomas and correlates with advanced disease. Activation of phosphatidylinositol-3-kinase (PI3-kinase) also occurs commonly in glioma and occurs in part through loss of the tumor suppressor PTEN. Because the EGFR and PI3-kinases are activated in glioma and in other human cancers, combination therapies directed against these kinases offer a mechanistic rationale to improve therapy. In published work, we showed that inhibition of EGFR cooperated with inhibition of PI3-kinase in the preclinical therapy of glioma. The PI3-kinases constitute a complex protein family classified according to structure and subunit composition. Despite numerous differences in up- stream, the physiological roles of individual PI3-kinase isoforms and the contributions of individual isoforms to specific malignancies remains poorly under- stood. The small-molecule PI3-kinase inhibitors LY294002 and wortman- nin have been instrumental tools to dissect basic elements of PI3-kinase signaling. As a consequence of indiscriminately inhibiting all PI3-kinases and a large number of related proteins, LY294002 and wortmannin are too toxic to be used in patients. Thus, the utility of small-molecule PI3-kinase inhibitors is in clinical practice development of new, more selective inhibitors that can be safely and effectively used in patients. To address the role of particular PI3-kinase isoforms in glioma, we have synthesized 12 isoform-selective inhibitors of particular PI3-kinase substrates likely to contribute to glioma and have characterized the IC50 values against 20 recombinant kinase targets. These agents represent the first new tools available in a decade for analysis of PI3-kinase signaling. We have screened all of these compounds against a panel of astrocytoma cell lines. Although most of these inhibitors were quite potent in blocking the PI3-kinase downstream target Akt, one only inhibitor (selectivity: p110α = DNA-Protein Kinase > p110g) induced significant growth arrest and apoptosis in the entire panel of human glioma cell lines and was most effective against cells wild-type for PTEN. This study demonstrates that the PI3-kinase catalytic subunit p110α plays an important role in proliferation and survival of glioma.

237. LOW-MOLECULAR-WEIGHT EGFR/KDR TYROSINE KINASE INHIBITOR OFFERS COMBINATORIAL BENEFIT WITH A RAPAMYCIN DERIVATIVE BASED ON PTEN STATUS

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Malignant gliomas are highly lethal tumors that display striking genetic heterogeneity. Novel therapies that inhibit a single molecular target may slow tumor progression, but tumors are likely dependent on multiple pathways leading to tumor transduction pathway. We recently reported the first completed trial of an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, gefitinib, in recurrent glioblastomas. Although a subset of patients experi- enced long-term control of tumor growth, the majority did not show significant tumor shrinkage. Analysis of tumor specimens demonstrated that progression of their tumors. The molecular mechanisms by which cancers develop resistance to EGFR inhibitors remains poorly understood. As EGFR may have significant impact on tumor growth through its pro-angiogenic effects, independent vascular endothelial growth factor receptor 2 (kinase domain region, KDR) activity may provide an important survival advantage with the withdrawal of EGFR effects. Additionally, PTEN-deficient glioma cell lines display increased sensitivity to mammalian target of rapa- mycin (mTOR) inhibition as compared with those with wild-type PTEN. AEE788 is a novel orally active tyrosine kinase inhibitor that decreases the kinase activity associated with EGFR and KDR. RAD001 [everolimus] is an orally available mTOR inhibitor structurally related to rapamycin. We hypothesized that combined inhibition of upstream EGFR and KDR recep- tors with AEE788 and inhibition of the downstream mTOR pathway with RAD001 would result in increased efficacy against gliomas compared to single-agent therapy. In vitro experiments showed that the combination of AEE788 and RAD001 resulted in increased rates of cell cycle arrest and...
238. HUMAN, TUMOR-FOUNDED NEURAL STEM CELLS AND TUMOR STEM CELL LINES FOR THE DIAGNOSIS AND THERAPY OF GLIOMASTOMAS
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We have recently provided the initial evidence that, unlike other brain malignancies, the lethal glioblastoma multiforme (GBM) contains neural precursors endowed with all of the critical features expected from NSCs. Similar, yet not identical, to their normal NSC counterpart, these precursors emerge as unipotent (astroglial) in vivo and multipotent (neuronal-astroglial-oligodendroglial) in culture. More importantly, these cells can act as tumor-founding cells down to the clonal level and can establish tumors which closely resemble the main histological, cytological, and architectural features of the human disease, even when challenged through serial transplantation. Thus, cells possessing all the characteristics expected from tumor neural stem cells from GBMs, including the typical infiltrating and migratory capacity expected from malignant glioma cells, appear to be involved in the growth and recurrence of adult human GBMs. Such features have never been observed previously in the use of common xenograft or allograft-based brain tumor models. Our report also describes tumor neural stem cells (TNSCs) that can be used to routinely establish single derived-GBM cell lines in a quick and reproducible fashion. Importantly, TNSCs from different patients retain their distinctive, line-specific proliferation and differentiation attributes which appear to be genetically determined, as shown by the establishment of clonal TNSCs, which possess stable properties identical to those of their parental bulk cultures. TNSCs remain unaltered after multiple in vitro passages and even serial in vivo orthotopic transplantation. Using these lines we are now exploring the possibility that the same key genetic, epigenetic, and extracellular cues that are involved in the maintenance of stem cells and their fate regulation may also be at work in TNSCs so as to prove that the body of knowledge that has emerged from studying basic brain stem cell physiology may be harnessed to identify new therapeutic targets and approaches in neuro-oncology. We shall present recent findings which show that some cues act upon neural stem cells. In our hands, TNSCs also provide an invaluable tool for the in vivo modeling and studying of GBMs, particularly in view of their patient-specific features. As a result, these cells may provide the means to improve diagnosis and develop patient-tailored therapies.

239. A BISPECIFIC IMMUNOTOXIN (DTAT13) TARGETING HUMAN INTERLEUKIN-13 AND UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTORS IN A MOUSE XENOGRAFT MODEL
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A bispecific immunotoxin (IT) DTAT13 was synthesized in order to target simultaneously the urokinase-type plasminogen activator receptor (uPAR)-expressing tumor neovasculature and IL-13 receptor expressing glioblastoma cells with the goal of intratumoral administration for brain tumors. The recombinant hybrid was created by using the non-integrating N-terminal portion of a uPAR (ATF) and the IL-13 receptor gene, the catalytic and translocation portion of diphtheria toxin (DT) for killing. The 71 kDa protein was highly selective for human glioblastoma in vitro showing no loss on binding compared with DTAT and DTIL13 controls. In vivo, DTAT13 induced the regression of subcutaneous glioma tumors when administered at 10 μg/day given on a five-dose schedule every other day. DTAT13 was able to target both overexpressed uPAR and the vasculature, as demonstrated by its ability to kill HUVEC. In preclinical studies indicated that DTAT13 was less toxic than DTAT or DTAT13. These findings indicate that bispecific IT may allow treatment of a broader subset of antigenically diverse patients while simultaneously reducing the exposure to toxin that is required if two separate agents were employed.

240. GENOME-WIDE ALLELIC IMBALANCE ANALYSIS OF PEDIATRIC GLIOMAS BY HIGH-DENSITY SINGLE NUCLEOTIDE POLYMORPHIC ALLELE (SNP) ARRAY
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In the Children’s Cancer Group high-grade glioma study CCG-945, out of 250 high-grade glioma cases diagnosed by local pathologists, 70 cases were reclassified as low-grade glioma after central consensus pathology review. This indicates a need for additional criteria other than morphology, such as genome-wide genotyping (allelic imbalance analysis), to improve the accuracy of histopathologic classification for these tumors. More importantly, the etiology and molecular pathogenesis of pediatric gliomas remain unclear. Brain tumor tissues were obtained under an IRB-approved protocol after informed consents were obtained from patients undergoing tumor resection at Texas Children’s Hospital, Baylor College of Medicine. Portions of the tumors were fixed in 10% formaldehyde and embedded in paraffin for sectioning and pathological diagnosis, and the remaining tissues were snap-frozen in liquid nitrogen for DNA extraction. All tumor tissues were obtained at initial diagnosis with no prior exposure to chemotherapy or radiation. Totally, 7 low-grade gliomas (3 JPA, 1 ganglioglioma, 1 astrocytoma) and 9 high-grade gliomas (GBM) were analyzed by SNP array that contains 11,360 SNP alleles spanning the human genome with a median intermarker distance of 105 kb. Sixteen pediatric gliomas were analyzed by SNP arrays. No loss of heterozygosity (LOH) was detected in any of the of the 11,362 SNP loci for the five juvenile pilocytic astrocytomas. The ganglioglioma has LOH on chromosome 9p while the astrocytoma has LOH in 28 SNP loci on chromosome 6q. On the other hand, high-grade gliomas are very heterogeneous in that the number of SNP loci with LOH varied from 32 to 2125. Significant LOH cytosand regions in GBM include 4q, 6q, 9q, 12, 13q, 14q, 17, 18p, and 19q. We also detected amplification of SNP loci near the genes EGFR and PDGFRα in two different cases of GBM. No observable allelic imbalance was detected in JPA, and allelic imbalance in other low-grade gliomas only involves a single chromosome. On the other hand, allelic imbalance in high-grade gliomas is quite variable and involves multiple chromosomes. The simultaneous measurement of DNA copy number changes and LOH by SNP arrays should enhance our ability to discover cancer-causing genes and to refine the diagnosis of pediatric gliomas.

241. DISSECTING INTRATUMORAL HETEROGENEITY IN UNTREATED GLIOBLASTOMA: TALES FROM THE EDGE T. Van Meter,1 C. Dumur,2 N. Hafez,2 C. Garrett,3 H. Fillmore,1 and W. Broaddus1 1Neurosurgery; 2Pathology, Virginia Commonwealth University, Richmond, Virginia, USA

Glioblastoma multiforme is the most aggressive and treatment-resistant adult primary brain tumor. The molecular changes that underlie tumor heterogeneity in glioblastoma have not been studied in detail. We have undertaken a detailed analysis of tissue prospectively collected intra-operatively using Stealth imaging-assisted tissue extraction. This method was used to isolate tissue samples from multiple intra-tumoral regions in untreated glioblastoma, corresponding to the enhancing tumor rim and areas of hypoxic tumor core. Affymetrix HG-U133A high-density oligonucleotide arrays were used to assess differences in gene expression profiles in the different regions. Our approach used an RNA extraction protocol paired with in-process histological scoring of tissue samples using H&E staining of frozen sections. Tumor gene expression profiles from different tumor core sampled and correlated with percent tumor, percent necrosis, and other histological features. In this report, we focused on genes upregulated in the enriching periphery of regions bearing >90% tumor versus normal brain, compared to the core regions. We have analyzed the resulting normalized data sets using a series of 3 algorithms (MBEI, MAS5 and RMA) to provide a consensus profile of transcriptomic differences between periphery and core samples. Previously, we have reported that EGFR and AKT signaling are upregulated in aJPA (ATF) and the IL-13 receptor gene, the catalytic and translocation portion of diphtheria toxin (DT) for killing. The 71 kDa protein was highly selective for human glioblastoma in vitro showing no loss on binding compared with DTAT and DTIL13 controls. In vivo, DTAT13 induced the regression of subcutaneous glioma tumors when administered at 10 μg/day given on a five-dose schedule every other day. DTAT13 was able to target both overexpressed uPAR and the vasculature, as demonstrated by its ability to kill HUVEC. In preclinical studies indicated that DTAT13 was less toxic than DTAT or DTAT13. These findings indicate that bispecific IT may allow treatment of a broader subset of antigenically diverse patients while simultaneously reducing the exposure to toxin that is required if two separate agents were employed.
242. CHROMOSOMAL TILE PATH ARRAY-CGH ANALYSIS IN ASTROCYTIC TUMORS LEADS TO IDENTIFICATION OF A NOVEL CANCER TUMOR SUPPRESSOR GENE ON CHROMOSOME 19q13.32
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Adult astrocytic tumors have complex and diverse genotypes. A number of oncogenes and tumor suppressor genes (TSGs) have been implicated in the development and/or progression of these tumors, including CDKN2A, CDKN2B, PTEN, TP53, EGFR, and others. In an attempt to identify novel TSGs, we have constructed chromosome 19q13.32 arrays that covers the entire chromosome 19q13.32. After screening tumors originating from primary glioblastoma and astrocytomas, we have identified a novel TSG at 19q13.32. This TSG has been named as DEPDC5, which is a member of the dead-related protein family.

244. PIK3CA IS MUTATED IN OLGIDODERGIOGLOMYS, ASTROCYTOMAS, AND MEDULLOBLASTOMAS AND ASSOCIATED WITH CHROMOSOME 1p, 19q LOH IN OLIGODENDROGLIOMA
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The phosphatidylinositol 3-kinase (PI3K) pathway plays a critical role in cell survival and death, and is often dysregulated in cancer. Mutations in PIK3CA, the gene encoding PI3Kα, are found in a variety of human cancers, including brain tumors. We have identified PIK3CA mutations in oligodendrogliomas, astrocytomas, and medulloblastomas.

Gene expression profiling of a set of 76 newly diagnosed high-grade astrocytomas reveals molecular subtypes of tumor that differ in both survival times and in the subsets of genes whose expression is most strongly associated with survival. Based on relative expression of 35 marker genes, tumors are segregated into 3 groups, each of which has an expression signature that characterizes a distinct set of tissues. One tumor subtype displays a median survival time (175 weeks) that is substantially longer than that of the other subtypes (61 weeks and 71 weeks) and is distinguished by a marker signature that resembles that of fetal brain or undifferentiated neural stem cells.

In oligodendrogliomas, we have constructed 22q tile path microarray for GHG (array-CGH) in order to precisely identify the chromosomal 22 abnormalities of astrocytic tumors. The technique has a number of advantages in that (1) it does not rely on naturally occurring polymorphisms, (2) the findings can be directly linked to published human genome sequences, and (3) it allows quantitative assessment of copy number at each clone. A tile path clone set for chromosome 22, which covers 82% of 22q with 443 BAC/PAC cosmids, has been obtained from the Wellcome Trust Sanger Institute. The array was constructed according to the published protocol using modified DOP-PCR method. A total of 126 astrocytomas consisting of 92 glioblastomas (GB), 29 anaplastic astrocytomas (AA), and 5 diffuse astrocytomas (A) were subjected to the study. These tumors have also been examined for allelic status using 28 microsatellite markers distributed along the entire 22q. Approval for the study has been obtained from the local ethical committee. The results showed good concordance between microsatellite and array-CGH data. As a result of combined analysis using chromosome 22 and 22q data, we identified 22q abnormalities in 38% of GB, 33% of AA and 5% of A. Among several candidate regions identified, we further investigated two overlapping homozygous deletions on 22q12.3 that spanned three clones. The region harbored three genes, YWHAH, CC20RF24, and DEPDC5. Homozygous deletions were confirmed by Southern hybridization and multiplex PCR. Gene-by-gene mutation analysis revealed 3 different protein truncating mutations in 2 glioblastoma cell lines and another somatic nonsense mutation in a primary glioblastoma and its xenograft in DEPDC5. We have thus demonstrated that chromosome 22 tile path array can accurately discriminate single copy number change.

245. MOLECULAR CLASSIFICATION OF OLIGODENDROGLIOMA IDENTIFIES TRANSCRIPTS ASSOCIATED WITH RESPONSE TO CHEMOTHERAPY
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Malignant gliomas are the most common primary central nervous system tumor in adults. One glioma subtype, the oligodendrogloma, has a markedly better prognosis than most others (median survival is 5–10 years). A common genomic aberration in oligodendrogliomas is a chromosome 19p deletion, which has been shown to be an important clinical marker. Recent findings suggest that oligodendrogliomas harbor mutations in the PTEN tumor suppressor gene, which encodes a lipid phosphatase.

Gene expression profiling of a set of 28 oligodendrogliomas and 6 control brains using Affyme- ter HG-U133A microarrays. The discovery of several gene expression modules that are significantly correlated with response to treatment (combined radiation therapy and chemotherapy). In order to better understand the molecular mechanisms that underlie chemosensitivity and potentially identify molecular pathways affected in glioblastomas, we compared mRNA expression profiles of 28 oligodendrogliomas and 6 control brains using Affymetrix HG-U133A microarrays. We first performed unsupervised clustering to group samples on the similarities in mRNA expression profile of the glioblastoma samples. Three subgroups were identified. Subgroup 1 consists of low-grade tumors and control brains; subgroup 2 consists of medulloblastomas with loss of 1p, while subgroup 3 consists of medulloblastomas with loss of 19q13.32.
We next performed supervised clustering to identify genes associated with loss of the short arm of chromosome 1. Both FISH and LOH-PCR were used to determine loss of 1p and 19q in our samples. Supervised clustering identified 95 probe sets as being differentially expressed between tumors that have lost one copy of 1p compared to those that have retained both copies. Interestingly, 81/95 (85%) of these probe sets are located either on chromosome 1p or on 19q, and the average difference in expression level is 0.35 ± 0.08. This suggests that these genes are differentially expressed upon chromosome 1p loss and may identify molecular mechanisms that underlie chemosensitivity. Finally, supervised clustering was performed to identify genes that are associated with chemotherapeutic response. We identified 16 probe sets that are significantly differentially expressed between chemosensitive and chemoresistant tumors. These genes can provide a diagnostic value as to whether the tumor will respond favorably to chemotherapy and may identify molecular mechanisms that underlie chemosensitivity.

A majority of adult low-grade gliomas (LGGs) grow slowly for many years and then, unpredictably, undergo malignant transformation. The clinical management of LGGs is controversial as there is no proven benefit for intervention prior to transformation. We have developed a reproducible and sensitive method of measuring tumor volumes on MRI scans in order to model growth rates of untreated adult LGGs and to determine whether acceleration of tumor growth occurs prior to transformation. Coronal-Oblique FLAIR and thin section Gd T1w sequences were obtained six months from 33 patients with untreated LGGs who were recruited into a multimodality imaging study. Tumor volumes were measured on 3-mm-thick sections with an interslice thickness of 1.5 mm from FLAIR images with a semi-automated intensity gradient-based thresholding program with manual editing used when required. The tumor was contoured on all covering slices, the contoured areas were saved as a region file, and their combined volume was calculated. Percentage tumor growth rates per year were derived from hierarchical regression modeling and compared between different tumor histologies and between non-transformers (NT) and transformers (T). Transformation was defined as clinical deterioration, or the appearance of new or increased enhancement on the Gd studies, and confirmed by biopsy of the enhancing region or resection of the tumor. Seventeen patients transformed and 16 remained clinically and radiologically stable (NT). The average annual growth rate in the NT group was 13% (95% CI, 9%–18%) and in the T group was 26% (95% CI, 21%–32%) up to six months prior to the transformation scan. This equates to a 12% higher growth rate in the T group (95% CI, 5%–18%; P < 0.0001). Within the T group, the average growth rate increased in the final six-months screening period to 57% per annum (95% CI, 19%–100%; P = 0.08 compared with earlier growth rates). Non-transforming astrocytomas grew at the same rate as oligodendrogliomas (14%–15% per year). We conclude that LGG which subsequently transform grow significantly faster prior to malignant transformation than non-transforming tumors. There is no difference in growth rates between astrocytic and oligodendroglial tumors. Through using hierarchical regression modeling and compared with different tumor histologies and between non-transformers (NT) and transformers (T). Transformation was defined as clinical deterioration, or the appearance of new or increased enhancement on the Gd studies, and confirmed by biopsy of the enhancing region or resection of the tumor. Seventeen patients transformed and 16 remained clinically and radiologically stable (NT). The average annual growth rate in the NT group was 13% (95% CI, 9%–18%) and in the T group was 26% (95% CI, 21%–32%) up to six months prior to the transformation scan. This equates to a 12% higher growth rate in the T group (95% CI, 5%–18%; P < 0.0001). Within the T group, the average growth rate increased in the final six-months screening period to 57% per annum (95% CI, 19%–100%; P = 0.08 compared with earlier growth rates). Non-transforming astrocytomas grew at the same rate as oligodendrogliomas (14%–15% per year). We conclude that LGG which subsequently transform grow significantly faster prior to malignant transformation than non-transforming tumors. There is no difference in growth rates between astrocytic and oligodendroglial tumors.
249. HIGH SPECIFICITY AND SENSITIVITY OF HIGH-RESOLUTION, BIPLANE DIGITAL SUBTRACTION ANGIOGRAPHY IN THE DIAGNOSIS OF PATIENTS WITH SUSPECTED GLIOBLASTOMA MULTIFORME: IMPACT ON THE MANAGEMENT OF NONSURGICAL CASES

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In a significant subset of patients suspected to have supratentorial glioblastoma multiforme (GBM) based on MRI, biopsy does not have therapeutic consequences, is performed to rule out a hypothetic curable lesion, is not always conclusive, and carries a significant risk of bleeding and clinical deterioration. Less invasive, but equally reliable diagnostic techniques are warranted for these patients with a limited life expectancy. We routinely perform preoperative digital subtraction angiography (DSA) in patients suspected to have a GBM. We investigated the diagnostic specificity and sensitivity of DSA in this patient population. Patients diagnosed between 1993 and 2002, with a supratentorial lesion compatible with a GBM based on MRI, with a preoperative high-resolution, biplane DSA (1024 × 1024 matrix), and with a histological diagnosis were included. Neuroradiologists blinded for the histological diagnosis analyzed the angiograms and assessed the image quality and the presence of tumor-blush, pathological vessels (PV), and an early venous drainage (EVD). They were asked to answer following questions about the lesion: Is it a tumor? Is it a malignant tumor? Is it a GBM? One hundred eighty-six patients were eligible for the study (42% supratentorial, 154 surgical resections). No major complications resulted from angiography. Preliminary data showed following results: (1) The presence of PV associated with EVD at DSA was 100% specific for the presence of a malignant tumor (77% a malignant glioma, 20% a metastasis, 3% a PNET). (2) PV associated with EVD at DSA was present in 82% of GBMs and 75% of all malignant tumors including GBM (sensitivity). We conclude that in patients with supratentorial, intracerebral lesions compatible with a GBM based on MRI, the presence of pathological vessels and of an early venous drainage in high-resolution biplane DSA is both extremely specific and highly sensitive for the diagnosis of a malignant tumor, mostly a malignant glioma. In these patients, especially those in whom no therapy is planned, the risk of biopsy can be avoided.

250. NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME: PROTON MR SPECTROSCOPIC CHARACTERISTICS AND CORRELATION WITH CLINICAL OUTCOME

Proton magnetic resonance spectroscopic imaging (1H MRSI) is a non-invasive means of analyzing metabolic integrity within both normal and diseased areas of the brain. The purpose of this study was to use 1H MRSI to determine absolute levels of lactate (Lac), lipids (Lip), acetyl aspartate (N-acetyl aspartate ratio index [CNI]) in two groups of glioblastoma multiforme (GBM) patients differentiated by time to progression (TTP) to assess for relationships between clinical outcome and metabolic markers. Twelve newly diagnosed GBM patients were recruited for this study. These patients were divided into two groups based on clinical status: rapid progression (RP, progression before 6 months; n = 8) and moderate progression (MP, progression after 6 months; n = 4). In addition to pre-operative anatomic MR imaging, all patients underwent 3D J-difference lactate-edited MRSI imaging. The lactate-edited method required two acquisitions in each phase encoding step and provided reliable quantification of lactate, lipids, and CNI. The metabolite levels were quantified by using a PRESS volume selection technique. The lactate-edited method was shown to be robust and reliable for quantification of brain metabolites. Using this technique, we were able to acquire high-resolution 3D images from each patient's 1H MRSI exam: number of voxels with significant z-scores, considered abnormal. The following measurement variables were derived from each patient's 1H MRSI exam: number of voxels with significant z-scores (sigZ), average z-score (avgZ), and Max z-score (maxZ). The average volume of the T2L hyperintense captured by the PRESS box was similar among both groups. MRSI voxels contaminated with lipid artifact were excluded from analysis. Results were as follows.

<table>
<thead>
<tr>
<th>Group score derived</th>
<th>from an average of each patient’s preoperative exam</th>
<th># voxels</th>
<th>avg Z</th>
<th>max Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lac</td>
<td>lip</td>
<td>cni</td>
<td>lac</td>
<td>lip</td>
</tr>
<tr>
<td>Rapid Progression</td>
<td>26.3</td>
<td>21.8</td>
<td>28.9</td>
<td>6.8</td>
</tr>
<tr>
<td>Moderate Progression</td>
<td>19.0</td>
<td>8.0</td>
<td>21.0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Overall, the presence of Lac and that of CNI were comparable in the RP and the MP groups. Lip, however, was found in higher quantities in the RP group than in MP. This trend was observed under all measurement variables, and was particularly notable under sigZ. Our preliminary data suggests that lipid may predict clinical outcome in GBM patients and that lipid is also a better predictor of outcome than Lac or CNI. The results of this study suggest that metabolic information derived from 1H MRSI may be a more reflective marker of clinical outcome in patients with GBM. Further study with larger sample size and longer follow-up will be conducted to further evaluate 1H MRS variables in predicting clinical outcome.

253. ADVANCES IN MANAGEMENT OF EPENDYMOMA

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Pediatric ependymomas are enigmatic tumors, and their clinical management remains one of the more difficult in pediatric oncology. There are a number of controversies in ependymoma, and these re highlighted in my address. Ependymomas are thought to arise from the ependymal lining of the ventricles and lining of the central canal, though the cell of origin is unknown. Indeed our understanding of the biology of this disease is still limited. While a number of prognostic factors have been identified, these are based on predominantly single-institution studies over long time periods covering many different institutions. No major conclusions can be drawn from this study with larger sample size and longer follow-up will be conducted to further evaluate 1H MRS variables in predicting clinical outcome.
With the exception of certain rare childhood glioma variants, pediatric gliomas are histologically similar to lesions that arise in adults. However, they have distinct molecular features, suggesting age-related pathways of tumorigenesis. Whereas high-grade gliomas in adults, particularly grade IV lesions, typically have amplification of EGFR and mutations of PTEN, such changes are rare in pediatric high-grade gliomas. In contrast, approximately 40% of pediatric malignant gliomas have p53 mutations, which appear to constitute an adverse prognostic factor. P16 or Rb deletions are observed in approximately half of tumors, and correlate with p53 mutations. Unlike the situation in adults, chromosome 1p and 19q deletions, although present in a subset of gliomas, are not associated with a favorable prognosis. Studies are in progress to determine whether prospective categorization of tumors by these molecular features and markers of drug resistance identifies prognostically distinct tumor subgroups in parallel with these analyses, studies are in progress to test new therapeutic approaches for these tumors. One approach that is being examined in several studies involves the use of concurrent chemotherapy with irradiation, in addition to standard post-irradiation chemotherapy. Studies are also in progress that aim to counteract drug resistance as a way of improving response to conventional chemotherapeutic agents. A second general strategy involves targeting of the growth signaling mediators that may contribute to tumor growth, such as PDGFR, EGFR, and VEGFR. Using small molecular inhibitors or drugs that target PI3K, a third approach to chemotherapy involves targeted inhibition of pathways that contribute to tumor angiogenesis and/or invasion. A fourth strategy applies convection-enhanced delivery of high-molecular-weight macromolecules targeted to receptors that are overexpressed on or very near the tumor. This last approach is in the early stages of clinical development, and preliminary results are encouraging. In future studies, patients will be treated with combination chemotherapy consisting of cyclophosphamide, doxorubicin, and etoposide. Clinically manifest BMs were treated with WBRT. Patients with asymptomatic BM did not receive cranial irradiation. The RR of asymptomatic BM to chemotherapy reflects the response of the primary tumor. Therefore, the current practice is to administer local irradiation to BMs that arise from SCCLCs, whereas chemotherapy alone is sufficient for BMs that arise from other malignancies. As a result of this approach, the median survival after diagnosis of asymptomatic BM was 3.8 months and after diagnosis of symptomatic BM was 2.6 months. Among patients who received chemotherapy as the sole therapy, the median survival was 2.6 months. The median survival after diagnosis of asymptomatic BM was 3.8 months, whereas for patients that for symptomatic BM was 8.0 months. The prevalence of asymptomatic BM to chemotherapy was 27%. We could not confirm the high RR found by for-
259. A PHASE 2 TRIAL OF INTRA-CSF ETOPOSIDE IN THE TREATMENT OF NEOPLASTIC MENINGITIS
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The objective of this study was to determine the toxicity and response rate of intra-CSF etoposide [VP-16] in the treatment of patients with neo-
plastic meningitis (NMM). NMM, a metastatic complication of both primary CNS and systemic cancer, occurs in 1% to 5% of patients with known cancer. Currently available treatment options are limited and provide only modest benefit. Twenty-two patients (median age 55 years) with clini-
cal and cytopathologically documented NM received intra-CSF VP-16. Tumor histologies included the following: lung (6 patients), breast (4), brain (4), non-Hodgkin's lymphoma (3), melanoma (3), colon (1), and prostate (1). Concurrent involved-field radiotherapy (16/22 patients) or systemic chemo-
therapy (13/22) was administered based on clinical indications. VP-16 was administered at a fixed dose (0.5 mg every day given 5 times per week every other week for 8 weeks [induction]). Patients were evaluated by CSF cytology and neurological examination at the conclusion of induction therapy. Responding patients continued to receive VP-16 (5 consecutive days every 4 weeks) with monthly evaluations. Seven of 22 patients (32%) treated with VP-16 had a cytopathological response and either stable or improved neurological status at the conclusion of induction. Six of 22 patients (27%) progressed during induction therapy and did not receive the 8-week induction course of therapy. In responding patients, duration of response ranged from 8 to 40 weeks (median 24 weeks). Toxicity was manifested as transient chemi-
cal arachnoiditis (4 patients); 10% of all survival. There was no evidence of myelosuppression nor were treatment-related hospitalizations or deaths seen. It is concluded that VP-16 has modest activity against NMM and easily managed toxicity.

260. PHASE 3 STUDY COMPARING RADIOTHERAPY WITH SUPPORTIVE CARE IN OLDER PATIENTS WITH NEWLY DIAGNOSED ANAPLASTIC ASTROCYTOMAS (AA) OR GLOBLASTOMA MULTIFORME (GBM): AN ANOCEF GROUP TRIAL

Despite evidence of increased incidence, the optimal treatment of mali-
nant astrocytomas in elderly patients is unsettled, ranging from palliative care to a vigorous approach with radiotherapy and chemotherapy. To start evaluating this issue, patients with AA or GBM, age 70 years or older, and a Karnofsky performance status of at least 70 were randomly assigned after biopsy or surgical excision to receive either supportive care (corticosteroids, anticonvulsants, physical therapy, and palliative support) or supportive care and local RT (30 Gy/28 fractions/38 days). Randomization was stratified by center. The primary end point was overall survival. The secondary end points were tolerance and quality of life. Between February 2001 and December 2004, 84 patients with a median age of 73 years (range, 70–85) were randomized in 11 medical centers. A sequential triangular design was used. It is concluded that VP-16 has modest activity against NM and easily managed toxicity.

261. THE ROLE OF FRACTIONATED STEREOTACTIC RE-IRRADIATION IN RECURRENT GLIOMA
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The present analysis evaluates the effectiveness of fractionated stereo-

tactic re-irradiation (FSRT) in recurrent glioma. From January 2002 to July 2003, 156 patients with recurrent gliomas were treated with FSRT. At primary diagnosis, 63 patients had WHO grade II tumors, WHO grade III astrocytomas were diagnosed in 40 patients, and 53 patients suffered from glioblastoma multiforme (GBM). All patients underwent neurosurgical interventions at primary diagnosis. Median time between primary diag-

cnosis and re-irradiation was 50, 31.5, and 10 months for grade II, III, and GBM, respectively. Using 3–4 non-coplanar fields formed with a multi-leaf collimator, a median dose of 36 Gy was applied in a median fractionation of 5x2 Gy/week depending on the size and the localization of the lesion. No concomitant chemotherapy was applied. Radiation was well tolerated by all patients. No severe side effects occurred. Median overall survival was 11.8 months for patients with grade II gliomas, 48 months for grade III, and 21 months for patients with GBM. Calculated from the initiation of FSRT, median survival was 23, 16, and 8 months for grade II, III, and IV gliomas. Main prognosticators for overall survival were histology, and extent of neu-

crosis. Here we report that stereotactically guided fractionated re-irradiation is a safe and effective treatment modality in selected cases with recurrent gliomas. Further investigations to continuously improve overall survival in patients with astrocytomas are warranted, especially with regard to newer radio-chemotherapeutic strategies.

262. LONG-TERM FOLLOW-UP OF PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA TREATED WITH A HIGH-DOSE METHOTREXATE-BASED REGIMEN WITH OR WITHOUT WHOLE BRAIN RADIOTHERAPY
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The primary objective of this study is to report the long-term outcome of patients with newly diagnosed primary central nervous system lymphoma (PCNSL) patients treated with high-dose methotrexate (MTX)-based chemotherapy with or without whole brain radiotherapy (WBRT). Our initial report of this regimen (J. Clin. Oncol. 18, 3144, 2000) projected an overall median survival of 60 months and an excellent outcome for patients younger than age 60. We have now followed all surviving patients for nearly 10 years and have detailed information regarding relapse and delayed treatment toxicity. There were 57 patients with a median age of 65 years (range, 22–65 years) and mean Karnofsky performance status of 75 (range, 30–100). Patients received five cycles of MTX 3.5 g/m² and vincristine 1.4 mg/m². Procarba-

zine 100 mg/m²/d for 7 days was given with the first, third, and fifth cycle of MTX. Three weeks after WBRT or cycle 5 of MTX, cytosine arabinoside 3 g/m² was given for two cycles for a total of 4 doses. Thirty-one patients received WBRT (45 Gy) and 26 older patients deferred WBRT in an effort to reduce treatment-related neurotoxicity. Thirty-seven patients have died, and the overall median survival was 51 months with a median follow-up of the 20 surviving patients 115 months (range, 12–144 months). Recurrent disease developed in 22/57 patients (39%) typically within 3 years of diag-

nosis; one patient developed an isolated systemic non-Hodgkin lymphoma 7.4 years after PCNSL diagnosis. Thirty percent (17/57) of all patients have developed treatment-related dementia; the risk was higher in patients age 60 and older who received WBRT (75%), but 26% of those patients <60 who received WBRT also developed cognitive deficits or dementia. Late neuro-
toxicity has not developed in any surviving patient who deferred WBRT. At last follow-up, a total of 20/57 patients (35%) are alive, including 74% of the patients under the age of 60 at diagnosis plus 19% of older patients who received chemotherapy alone. Cause of death was tumor in 23 patients (40%), acute toxicity during salvage treatment in 2 (3.5%), late toxicity in 9 (16%), other cancers in 2 (3.5%), and unknown in one (1.8%). Long-term follow-up confirms our initial observation of excellent overall survival with this regimen. Younger patients do particularly well, but have a significant risk of developing late neurotoxicity. An subset of older patients who deferred WBRT enjoyed prolonged survival and an excellent quality of life without evidence of treatment-induced neurotoxicity.

263. MODIFIED BORON NEUTRON CAPTURE THERAPY (BNCT) FOR MALIGNANT GLIOMAS USING EPITHERMAL NEUTRON AND TWO BORON COMPOUNDS WITH DIFFERENT ACCUMULATION MECHANISMS: EFFECTIVENESS OF RADIATION PHOTOMICROGRAPHS AND IMAGES
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Boron neutron capture therapy (BNCT) is tumor-specific radiotherapy. To improve the effectiveness of BNCT for malignant gliomas, we utilized epithermal neutron and two different boron compounds from January 2002 to December 2003. Only one glioblastoma, one gliosarcoma, one anaplastic astrocytoma, and one ana-

plastic oligoastrocytoma (6 were new and 7 were recurrent)—were treated with this modified BNCT from January 2002 to December 2003. Only one glioblastoma patient had no postoperative enhanced lesion. The patients received 11F-labeled BPA PET to assess the accumulation and distribution of BPA before neutron irradiation. Irradiation time was determined not to
TP-38 is a recombinant chimeric protein composed of the epidermal growth factor receptor (EGFR) binding ligand (TGF-a) and a genetically engineered form of the Pseudomonas exotoxin PE-38. We report preliminary results of a randomized, phase 2 study conducted at multiple European centers. Either of two dose levels of TP-38 (30 ng/ml or 100 ng/ml) was administered to patients with recurrent glioblastoma in a single treatment consisting of a continuous, intratumoral infusion. This was a nonrandomized study; patients did not undergo tumor resection immediately prior to treatment. Three catheters were stereotactically placed in investigator-determined locations within the enhancing tumor area. The infusion rate was 0.2 ml/min per catheter. Each catheter delivered 13.4 ml over 67 h. The total volume infused was approximately 40 ml, and the total dose of TP-38 infused was approximately 2 μg or 4 μg. Patients were followed until death. Tumor responses were assessed by MRI at every 8 weeks, beginning 4 weeks after treatment. Safety was closely evaluated. Time to progression, progression-free survival, and overall survival were measured endpoints. The protocol was generally well tolerated, although the majority of patients developed grade 1 or 2 local redness and swelling at the injection sites. Clinical responses were 4 cases with partial response, 9 with stable disease, and 10 with progressive disease. Mean survival time has not been reached at the mean observation time 217 days. Personalized peptide vaccination is well tolerated and has the ability to induce immune responses in the majority of malignant glioma patients, along with several cases of major tumor regression. These results would encourage the phase 2 clinical study of personalized peptide vaccination for patients with recurrent malignant glioma.
267. THE TGF-BETA2 SPECIFIC ANTISENSE OLGONUCLEOTIDE AP 12009 AS IMMUNOTHERAPY IN HIGH-GRADE BRAIN TUMORS: A CLINICAL PHASE IIB STUDY
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The outcome for young children with non-cerebellar PNET has been dismal with conventional chemotherapy attempting to either delay or avoid irradiation. Between 1991 and 2002, 42 children (median age 2.9 years, range, 0.2–7 years) were treated with two serial studies: “Head Start” I (1991–1997) and “Head Start” II (1997–2002) in an effort to avoid or reduce radiation. Head Start I induction chemotherapy included 5 cycles of vincristine, cisplatin, cyclophosphamide, and etoposide at 3- to 4-week intervals, followed by consolidation with thiopeta, carboptin, and etopo- side with AsuCR. Head Start II therapy was identical, except for the addition of high-dose (400 mg/kg) methotrexate during each induction cycle for patients with metastatic (M1) disease. No irradiation was administered to patients. The 1-, 2-, and 3-year Kaplan Meier analyses of event-free survival (EFS) are 66.2 ± 4.4, 42.8 ± 7.9, and 40.0%, and of overall survival (OS) are 71 ± 4, 49.6 ± 8.6, and 43.4%. A significant difference in outcome was noted for children 36 months; 1- and 5-year OS for children 36 months are 74% and 67%, a trend for improved survival with radical resection was noted: 5 of 13 patients with less than a radical resection survive without disease compared with 14 of 24 with radical resection. All 4 children with brainstem PNET died of tumor progression. Patients with M1 disease at diagnosis fared poorly: Of 5 pineoblastoma, 1 brainstem PNET, and 1 other supratentorial PNET, 2 survive without disease. The outcome for children with non-cerebellar PNET with this intensive chemotherapy strategy still remains poor for children.

268. PRE-RADIATION CHEMOTHERAPY MAY IMPROVE SURVIVAL RATE OF DIFFUSE BRAINSTEM GLIOMAS: FINAL RESULTS OF BSG98 TRIAL
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Despite numerous attempts (standard RT, hyperfractionated RT, standard RT, high-dose chemotherapy), the usual median survival of patients with diffuse brain stem tumor does not exceed 9 months. We prospectively proposed to delay radiotherapy as long as no progression was observed under multidrug chemotherapy. As soon as MRI showed a diffuse BSG, cycles of chemotherapy were initiated. Each cycle included three monthly courses of alternating BCNU and CDPP (40 mg/m² for 4 days in continuous infusion), course and high-dose MTX (12 g/m²). Cycles were delivered until progression, or to a maximum of 4 (1 year of treatment). Standard radiotherapy then was delivered to the tumor with maintenance hydroxyurea. Twenty-three patients were prospectively included. We compared with a historical control group of 14 patients treated in the same institution, by front-line radiotherapy and/or procarbazine or carboptin. The median number of cycles was 3 (1 to 4). Iatrogenic severe infections occurred in 4 patients, and 11 patients required platelet transfusions. The median survival was significantly increased as compared to historical controls (17 months [95% CI, 9–23 months] vs. 9 months [95% CI, 8–10 months]; P = 0.015), and survival from time of radiotherapy is similar in both groups, though the number of days in hospital was prolonged (57 vs. 25 days; P = 0.001). Steroids could be at least transiently withdrawn in half of the patients of both groups (P = 0.79). We conclude that front-line chemotherapy alternating hematotoxic and non-hematotoxic schedules significantly prolongs overall survival of children. However, the price to be paid (infections and hospitalization) deserves honest discussion with the children and their parents.

269. OUTCOME OF INTENSIVE INDUCTION CHEMOTHERAPY FOLLOWED BY CONSOLIDATIVE MYELOABLATIVE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL RESCUE (AUSCR) IN YOUNG CHILDREN WITH NEWLY DIAGNOSED NON-CEREBELLAR PRIMITIVE NEUROECTODERMAL TUMORS (PNET): THE “HEAD START” I AND II PROTOCOLS
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From 1993 to 2002, while 63 children were electively treated with the Italian post-surgical protocol for EFD (hyperfractionated radiotherapy [HFRT] + VCR/CTX/VP16), other patients were considered for adjuvant treatment after 2 (n = 12) or 3 (n = 2) excisions of the tumor whose treatment had been considered exclusively surgery by the referral neurosurgeon. Mean time to local progression had been 14 months, mean age at diagnosis, 5 years. Tumor originated in posterior fossa (PF) in 10 children and was supratentorial (ST) in the other 4; 11 tumors had been completely excised within 5 years. Tumor originated in posterior fossa (PF) in 10 children and was supratentorial (ST) in the other 4; 11 tumors had been completely excised at both surgical report and radiological evaluation, 3 had macroscopic resi- dues. Histological diagnosis was classic EFP in 9 pts and anaplastic in 5. Eight children were referred NED and 5 ED after second or further surgery, 5 had low cranial nerves palsy (1 requiring tracheostomy), 1 had recurrent surgery-related meningitis, and 2 had persistent hydrocephalus. All children were treated with RT to tumor bed (6 HFRT: 70.4 Gy, 8 standard RT: 54–59.4 Gy) and 5 also with pre-RT CT. Five of 14 pts (5/10 with PF tumors) had a further relapse at a mean of 6 months after last surgery; all have died, thus obtaining a PFS of 66% and an OS of 74% at a mean follow-up of 3 years after relafer. Considering only pts with PF tumors, PFS and OS were 53% and 61%, respectively. Patients with relapsing EFP after surgery only, especially if originating in PF, have a more severe prognosis despite surgery completeness and non-anaplastic subtype than pts receiving adjuvant treatment after first diagnosis (PF tumors 3 years, PFS: 53% vs. 63%, P = 0.03) moreover, subsequent surgical acts for tumor re-growth are followed by severe neurological sequelae.

Abstracts from the World Federation of Neuro-Oncology Meeting
271. OUTCOME OF INTENSIVE INDUCTION CHEMOTHERAPY FOLLOWED BY CONSOLIDATIVE MYELOABLATIVE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL RESCUE (AUSCR) IN YOUNG CHILDREN WITH NEWLY DIAGNOSED EPENDYMOMA

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The outcome of young children with ependymoma has been dismal with conventional chemotheraputic attempting to either delay or avoid irradiation. This study investigates the efficacy of high-dose chemotherapy followed by stem cell rescue in pediatric patients with ependymoma. The patients were 28 children from “Head Start I” (1991–1997) and “Head Start II” (2002–2007). Overall, the median age at diagnosis was 2.4 years. Twenty-four patients with local disease received an induction regimen of 5 cycles of chemotherapy (cisplatin, vincristine, etoposide, cyclophosphamide at 3–4 week intervals). All patients with leptomeningeal dissemination received the same drugs with the addition of high-dose methotrexate and leucovorin rescue. After induction, individuals in both groups without evidence of disease proceeded to narrow ablative chemotherapy (thiotepa, carboplatin and etoposide) with autologous stem cell rescue. Twenty patients (71%) had supratentorial tumors and eight infratentorial and leptomeningeal disease. Seventeen patients (61%) had a gross total resection (GTR) at diagnosis. The 1-, 2-, and 5-year Kaplan Meier analyses of progression-free survival (PFS) of these patients were 70 ± 9%, 43 ± 10%, and 14 ± 7%. The 1-, 2-, and 5-year overall survival (OS) were 78 ± 8%, 63 ± 9%, and 38 ± 10%. Survival data did not differ significantly for patients 3 years old at diagnosis. Thirty-seven percent of the patients with supratentorial tumors are long-term survivors compared to 55% of the patients with posterior fossa tumors. Half of the patients who underwent a GTR survived compared to 36% of the ones for whom GTR was not achieved. Radiation was administered to 15/28 patients overall: 75% of patients with prolonged survival received RT. Two of the nine patients with disseminated disease are long-term survivors. The toxic mortality within this group of 28 patients was 14%. Outcome with this treatment strategy does not appear superior to other strategies seeking to avoid or delay RT.

272. GENE THERAPY

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Gene therapy is a new medicine based on a technique for correcting defective genes responsible for disease development. It holds potential for treating or even curing formidable diseases such as monogenic metabolic disorders, cancers, infectious diseases, and vascular diseases. More than 1000 clinical trials using 60000 patients were identified worldwide in 2004, but almost all trials are aimed at establishing the safety of gene therapy rather than the effectiveness. In fact, gene therapy still faces many scientific or ethical obstacles before it can become a good practical medicine for mankind. A surprising breakthrough in the success of gene therapy came in 1990 when it was demonstrated that upregulation of intracranial tumors by the use of SDSC from the same patients. Another major blow came in 2003, when the Food and Drug Administration (FDA) placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells, because children treated with French gene therapy for X-linked severe combined immunodeficiency disease (X-SCID) had developed a leukemia-like condition. In malignant glioma, on the other hand, gene therapy using herpes simplex virus thymidine kinase gene/ganciclovir is the first clinical trial in the world, which started in 1992. Thereafter, immuno-gene therapies using cytokine genes such as interleukin or interferon (IFN) were opened one after another. For the past five years, 7 of 11 clinical trials in USA have included immuno-gene therapies. We have been also developing a new immuno-gene therapy in Japan. This therapy using interferon (IFN)- and cationic liposomes is a first original protocol developed in Japan. A pilot clinical trial of the therapy began in 2000, and thereafter the safety and effectiveness were confirmed. In addition, researchers belonging to Experts on the Gene Therapy Advisory Committee in the UK have been given approval to carry out a large clinical trial in 2004, which involves injecting oncolytic herpes simplex into the gliomas. Using this protocol, the first patient to receive treatment had a long survival more than 7 years. From this evidence, it is considered that gene therapy for malignant brain tumors is one of the most promising strategies in all gene therapies. In this lecture I introduce the current status of gene therapy research and clinical trials for malignant brain tumors, including ours. In addition, I comment on the status of gene therapy regulation in Japan, a new molecular targeting therapy based on the results of genetic analysis in patients treated with our IFN-gene therapy and the combination of gene therapy and cell therapy. In the near future I believe that gene therapy can offer hope to patients with malignant brain tumors, including glioma.

Malignant gliomas are highly invasive brain tumors. This invasive tendency renders these tumors surgically incurable and associates them with a high mortality rate. Glioma cell invasion is poorly understood, and we believe that increased understanding of this process will lead to the development of novel therapies. Through the use of a serial in vivo selection procedure, we have isolated highly invasive and highly noninvasive cell subpopulations from the human glioma cell line U87. Microarray comparison of the two subpopulations was then used to identify novel genes not previously implicated in glioma invasion. One of the differentially expressed genes was the p75 neurotrophin receptor. This receptor is present at both the RNA and protein level in the invasive cell population, but is undetectable in the noninvasive population. Importantly, we have also observed that treatment of these cells with p75 ligands increases the migration and invasion of the invasive, p75-positive cells, but has no effect on the noninvasive, p75-negative population. In addition, we found that p75 is present in other glioma cell lines and that a strong positive correlation exists between levels of p75 expression and neurotrophin-induced migration in these cells. Subsequently we found that the p75 levels in the original U87 cell line and demonstrated that this upregulation conferred an increased migratory and invasive ability in vitro. Conversely, downregulation of p75 in a human glioma cell line expressing high levels of p75 decreased the migration and invasion of these cells in vitro. Finally, we demonstrated that p75 is overexpressed in human glioma patient specimens. These data suggest that p75 is present, functional, and involved in glioma migration and invasion. Future experiments are aimed at identifying the downstream signaling molecules that mediate these effects.

274. HUMAN SKIN–DERIVED STEM CELLS INHIBIT BRAIN TUMOR GROWTH

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Neural stem cells (NSC) implanted into rodent intracranial gliomas distribute themselves and surround infiltrating tumor cells. A number of questions are left unanswered (the difficulty of isolating NSC from adult tissues, an ethical issue linked to the use of fetal derivatives, and the advantages of NSC in allogenic transplantation). We isolated human skin-derived stem cells (SDSC) from samples of skin obtained from glioma patients and studied their behavior in the presence of brain tumors. We initially studied the ability of SDSC targeting ability came in 1990 when it was demonstrated that upregulation of intracranial tumors by the use of SDSC from the same patients. Another major blow came in 2003, when the Food and Drug Administration (FDA) placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells, because children treated with French gene therapy for X-linked severe combined immunodeficiency disease (X-SCID) had developed a leukemia-like condition. In malignant glioma, on the other hand, gene therapy using herpes simplex virus thymidine kinase gene/ganciclovir is the first clinical trial in the world, which started in 1992. Thereafter, immuno-gene therapies using cytokine genes such as interleukin or interferon (IFN) were opened one after another. For the past five years, 7 of 11 clinical trials in USA have included immuno-gene therapies. We have been also developing a new immuno-gene therapy in Japan. This therapy using interferon (IFN)- and cationic liposomes is a first original protocol developed in Japan. A pilot clinical trial of the therapy began in 2000, and thereafter the safety and effectiveness were confirmed. In addition, researchers belonging to Experts on the Gene Therapy Advisory Committee in the UK have been given approval to carry out a large clinical trial in 2004, which involves injecting oncolytic herpes simplex virus into the gliomas. Using this protocol, the first patient to receive treatment had a long survival more than 7 years. From this evidence, it is considered that gene therapy for malignant brain tumors is one of the most promising strategies in all gene therapies. In this lecture I introduce the current status of gene therapy research and clinical trials for malignant brain tumors, including ours. In addition, I comment on the status of gene therapy regulation in Japan, a new molecular targeting therapy based on the results of genetic analysis in
275. INFLUENCE OF ANTI-ANGIOGENIC SUBSTANCES ON NEURAL PROGENITOR CELL MIGRATION, DIFFERENTIATION, AND TUMOR HOMING

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Neural progenitor cells (NPC) have been used to track solid brain tumors in vivo as well as to express a therapeutic molecule intratumorally. In addition to their antimetastatic properties, a restorative potential of these NPC is hoped for. We have investigated the use of local expression of the angiogenic inhibitor endostatin in a model of hematogenous cerebral melanoma metastases using neural progenitor cells as therapeutic vehicles. Concomitantly, we have investigated the effects of endostatin and of additional angiogenesis inhibitors (angiotatin, Su5416, Su5614, and Cox2-inhibitor) on in vivo migration and differentiation. In a second step, the therapeutic potential of endostatin-transfected NPCs was investigated. Migration of NPCs was tested with a modified Boyden-chamber assay and a modified wounding assay. Endostatin levels were measured by ELISA. Proliferation and differentiation patterns were analyzed by immunohistochemistry using antibodies against GFAP, NeuN, Huc, MBP, Glic, nestin, vimentin, and Ki67. The primary NPC from GFP-transgenic mice as well as the mcy-immortalized C172 NPC line were exposed to murine recombinant most endostatin and angiotatin and the tyrosine kinase inhibitors Su5416 and Su5614. The cerebral metastasis model used murine melanoma cells either injected into the internal carotid artery of mice or placed stereotactically into the frontal lobe and concomitantly infused with endostatin. Survival was recorded, and the brains were subjected to immunohistochemical analysis. Endostatin and Su5614 led to a significant reduction in migration, whereas angiotatin and Su5416 did not alter migration. Phenotypic and proliferative changes compared to control cells were not observed. Endostatin-transfection of NPCs did not result in prolonged survival although endogenous endostatin expression by tumor cells demonstrated survival advantage. Upon inspection of tumors, a reduced migration of NPCs into metastases was noted if compared to non-endostatin transfected NPCs. Endostatin inhibited NPC migration. This observation concurs with the in vivo findings of reduced NPC homing toward the tumor and therefore abolishing the therapeutic properties of NPCs using endostatin anti-angiogenic therapeutic paradigm.

276. ANTIANGIOGENIC AGENT THALIDOMIDE INCREASES THE ANTITUMOR EFFECT OF SINGLE HIGH-DOSE IRRADIATION (GAMMA-KNIFE RADIOSURGERY) ON THE RAT ORTHOTOPIC GLIOMA MODEL

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We investigated the effect of gamma-knife radiosurgery (GKR) on a rat model of glioma with or without simultaneous administration of temozolomide (TMZ) and thalidomide. Combined GKR (20 Gy single maximal dose) and thalidomide (for 3 days including the radiosurgery day) was delivered on the 18th day after stereotactic implantation of C6 glioma cells. The animals were sacrificed 24 h after GKR for evaluation of apoptosis, PCNA index, microvessel density, and expression of basic fibroblast growth factor (bFGF) and of vascular endothelial cell derived growth factor (VEGF). To determine the tumor size reduction, other groups of animals were sacrificed at 5 days after GKR (with or without pharmacotherapy), which was delivered 14 days after the tumor inoculation. Compared with the rat which received GKR alone, there was significant increase of tumor cell apoptosis in GKR + TMZ. The amount of thalidomide uptake was most prominent in the GKR and thalidomide combination group. In addition to the reduction of bFGF and VEGF expression, reduction of MVD and induction of apoptosis by thalidomide may be associated with the prominent shrinkage of tumor among the GKR treatment groups. Our data suggest that radiosurgery combined with antiangiogenic therapy may be the most promising combination to control malignant glioma.

277. THE ROLE OF AUTOTAXIN IN GLIOMA INVASION

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One of the salient features of malignant astrocytomas is the propensity of these cells to locally invade brain adjacent to it. This is the case of invasion of tumors that the tumor invariably recurs. A better understanding of the molecular mechanisms of this locally invasive behavior could yield potential therapeutic targets aimed exclusively at this tumor cell subpopulation. To this end, a gene expression profile of GBM invasion was created by comparing mRNA from invasive cells to that of matched noninvasive cells from the tumor core. Differentially expressed genes between the two laser capture microdissected cell populations revealed multiple genes related to invasion. Among these was the autocrine motility factor Autotaxin (ATX), which is secreted by invasive melanoma and as well as by metastatic breast carcinoma and other malignant tumors. Recently, its role as a lysophospholipase D was identified, catalyzing lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA). LPA stimulates motility in various cell types, including glioma cells, which express high levels of LPA1 receptor. Evaluation of ATX expression in a model glioma revealed a high expression in the invading cells of astrocytic tumors of all grades. To investigate the functional role of ATX in astrocytoma invasion, stable transfectants carrying wild-type ATX and a catalytically inactive form (H316Q-U2S1) were created by using the U251 glioma cell line. ATX-U2S1 migrated significantly faster than H316Q-U2S1 in a radial migration assay, an effect that was not reversed by LPA dependent motility stimulation. Cells expressing high levels of ATX also proved more adherent to laminin, specifically in the presence of its substrate LPC. To test the property of invasion we used an orthotopic rat brain slice assay, which showed ATX-U2S1 to be more highly invasive than H316Q-U2S1 or control cells. ATX knockdown with siRNA resulted in lowered invasion of spheroids in a collagen I matrix, an effect that was reversed with the addition of LPA. Changes in motility that are induced by LPA receptors result in cytoskeletal rearrangements brought about by the rho family of GTPases. We examined the actin cytoskeleton in the poorly motile H316Q-U2S1 cells and found focal adhesion and stress fibers, enhanced fociopoia formation and reduced membrane ruffles, suggesting modulation of rac signaling. Further studies consist of elucidating whether the motility inhibition of the secreted protein H316QAUTX functions in a paracrine or autocrine manner.

278. INTERPLAY BETWEEN EPHB2 AND EPHRIN-B ON GLIOMA CELLS PROMOTES GLIOMA MIGRATION AND INVASION

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The receptor tyrosine kinases of the EphB family and their ephrin-B ligands play a key role in neurodevelopmental processes such as boundary formation, vasculogenesis, and cell migration. The Eph receptors transmit forward signals via their kinase domain and reverse signals via their transmembrane ephrin-B ligands. EphB2 are upregulated in glioblastoma, especially in invading glioma cells (Nakada et al., Cancer Res. 64, 2004). Here we use a soluble EphB2 ectodomain fusion protein (EphB2Fc) to demonstrate that ephrin-B transduces signals that regulate cell migration and invasion. EphB2Fc induced ephrin-B tyrosine phosphorylation, migration, and invasion in in vitro and in ex vivo rat brain slice model using U87 and U251 glioma cells. Expression showed single maximal dose irradiation of glioblastoma of motile U87 and U251 cells. Activation of ephrin-B by EphB2Fc induced phosphorylation of Akt. The phosphorylidyinositol 3-kinase (PI3-K) inhibitor LY294002 reduced EphB2Fc stimulated migration and invasion concomitant with phosphorylation of Akt. Human brain tumors of the P3-K/Akt pathway that express higher expression of ephrin-B in glioblastoma than in low-grade astrocytomas or normal brain. Immunohistochemistry showed phosphorylated form of ephrin-B localization primarily in glioblastoma cells. Taken together, EphB2 stimulation by EphB2Fc increases "outside-in" signals that affect migration and invasion in glioma through the P3-K/Akt pathway. This study was supported by the American Brain Tumor Association (MIN), N042262 and N043446.

279. HIF-1A EXPRESSION IS ASSOCIATED WITH GLIOMA CELL INVASION

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Hypoxia is associated with adverse outcome for a number of solid tumors, including gliomas. We reported expression of hypoxia inducible factor-1a (HIF-1a) in two different microenvironmental areas of gliomas: (1) pseudopalisading cells at the tumor core, and (2) invasive tumor cells located at brain adjacent to tumor (BAT). Using CA9, a carbonic anhydrase as a surrogate marker for tumor hypoxia, we showed by immunohistochemistry (IHC) that HIF-1a expression within these two brain tumor areas may be driven by different mechanisms (possibly leading to distinct cellular responses): Hypoxic cells within the pseudopalisades exhibited staining for HIF-1a and CA9, whereas all the invading HIF-1a positive glioma cells in the BAT were negative for CA9. These results suggest that upregulation of
HIF-1α in invading glioma cells assessed by IHC might be controlled by type protein tyrosine phosphatase ' (RPTP'), a specific contactin ligand that mediates adhesion to extracellular matrix (ECM) molecules. Expression of the receptor–ligand complex was analyzed in U-87 MG glioma xenografts using immunohistochemistry (IHC). HIF-1α was found to be strongly upregulated in invading glioma cells, and fluorescence in situ hybridization (FISH) analysis showed that HIF-1α is expressed in a subset of glioma cells, while HIF-1α, a gene known to be induced by HIF-1α under hypoxic conditions, was not detected in these cells. Interestingly, HIF-1α expression in glioma cells in response to microenvironmental cues may point to exploitable targets in the invasive cells.

### 280. THE SOLUBLE DECOY RECEPTOR FOR VEGF, VEGF TRAP, INDUCES ANTITUMOR EFFECT IN VIVO

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Pathological angiogenesis is a hallmark of cancer, and specifically of glioblastomas, the most malignant and common form of primary brain tumors. VEGF is the key molecular player involved in the mechanism of vascular growth. We undertook this project to test VEGF Trap, a new antiangiogenic agent that acts as a soluble decoy receptor for VEGF. This molecule incorporates domains of both VEGFR-1 and VEGFR-2 and binds VEGF with high affinity. U-87 MG human glioma cells were implanted in the brain of nude mice, and VEGF Trap was administered (25 mg/kg sc, twice a week for a total of 3 weeks) at days 0, 4, and 10 after cell implantation. hFc and PBS were used as control treatments. Serum was collected three days after the initial dose to assess circulating levels of VEGF Trap. Serial temporal examination of the brains of untreated mice showed that tumors grew to a volume of 0.3 mm³ and exhibited a very low microvascular density (MVD = 6 vessels/0.5 mm²), with central necrosis within four days of implantation, and peripheral reactive vasculature. At that time point, treatment of glioma-bearing animals with VEGF Trap increases significantly the survival time of animals compared to that of control-treated animals (log-rank test). Another group of animals was treated starting day 10 of the experiment, at the time that tumors were masses of cells with volumes of 30–45 mm³ and had fully developed vasculature (MVD = 30–35 vessels/0.5 mm²). At this stage of the disease, VEGF Trap treatment induced a significant prolongation of animal survival (P < 0.0001, log-rank test). VEGF Trap was detected in the serum of the animals at levels of about 30 μg/ml or greater. Taken collectively, our data indicate that treatment with VEGF Trap results in prolongation of survival in this intracranial human glioma xenograft model.

### 281. DOMINANT-NEGATIVE INHIBITION OF THE RECEPTOR TYROSINE KINASE RECEPTOR AXL SUPPRESSES GLIOMA CELL MIGRATION AND INVASION AND PROLONGS SURVIVAL

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Receptor tyrosine kinases (RTKs) play an important role in growth and progression of brain tumors. Our previous work has shown that the RTK AXL, whose biological function has remained obscure so far, is overexpressed by glioma cell lines and human astroglial tumors. The aim of the present study was to analyze the role of AXL in glioma biology. Two glioma cell lines expressing high levels of AXL (i.e. SF126) and the other lacking intrinsic AXL (i.e., SF767) were transfected to overexpress either the human wild-type form (AXL-WT) or a truncated, dominant-negative mutant form (AXL-DN). Glioma cell morphology and cell behavior with respect to proliferation, aggregability, migration, and invasion were assessed in vitro. To study the relevance of AXL for tumor growth, the glioma cell lines were implanted subcutaneously and into the brains of nude mice. Finally, glioma cells were implanted into the dorsal skinfold chamber model to assess tumor cell behavior and invasive and proliferative gene expression in vivo by intravital multi-fluorescence microscopy. SF126-AXL-DN cells were characterized by reduced cell-to-cell contacts, a moderately reduced proliferative activity, and most importantly by a severe impairment in tumor cell migration and growth, although with weaker effects than DC101, and tumor cell invasion into the adjacent tissue was suppressed. Finally, survival following intracerebral implantation of SF126-AXL-DN cells was significantly prolonged compared to SF126-WT cells. In contrast, treatment of AXL signaling in SF767 cells had no significant effects. Our study provides the first evidence that the tyrosine kinase receptor AXL modulates migration and invasion of human glioma cells and that inhibition of AXL signaling suppresses tumor expansion and prolongs survival by blocking tumor cell invasion. Thus, AXL may represent a novel molecular target for the treatment of malignant glioma.

### 282. INHIBITION OF GliOBLASTOMA ANGIogenesis AND INVASION BY COMBINED TREATMENTS DIRECTED AGAINST VEGFR-2, EGFR AND VE-CADHERIN

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Increasing evidence suggests that inhibition of tumor angiogenesis can influence tumor cell invasion and metastasis. We previously showed that systemic antagonization of vascular endothelial growth factor receptor-2 (VEGFR-2) with the monoclonal antibody (mAb) DC101 inhibited glioblastoma growth in an orthotopic model, but caused increased tumor cell invasion along the preexistent vasculature. In human glioblastoma cells, signaling through the epidermal growth factor receptor (EGFR) predominantly stimulates tumor cell invasion. Therefore, we attempted to inhibit tumor cell invasion caused by DC101 therapy by combined systemic treatment with a mAb against EGFR (C225). In addition, we analyzed whether antagonization of vascular endothelial (VE)-cadherin as a different angiogenic target can also inhibit glioblastoma cell invasion. Whether this also stimulates invasion. Treatments were either initiated on day 1 after intracerebral tumor cell injection or on day 6 when tumors were already established. Increased tumor cell invasion caused by DC101 monotherapy was inhibited by 50%–66% through combined treatment with C225 and DC101. C225 inhibited glioblastoma cell migration in vitro, but had no effect on the volume of the main tumor mass or on tumor cell prolifera- tion or apoptosis in vivo, neither alone nor in combination with DC101. The anti-VE-cadherin mAb E4G10 also inhibited tumor angiogenesis and growth, although with weaker effects than DC101, and the effects of E4G10 were dependent on early initiation of treatment. E4G10 treatment caused increased tumor cell invasion along the host vasculature, although also with a weaker effect than DC101. Our findings show that anti-angiogenic glioblastoma therapy targeting either VEGFR-2 or VE-cadherin can inhibit tumor growth, but can increase tumor cell invasion in an orthotopic model. The increased tumor cell invasion caused by DC101 treatment can be inhibited by simultaneous antagonization of EGFR, which in the context of human glioblastomas has been implicated in tumor cell invasion.

### 283. CONTACTIN IS EXPRESSED IN HUMAN ASTROCYTIC GLIOMAS AND MEDIATES REPULSIVE EFFECTS

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Using subtractive cloning, we identified contactin as overexpressed in glioblastomas compared with normal brain. Contactin is a cell surface adhesion molecule that is normally not expressed by astrocytes. It is expressed by neurons and oligodendrocytes at particularly high levels during development. Contactin can mediate adhesion or repulsive intercellular interactions depending on the molecular context. We analyzed the expression of contactin in human astrocytomas and determined its functional relevance for glioma cells. Western blotting, immunohistochemistry and confocal immuno-chemistry were used to analyze contactin expression in astrocytomas and astrocytes. Adhesion and migration assays were performed to study effects of contactin on glioma cell migration. Contactin DN was transfected into glioma cells to determine the effect of contactin overexpression on attachment to extracellular matrix (ECM) molecules. Expression of the receptor–protein tyrosine phosphatase, (RPTP), a specific contactin ligand that is also overexpressed in glioblastomas, was downregulated by stable siRNA
transfection to study interactions with contactin overexpressing cells. Contactin expression was detected in GFAP positive tumor cells but was absent in normal astrocytes. Levels of contactin in gliomas were associated with glioblastoma malignancy grade. Cells adhered to contactin or was stimulated in its motility by contactin. In contrast, increasing coating concentrations of contactin caused progressive cell proliferation or adhesion to various ECM molecules. Contactin expression also did not alter the adhesion to cells expressing normal or downregulated levels of RPTP. The expression of astrocytic as well as neuronal markers were unaltered by contactin overexpression, confrontation of glioma cells with contactin has repulsive effects, which may contribute to the diffuse infiltration pattern characteristic of these cells in human brain.

We previously identified the receptor-type protein tyrosine phosphatase (RPTP) adaptor protein contactin in several cell lines of human glioblastoma cells by cDNA transfection had no effect on cell proliferation or adhesion to various ECM molecules. Contactin expression also did not alter the adhesion to cells expressing normal or downregulated levels of RPTP. The expression of astrocytic as well as neuronal markers within glioma cells may reflect an ability of these cells for multilineage differentiation, a phenotype that has recently been described also for the so-called glioma stem cells. While adhesive and proliferative properties of glioma cells are unaltered by contactin overexpression, confrontation of glioma cells with contactin has repulsive effects, which may contribute to the diffuse infiltration pattern characteristic of these cells in human brain.

284. INHIBITION OF Glioblastoma Growth BY SiRNA-MEDIATED ANTAGONIZATION OF RPTPβ
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Gangliosides are acidic glyco-sphingolipids characterized by the presence of one or more sialic acid residues. In neoplastic tissues simple ganglio- side expression (RPTP) is upregulated in classical glioblastoma, shows a limited degree of infiltrative behavior, both in vivo and in vitro), we have examined the modulatory effect of 4 imino sugars with structural similarities to NB-DGJ on ganglioside expression (TaqMan RT-PCR) and angiosuppressive action of CPT-11, because it is a mediator of hypoxic condition, VEGF induction, and radiation/chemoresistance. We evaluated angiosuppressive action of CPT-11, which inhibits haptotaxis toward PTN is specifically mediated by upregulated expression of MMPs/TIMPs as well as invasion. Results were more marked in the invasive IP8-18 line than in RPTP. These imino sugars not only show that targeting of ganglioside synthesis pathways may prove of therapeutic interest but also may have a potential role to play in clinical neuro-oncology. This work was supported, in part, by Oxford Glycosciences.

285. REGULATION OF PATHOLOGICAL VASCULARITY OF MALIGNANT ASTROCITOMAS BY ANGIOPOIETIN-1
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A histopathological hallmark of malignant astrocytomas is microvascular proliferation and formation of vascular entities referred to as “gliomuloid bodies.” The significance of gliomuloid bodies and the molecular mechanisms driving the abnormal vascular architecture in human malignant gliomas are not known. Vascular endothelial growth factor-A (VEGF-A) is known to be the main angiogenic regulator of tumor angiogenesis in malignant astrocytomas. However, a direct link between VEGF-A and gliomuloid bodies in tumor models, in particular astrocytomas, has not been established. Angiopoietin-1 and Angiopoietin-2 (ANG1 and ANG2) were proposed as key angiogenic regulators, the expression of which by the use of a highly invasive astrocytoma culture (IPSB-18) and a cultured (IPPT) giant cell variant glioblastoma (a tumor which, although histologically similar to classical glioblastoma, shows a limited degree of infiltrative behavior, both in vivo and in vitro), we have examined the modulatory effect of 4 imino sugars with structural similarities to NB-DGJ on ganglioside expression (immunochemistry, flow cytometry, and HPLC) as well as on invasive behavior in glioma by the use of time-lapse microscopy and Transwell assay. We also assessed MMP-2 and -9 and TIMP gene expression (TaqMan RT-PCR) before and after treatment. All agents inhibited ganglioside expression (in particular GD3), although differentially towards different agents. Inhibitory effect was seen on expression of MMPs/TIMPs as well as on invasion. Results were more marked in the invasive IP8-18 line than in RPTP. These imino sugars agents not only show that targeting of ganglioside synthesis pathways may prove of therapeutic interest but also may have a potential role to play in clinical neuro-oncology. This work was supported, in part, by Oxford Glycosciences.

286. IMINO SUGAR-BASED GANGLIOSIDE INHIBITORS DOWNREGULATE INVASIVE BEHAVIOR IN MALIGNANT GLIOMA IN VITRO
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Hypoxia inducible factor-1 (HIF-1) is an important molecular target because it is a mediator of hypoxic condition, VEGF induction, and radiation resistance. We evaluated angiogenic suppressive action of CPT-11, YC-1, and 2ME for malignant gliomas in vitro and in vivo. S. Takano, H. Kamiyama, K. Tsuobi, and A. Matsumura; Department of Neurosurgery, Institute of Clinical Medicine, University of Tsukuba, Tsukuba City, Japan

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287. ANGIOSUPPRESSIVE THERAPY TARGETING HIF-1/VEGF FOR MALIGNANT GLIOMAS IN VITRO AND IN VIVO
S. Takano, H. Kamiyama, K. Tsuobi, and A. Matsumura; Department of Neurosurgery, Institute of Clinical Medicine, University of Tsukuba, Tsukuba City, Japan

Hypoxia inducible factor-1 (HIF-1) is an important molecular target because it is a mediator of hypoxic condition, VEGF induction, and radiation resistance. We evaluated angiogenic suppressive action of CPT-11, YC-1, and 2ME for malignant gliomas in vitro and in vivo. S. Takano, H. Kamiyama, K. Tsuobi, and A. Matsumura; Department of Neurosurgery, Institute of Clinical Medicine, University of Tsukuba, Tsukuba City, Japan
tory concentration was more than 25 μM. In vivo, CPT-11 even at low dose inhibited malignant glioma growth, inhibiting tumor cell proliferation and enhancing tumor cell apoptosis, in addition inhibiting angiogenesis, and decreasing microvessel density of tumor vessels and administration of probe, and quantitative maps of enzyme expression were generated. Following completion of imaging, enzyme expression was determined on tissue sections. Quantitative MR maps successfully demonstrated enzyme expression within the transfected tumors, but not the control tumors. The image data was correlated with expression of the enzyme on tissue sections. These studies also demonstrated the ability of these probes to detect temporal changes in the molecular mediators of angiogenesis. These novel probes promise the successful MR imaging of the expression of various molecular targets localized to gliomas in vivo and should find numerous applications, including the evaluation of novel, targeted therapeutic agents.

A major obstacle in the treatment of gliomas is the invasive capacity of the tumor cells. Previous studies have demonstrated the capability of neural stem cells (NSCs) to home to sites of glioma cell invasion using chemotactic guidance signal for NSC tropism to disseminated tumor cells. Our data indicate that the ECM of malignant gliomas is a significant modulator of NSC migration. ECM proteins preferentially expressed in areas of glioma cell invasion may serve as additional local guidance signal for NSC tropism to disseminated tumor cells.

291. INHIBITION OF HIF-1A BY SHORT HAIRPIN RNA TECHNOLOGY INHIBITS BOTH IN VITRO AND IN VIVO HUMAN GLIOMA CELL GROWTH

R. Griesinger and D. Gillespie; Neurosurgery, Huntsman Cancer Institute, Salt Lake City, Utah, USA

Hypoxia-inducible factor-1α (HIF-1α) is the major regulator of vascular endothelial growth factor (VEGF). VEGF is thought to be the principal mediator of peritumoral edema and angiogenesis in malignant gliomas. Interruption of VEGF secretion could result in growth inhibition of these tumors. We examine the role of inhibition of HIF-1α using short hairpin RNA (shRNA) techniques in the growth of human gliomas. Malignant glioma cell lines were stably transfected with vectors expressing either shRNA directed against the HIF-1 gene or with control plasmids containing nonsense shRNAs. HIF-1 and VEGF expression was examined by immuno- histochemistry, Western blot, and RT-PCR. In vitro and in vivo growth studies were carried out on these cells. Measures of proliferation, angiogenesis, tissue perfusion, and hypoxia were performed on cells in culture as well as on xenograft tumors. HIF-1α shRNA transfected cells demonstrate inhibition of both HIF-1α expression and VEGF secretion. In vitro growth is slightly decreased by shRNA directed toward HIF-1α, while vivo growth is significantly decreased in these cells compared to control cells. Interestingly, the plasmid expressing the anti-HIF-1 shRNA is still present in the xenograft cell after 75 days of implantation in the mouse. Cell proliferation and angiogenesis are decreased in the tumors containing the shRNA inhibiting HIF-1. Preliminary results of intratumoral and intravenous use of anti-HIF-1 shRNAs in established xenograft human gliomas is discussed. Inhibition of HIF-1α results in inhibition of VEGF secretion, glioma tumor growth and angiogenesis. Further studies are necessary, but this study suggests potential clinical applications of targeting HIF-1α mediated growth for inhibition of glioma growth and angiogenesis.
Malignant glioma is hallmarked as one of the most invasive tumors in the human. According to recent literature, 18 months would be the best long-term median survival. Twist, which has been noted to play an essential role on carcinoma metastasis, expresses in malignant glioma cell lines and the cell invasion function is unclear. We investigated an interaction between TWIST and the invasiveness of malignant glioma. Malignant glioma cell lines U87MG and U251MG, which have already confirmed TWIST expression, were employed to explore the inhibitory effects of TWIST gene silencing. After silence of the TWIST gene in the mRNA and protein levels, by using specific siRNA and Western blot analysis, we suggest the presence of repellents and/or inhibitors in the serum-containing conditioned medium from glioma cell lines (Werbowetski et al., J. Neurobiol. 60, 71, 2004). We have, therefore, developed and optimized a functional screening assay using protein purification to isolate potential inhibitors or repellents of glioma tumor invasion in three-dimensional collagen gels from both endogenous and serum-derived sources. Serum-containing conditioned medium from C6 rat astrocytoma spheroids from spinner culture was concentrated and applied to a Resource Q anion exchange column on an AKTA FPLC. Fractions were collected and applied to C6 spheroids implanted in a collagen matrix, and those that inhibited invasion were pooled and applied to a heparin sepharose affinity column. Fractions were again collected and subjected to the functional screening assay, and the most inhibitory fractions were sent for mass spectrometry. Mass spectrometry analysis of inhibitory fractions identified inter alpha trypsin inhibitor heavy chain-2 (ITI-H2). This protein is secreted by the liver, secreted into the serum, and acts alone or bound to the high-density lipoprotein to stabilize the extracellular matrix and/or inhibit serine protease activity in the complex oocyte complex and in a variety of tumor cells in hyaluronic acid-rich environments to negatively regulate cell motility. An antibody raised against ITI-H2 and tested using Western blot analysis suggests that ITI-H2 is present as both a single protein and a bikunin-bound form in the inhibitory fractions. Stable cell lines of ITI-heavy chain and bikunin overexpressing glioma and HEK 293 cell lines are currently being tested to further validate our model both in vitro and in vivo. Our studies identify a role for inter alpha trypsin inhibitor in malignant glial cell invasion and warrant further study of serum proteins as readily available sources of invasion inhibitors. The identification of serum-derived inhibitors that have potential therapeutic value in a variety of infiltrative and metastatic tumors in addition to malignant glioma.

**294. INTER ALPHA TRYSIN INHIBITOR HEAVY CHAIN 2: A NOVEL INHIBITOR OF GLIOMA CELL INVASION IN THREE-DIMENSIONS**

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**295. BACITRACIN INHIBITS GLIOMA CELL MIGRATION AND INVASION IN VITRO**

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**296. SLIT2-ROUNDBOUT 1(ROBO1) SIGNALING INHIBITS MEDULLOBLASTOMA BUT NOT GLIOMA CELL INVASION IN THREE-DIMENSIONS**

T.E. Werbowetski, M. Seyed Sadr, N. Jabado, A. Angers-Loustau, R. Rijekvig, J. Antel, D. F. Of Del Maestro, T.E. Werbowetski, M. Seyed Sadr, N. Jabado, A. Angers-Loustau, R. Rijekvig, J. Antel, D. F. Of Del Maestro, 1Neurology and Neurosurgery and 2Neuroimmunology Unit, Montreal Neurological Institute, Montreal, Canada; 3Department of Pediatrics, Montreal Children’s Hospital Research Institute, Montreal, Canada; 4Department of Anatomy and Cell Biology, University of Bergen, Bergen, Norway; 5Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

Chromotopic cues such as the Slt. Netrin and Semaphorin families guide the migration of neuronal and glial cell precursors during neural development. Recently, Slt. and its receptor Roundabout (Robo) have been implicated in tumor angiogenesis and leukocyte migration. It is not known if these molecules contribute to directing the invasion of brain tissue by...
C.D. James1; 1Neurology, Mayo Clinic Rochester, Rochester, Minnesota; and heterotopic (subcutaneous flank) xenografts, EGFR-amplified tumors, those with amplification of EGFRvIII (deleted for codons 6-8), were tested. We found that PLC-gamma activity may be an important mediator of glioblastoma invasion (Song et al., Proc. Natl. Acad. Sci. USA 100, 13970, 2003). We also demonstrated that EGFR activation in vitro stimulates glioma motility/invasion by way of increased PLC-gamma activity in vivo at two levels. First, in both orthotopic (intracranial) and heterotopic (subcutaneous flank) xenograft tumors demonstrated PLC-gamma activation, in contrast to tumors not amplified for EGFR. We further evaluated the relationship between EGFR and PLC phosphorylation in vivo at two levels. First, in both orthotopic (intracranial) and heterotopic (subcutaneous flank) xenograft tumors, EGFR-amplified xenograft tumors demonstrated PLC-gamma activation, in contrast to tumors not amplified for EGFR. We also evaluated 89 human glioblastoma specimens and found a very significant correlation between EGFR amplification and PLC gamma phosphorylation: 16/31 EGFR amplified tumors versus 6/38 EGFR nonamplified tumors (P < 0.0001). Of the EGFR amplified tumors, those with amplification of EGFRvIII (deleted for codons 6-273) showed a higher association with PLC gamma phosphorylation (8/10) than tumors with wild-type EGFR amplification (8/21). Taken together, our results support that a relationship exists between high-level, amplification of EGFR receptor and PLC-gamma activity in vivo and that PLC-gamma activity may be an important mediator of glioblastoma cell invasion. Hence, EGFR (especially the vIII form) as well as PLC gamma activity may be important targets for anti-invasive therapeutics.

299. CELL INVASION OF HUMAN PITUITARY ADENOMA CELL LINE, HP-75 IN HYPOXIA
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Pituitary adenoma tissue is known as hypovascular, and concomitantly the partial oxygen pressure is lower than the surrounding normal organs likely in the other cancer tissues. In this study, we investigated whether hypoxia influences the cell invasiveness of the human nonfunctioning pituitary adenoma cell line, HP-75. HP-75 cells were exposed to hypoxia (1% for 24 h). The subsequent mRNA expression of genes was examined by cDNA microarray. The results were verified by real-time RT-PCR. Gelatin zymogram and reverse zymogram were employed to determine enzyme activities of MMP and TIMP. Cell motility, chemotaxis, and haptotaxis experiments were further studied. Cyclic DNA microarray and real-time RT-PCR indicated that laminin β2 chain mRNA was specifically upregulated by hypoxia (4.16-fold), but not in the other genes relating to cell motility and invasion involving extracellular matrix, cell adhesion molecules, and MMP families. Immunofluorescent study also demonstrated the increased expression of laminin β2 chain at the protein level followed by hypoxic induction. Gelatin zymogram and reverse zymogram, showing MMP and TIMP activities, were not particularly changed. Meanwhile, cell adhesion and chemotaxis to laminin, collagen type I, and fibronectin were not modulated by hypoxia. Cell motility was not ameliorated by hypoxia. Cell-to-cell adhesion was significantly elevated (9.6-fold). These results highly suggest that hypoxia induces elevated cell invasion and cell to cell mediated by elevated expression of laminin β-2 molecule that is specifically bound to collagen type IV.

300. HEMANGIOGENIC PHENOTYPES AS SURROGATE BIOMARKERS IN BRAIN TUMOR TREATMENT
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No biomarkers are currently available to indicate theangiogenic propensity of brain tumors nor to evaluate their response to therapy. Nevertheless, many different antiangiogenic drugs are currently being evaluated in clinical trials. Here we describe a novel in vitro functional assay, HUVEC-based angiogenic scale (HBAS), to assess the overall angiogenic activity in the plasma of patients with glioma and meningioma. Analysis of a cohort of 50 consecutive patients with these tumors showed that the majority had higher HBAS at baseline prior to surgical resection than age-matched normal subjects. The plasma HBAS also correlated with increased plasma and cellular vascular endothelial growth factor-A (VEGF-A) levels by ELISA. The cell lysate fraction from platelets was found to have the highest level of pro-angiogenic activity. In addition, comparison of hemangiogenic cellular markers indicated a switch toward increased circulating CD133 VEGFR2 endothelial progenitor cells (by flow cytometry) and circulating hematopoietic stem/progenitor cells (by methylcellulose colony forming assays) in subsets of patients with active glioma or meningioma. This hemangiogenic switch also correlated with an increased plasma level of stromal-derived factor-1 alpha (SDF-1a), suggesting that mobilization of these hemangiogenic progenitors from the bone marrow to the brain tumor neo-angiogenic niche may be at least partially mediated by the chemokine SDF-1a/CXCR4 pathway. Work is underway to study the correlation between the hemangiogenic phenotypes and the clinical outcome of these patients. Taken together, collective assessment of hemangiogenic biomarkers will provide novel tools to evaluate early outcomes and points for future clinical trials for patients with glioma and meningioma.
In a number of recent publications, VEGF has been implicated not only as a stimulator of angiogenesis, but also as a survival factor for both endothelial cells and GBM cells after irradiation. GBM is a highly resistant tumor with a high VEGF secretion. We hypothesize that VEGF protects endothelial cells and GBM cells against damage of ionizing radiation. Four different types of endothelial cells were used, commercially available HUVECs (from Clonetics), a HUVEC cell line (EC-RF24), primary HUVECs and bovine retinal endothelial cells (BRC), and two GBM cell lines (Gli-O6 and U87). Cells were irradiated with 0, 2, or 5 Gy under low-serum conditions with three different concentrations of VEGF added to the medium. Cell survival after single dose irradiation was measured by the XTT proliferation assay after 96 h. In addition, four human GBM cell lines (U87, Gli-O6, U251, and U251-NG2) were irradiated with various single dose fractions between 0 Gy and 20 Gy. The VEGF concentration in the medium was measured by ELISA at 0, 24, 48 and 72 h after gamma-irradiation. All cells tested showed already inhibited cell proliferation after single dose irradiation of 20 Gy. Although VEGF stimulated endothelial cell proliferation in a dose dependent manner, the cell-killing effect of irradiation was not affected. The variation in radiosensitivity of endothelial cells was smaller than that of GBM cells, with U87 being the most radioresistant. Interestingly, in all GBM cell lines we found a dose-dependent increase in the VEGF-secretion after ionizing radiation, with U87 having a 4-times higher VEGF secretion than the other tested cell lines, with a twofold increase in VEGF secretion 24 h after a single dose of 20 Gy. We could not demonstrate a protective effect of VEGF on cell death after ionizing irradiation of endothelial cells, nor GBM cells. We used four different types of endothelial cells to model brain tumor endothelial cells. The endothelial cells showed proliferative effects of VEGF, suggesting an active VEGF-R1 system. The discrepancy with the literature will be discussed. We conclude that VEGF is not a survival factor for endothelial or GBM cells.
leads to heightened (almost certain) tumor recurrence. A three-dimensional spheroid invasion assay was employed to determine the transcriptome of invasive glioma cells (U87WT and U87/EGFR) compared to their non-invasive counterparts from the spheroid center. Cells from invasive rim and spheroid core were collected by laser capture microdissection as three biological replicates representing each cell line; mRNA was isolated and underwent oligonucleotide microarray analysis. Mitogen-activated protein kinase (MAPK) signaling, since it MAP2K3, a member of the MAPK kinase family, was identified to be significantly upregulated in invasive cells. MAP2K3 is involved in stress signaling, tumor cell invasion, and apoptosis resistance; MAP2K3 activates p38 by phosphorylation. Immunofluorescence on sections from patient tissue microarray identified to be signficantly upregulated in invasive cells. MAP2K3 is involved in stress signaling, tumor cell invasion, and apoptosis resistance; MAP2K3 activates p38 by phosphorylation. Immunofluorescence on sections from patient tissue microarray revealed increased levels of MAP2K3 and phosphorylated p38 in invasive cells. Inhibition of these genes by siRNA and small molecules decreased invasiveness of spheroids in vitro while sensitizing them to apoptosis induction, confirming significance of this pathway for glioma invasion. Gene-staining revealed strong intensity for MAP2K3 and phosphorylated p38 in 100% of invasive glioma cells, while cells from the core exhibited weak or no staining; noncanonical brain revealed to be strong for MAP2K3. This data suggests that MAP2K3 and p38 are potential targets for anti-invasive therapies in combination with cytotoxic agents.

306. MICROARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS OF ASTROCYTIC TUMORS OCCURRING IN A LI-FRAUMENI-LIKE SYNDROME FAMILY K. M. Reis,1 B. Carvalho,1 J. Amorim,2 S. A. Ribeiro,1 F. Parada,1 R. Almeida,1 and B. Ylstra2; 1Life and Health Sciences Research Institute (ICVS), Health Sciences School, University of Minho, Braga, Portugal; 2Department of Pathology, Free University Medical Center, Amsterdam, The Netherlands; Departments of 1Internal Medicine, 2Pathology, and 3Neurosurgery, Hospital S. Marcos, Braga, Portugal; 4Macroscopic Core Facility, Free University Medical Center, Amsterdam, The Netherlands

Li-Fraumeni syndrome (LFS) is a rare autosomal dominant disease. It is characterized by a familial clustering of a wide range of cancers, predominantly breast cancer, sarcomas, brain tumors, and adrenal cortex cancer, diagnosed before the age of 45 years. Two distinct forms of LFS can be recognized, a classic LFS and a Li-Fraumeni-like syndrome (LFL). In the majority of the LFS families, the underlying genetic defect is a germ line mutation in TP53. Here, we describe a LFL family characterized by the presence of brain tumors in two siblings, a 26-year-old man with three metastatic astrocytic tumors (two diffuse astrocytomas and one glioblastoma, which occurred within a 4-year interval) and his 30-year-old sister with one diffuse astrocytoma. The advent of microarray-based comparative genomic hybridization (array-CGH) technology allows the analysis of whole chromosomal aberrations in a single experiment with high spatial resolution. The aim of this study was to assess the presence of TP53 germ line mutations in this LFL family and to relate it with the chromosomal aberrations of the astrocytic tumors. TP53 germ line mutation analysis of exons 5–8 was performed by PCR followed by direct sequencing of DNA isolated from peripheral blood of affected siblings. Affymetrix-embedded histological sections of the four astrocytic tumors, DNA was isolated and used for microarray-CGH analysis. The array-CGH consisted of about 5000 BAC clones with an average resolution of 1 Mb. Differentially expressed exons were identified by the formalin-fixed paraffin-embedded histological sections of the four astrocytic tumors. An identical germ line mutation was identified in both siblings. The microarray-CGH analysis of the three metastatic tumors of the male has as follows: The first diffuse astrocytoma did not show clear chromosomal alterations; the second diffuse astrocytoma showed some chromosomal alterations, including losses on chromosome 6q, 10q, and 13 and gain on 5q region; the glioblastoma showed the aberrations detected in the previous astrocytoma and contained additional abnormalities, including losses on 2p, 3p, 5p, 10q, 11p, 13p, 14q, 16q, and 22, as well as gain on 5p and 10p. The microarray-CGH analysis of the diffuse astrocytoma of the female showed no clear chromosomal aberrations. This study reports a TP53 germ line mutation in an LFL family associated with brain tumors in young adults. Microarray-CGH analysis of tumors shows the presence of chromosomal alterations, such as loss on 2p, 3p, 5p, 10q, 11p, 13p, 14q, 16q, and 22, as well as gain on 5q regions that are not usually present in sporadic astrocytic tumors.

307. CHANGES IN PROTEIN EXPRESSION FOLLOWING RADIOTHERAPY IN EXPERIMENTAL MALIGNANT GLIOMA C. Wibom,1 F. Pettersson,2 M. Johansson,1 R. Henriksen,1 and A. T. Bergenheim; 1Oncology; 2Organic Chemistry; 3Neurosurgery, University Hospital Umeå, Umeå, Sweden

The outcome of modern treatment for glioblastoma (GBM) has so far been disappointing. In order to develop new GBM treatment modalities, as well as to improve present ones, a more detailed understanding of the biological effects of different treatments must be acquired, together with new tools for a more rapid assessment of these effects. Today, radiotherapy is one of the mainstays of GBM treatment. This study aims to characterize diffusion patterns in protein expression in brain tumors following radiotherapy in an experimental rat glioma model. RT4C cells were stereotactically implanted into the right nucleus caudatus of 24 BD IX-rats. One group received radiotherapy delivered as a 12- Gy single fraction on day 12 after implantation, 1, 5, 7, and 12. Three animals from each group were sacrificed, and tumor tissue from each animal was analyzed with regard to protein expression using surface-enhanced laser desorption/ionization-time of flight–mass spectrometry (SELDI-TOF-MS). Mass spectrometric data was analyzed with principle components analysis (PCA) to detect differences between the groups as well as possible temporal changes. Using PCA, regions of interest within mass spectograms of 2.5–50 kDa were identified and further characterized through comparisons of mean peak intensities of the groups. Univariate T-test statistics revealed several peaks whose intensity significantly changed after radiotherapy. The prompt changes in the protein expression are a novel observation and might be of value to understand biological events following irradiation. The SELDI-TOF-MS technique in conjunction with PCA seems to be a well suited tool to study these changes and can detect specific proteins displaying differentiated expression levels. In a further perspective these findings may prove to be useful in the development of new GBM treatment schedules.

308. MGMT METHYLATION STATUS AND EXPRESSION LEVEL DO NOT CORRELATE WITH SENSITIVITY TO CCNU IN SHORT-TERM CULTURES DERIVED FROM MALIGNANT ASTROCYTOMA T. Warr,1 R. Poh,2 B. Suarez-Merino,1 S. Ward,1 P. Warren,1 J. Darling,1 and D. Thomas; 1Department of Molecular Neuroscience and 2Division of Neurosurgery, Institute of Neurology, University College London; London; University of Wolverhampton, Research Institute of Healthcare Science, Wolverhampton; UK

Adjuvant chemotherapy using DNA-damaging agents has largely failed to make a significant impact on the outcome of patients with malignant astrocytoma. One of the primary mechanisms of resistance to nitrosoureas such as CCNU is mediated through O6-methylguanine–DNA methyltransferase (MGMT). This DNA repair enzyme removes the cytotoxic alkyl adducts from O6-guanine, and hence the level of MGMT activity in tumor cells is related to their sensitivity to nitrosoureas. It has been proposed that functional inactivation of MGMT through hypermethylation of the gene promoter region could be predictive of chemosensitivity. We have previously reported differential sensitivity to CCNU in a panel of 17 short-term cultures derived from malignant astrocytoma. In this study, we determined the methylation status of MGMT using methylation-specific PCR in these 17 cultures. We also assessed the amounts of MGMT mRNA and protein present in each culture using real-time quantitative PCR and immunohistochemistry with a commercial antibody against MGMT. There was good correlation between MGMT promoter methylation and presence of MGMT mRNA and protein in all but 2 cases. In both these cultures, MGMT mRNA and protein were not detected even though the MGMT promoter was unmethylated. However, there was no correlation between sensitivity to CCNU and MGMT status. In the 2 most resistant cultures, the MGMT gene was methylated and was not expressed. Similarly, in 4/5 of the most sensitive cultures, MGMT was unmethylated, and in 2 of these cases, there was commensurate MGMT expression. However, in the remaining 2 cultures, MGMT expression was not detected, indicating that an alternative mechanism to gene methylation is responsible for MGMT inactivation. This study highlights that the resistance of malignant astrocytoma to nitrosoureas may be more complex than simple reliance on MGMT activity and prediction of response to such agents by MGMT methylation status should be used with caution.

309. SHARED EPIGENETIC MECHANISMS OF GENE INACTIVATION IN HUMAN AND MOUSE GLIOMAS J. F. Costello,1 C. Hong,2 P. Jun,1 A. Maunakea,1 W. A. Weiss,1 and A. W. Bollen; 1Neurological Surgery, 2Pathology, and 3Neurology, University of California San Francisco, San Francisco, California, USA

Human tumors arise from the deleterious effects of genetic and epigenetic mechanisms on gene expression. In several mouse models of human tumors, the tumorigenic phenotype is reversible, suggesting that epigenetic mechanisms also contribute significantly to tumorgenesis in mice. It is not known whether these are the same epigenetic mechanisms in human and mouse tumors, or whether they affect homologous genes. Using an integrated approach for genome-wide methylation (epigene) and copy number (genetic) analyses, we identified SLC5A8 on chromosome 12q23.1

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and PRKWNK2 on chromosome 9q22.31 that were affected primarily by aberrant methylation in human astrocytomas and oligodendrogliomas. SLC5A8 encodes a sodium monocarboxylate cotransporter, and PRKWNK2 encodes a serine/threonine kinase. Both genes are highly expressed in normal brain but significantly downregulated in primary gliomas. Bisulfite sequencing analysis showed that their promoter CpG islands were unmethylated in normal brain, but extensively methylated in brain tumors, consistent with the tumor-specific loss of gene expression. In glioma cell lines SLC5A8 and PRKWNK2 expression was also suppressed but could be reactivated with a methylation inhibitor. Expression of exogenous SLC5A8 or PRKWNK2 in glioma cells inhibited colony formation, suggesting they may function as growth suppressors in vitro. Remarkably, 9 of 10 murine oligodendrogial tumors from p53+/−/ot ink4a/raf−/− animals transgenic for S100b-v-erbB demonstrated a similar tumor-specific downregulation of mSLC5A8, the highly conserved mouse homologue of SLC5A8. Thus, the murine PRKWNK2 was also methylated and downregulated in a proportion of the mouse gliomas. Taken together, these data suggest that SLC5A8 and PRKWNK2 function as growth suppressors in vitro and that epigenetic mechanisms are their primary cause of gene silencing in human gliomas. The shared epigenetic inactivation of both SLC5A8 and PRKWNK2 in mouse and human gliomas indicates an additional degree of commonality in the origin and/or pathway to tumorigenesis between primary human tumors and these mouse models of gliomas.

310. GENETIC ALTERATIONS IN DESMOPLASTIC MEDULLOBLASTOMAS: EVIDENCE FOR MONOCONAL TUMOR ORIGIN AND IDENTIFICATION OF NOVEL AMPLIFIED AND/OR EXPRESSED PROTO-ONCOGENES

Desmoplastic medulloblastomas (dMBs) are histologically characterized by two distinct tumor components, the so-called pale islands and the desmoplasic areas. Previous molecular studies have shown that dMBs frequently carry PTCH mutations. However, little is known about other genetic and chromosomal aberrations associated with these tumors. We investigated total tumor DNA of 23 sporadic dMBs using comparative genomic hybridization (CGH). Chromosomal imbalances were identified in 17 tumors (74%). The number of aberrations detected per tumor varied from 1 to 12, with an average of 4.61 ± 0.73 (mean ± SEM). Recurrent chromosomal gains were detected on chromosomes 3 and 9 (6/23); 2 and 20 (5/23); 6, 7, 17, and 22 (24/23 each); and 1 (3/23). Recurrent losses were found on chromosomes X (8/23); Y (6/13 male patients); 9 and 12 (4/23 each); as well as 10, 13, and 17 (3/23 each). Amplifications were detected in 4 tumors and mapped to 1p12, 1p5, 9p, 12p13, 13q33–q34, and 17q22–q24. To address the question of clonality of the two components in dMBs, we performed CGH analysis on microdissected pale islands and desmoplasic areas. In 5/6 informative tumors both histological components shared common chromosomal imbalances, indicating an origin from a single progenitor cell. Genomically characterized amplifications detected on 1p35, 9p, and 17q22–q24 in 2 dMBs using matrix-CGH on genomic arrays of 6,000 large insert clones. Subsequent molecular analyses of amplified candidate genes identified by matrix-CGH confirmed amplification of several genes on 17q23 in three dMBs and the JMJD2C gene on 9p24 in 1 dMB, respectively. Expression analysis suggested RPS6KB1 as the most important target on 17q23, which was found to be markedly overexpressed in 10/11 medulloblastomas investigated. Taken together, our study provides strong genetic evidence for a monoclonal origin of dMBs and implicates RPS6KB1 and JMJD2C as novel proto-oncogenes that are aberrantly activated in these tumors.

311. AMPLIFICATIONS AND DELETIONS IN THE GLIOBLASTOMA GENOME: FROM NOVEL LOCI TO CANDIDATE GENE AND NON-GENE TARGETS

Array-based comparative genomic hybridization (ACGH) offers increasing resolution of amplification and deletion events in the tumor genome. The targets of less common events, along with the significance of these events, are the subject of intense investigation. We present the combined analysis of ACGH and expression profiling of 40 primary glioblastomas and 20 cell lines in an effort to identify narrow, high-copy-number aberrations which provide a limited number of candidate gene and non-gene targets, such as micro-RNAs. Profiles were generated using cDNA microarrays (Agilent) providing an interval resolution of approximately 100 kb. A changepoint algorithm (circular binary segmentation) was used to identify discrete copy number aberrations (CNAs) and their boundaries. Locus boundaries are systematically defined by grouping CNAs across multiple profiles. Within each locus, one or more minimal common regions (MCRs) of overlapping alteration are automatically identified, each potentially harboring a distinct cancer-relevant target. Twenty cell lines and 14 tumors were additionally profiled for RNA expression (Affymetrix). Expression data were mapped to genome position, and each gene was tested for copy-number-driven expression by calculating the shift in expression in samples with CNA. Significance was estimated by permutation testing. One hundred sixty-four discrete autosomal loci were identified, 111 present in more than one sample. Many MCRs were of low-copy number alteration. Fifty-five MCRs met high-confidence criteria: high-level alteration and/or high recurrence. Average size of this subset was 3.5 Mb, spanning an average of 36 genes. Fifteen of these MCRs were present only in cell lines; 40 were identified in tumors as well (21 amplifications, 19 deletions). Supporting the validity of the approach, all common CNAs previously described in high-grade gliomas were identified, including amplifications of PDGFRα, EGFR, MDM2, and CDK4 and deletions of p16/ink4a and PTEN. Aside from these, the majority of loci were novel, either not previously described in glioma or with a characterised target gene. Eighteen loci spanning from 2 to 4 Mb included only 20 genes. Each of these smaller loci was subject to quantitative PCR validation. We identified several candidate targets, including TERT, not previously identified as a target of amplification. Genes within the 52 loci were evaluated for expression, and 320 out of 1740 were found to show a significant effect of copy number on gene expression. Results are contrasted with identical analyses of 300 samples of 4 other non-glioma tumor types. High-grade gliomas harbor infrequent novel chromosomal copy aberrations with a mark potential cancer-relevant genes. Expression profiling allows further narrowing of this list of targets by characterizing the response to gene dosage.

312. COMPREHENSIVE GENETIC CHARACTERIZATION OF PLEOMORPHIC XANTHOASTROCYTOMAS

Pleomorphic xanthoastrocytomas (PXAs) are rare astrocytic neoplasms corresponding histologically to WHO grade II. They usually show circumscript growth and favorable prognosis despite exhibiting a high degree of cellular pleomorphism. PXAs mainly affect children and young adults. Here we present genomic profiling experiments of 50 PXAs. Chromosomal CGH revealed a distinct pattern of chromosomal imbalances. The hallmark alteration detected was a loss of 9p21, the region harboring CDKN2A and CDKN2B. Other copy number changes were less common and included losses on 1p36.3 (3% of tumors), 1q (8% of tumors), and 9p (10% of tumors). The 9p losses were found on chromosomes 17q21-q22. No other consistent loss has been reported previously. The loss of CDKN2A/2B has already been described as a hallmark alteration detected in 50% of PXAs. Tumors were grouped in 16 potentially cancer-relevant genes. Expression profiling allowed further narrowing of this list of targets by characterizing the response to gene dosage.
313. ALTERATIONS OF p53 AND p73 OCCUR INDEPENDENTLY OF ONE ANOTHER IN ALL GRADES OF PEDIATRIC DIFFUSE ASTROCYTOMA A. Prowald, 1 S. Urbschat, 2 S. Wemmert, 1 R. Ketter, 1 E. Meese, 1 K.D. Zang, 1 and W.-I. Steudel 1; 1Neurochirurgie, Neuroonkologie, Universitätskliniken des Saarlandes, Homburg/Saar; 2Institut für Humangenetik, Universität des Saarlandes, Saarbrücken; 1IGD Saar, Institut für genetische Diagnostik, Homburg/Saar; Germany 

In nearly half of sporadic low-grade meningiomas, no chromosome aberration can be detected. In the majority of the other half, chromosome 22 is lost. In higher grade meningiomas, this loss is followed by characteristic secondary chromosome aberrations. Regarding the molecular findings in schwannomas, homozygous loss or mutation of the NF2 gene located on chromosome 22 was supposed also to be the primary event in meningioma development. However, in nearly all high-grade but in only a minority of low-grade meningiomas, the loss of NF2 protein is observed. Therefore, the hypothetical combined heterozygous loss of or inactivation of two or more tumor suppressor genes (at least one of them located on chromosome 22) as well as the homozygous loss of a regulatory gene on chromosome 22 different from NF2 was discussed. In a search for microdeletions or/and structural recombinations of chromosome 22, we investigated primary cell cultures of 43 meningiomas by conventional G-band (26 without, 17 with loss of chromosome 22). Twenty-seven tumors were analyzed with spectral karyotyping (SKY) and 16 with fluorescence in situ hybridization (FISH) with DNA probes for the chromosomal regions of 22q11.2, 22q11.2.3, 22q12.1, 22q12.1, and 22q13.3. SKY analysis confirmed G-banding data for chromosome 22 and could specify marker chromosomes and translocations containing material from chromosome(s) 22. Confirming our assumption, microdeletions on chromosome 22 were detected by FISH in 6/8 cytogenetically nonarranged meningiomas. Surprisingly, in 2/8 cases we observed gains of the 22q13.3 region and in 2/8, gains of the 22q12.1 region. Here we present first evidence for an uncommon mechanism during early meningioma development at least for a meningioma subgroup: (i) deletion and translocation of sequences from chromosome 22 to different chromosomes, (ii) deletion of the original sequences on chromosome 22, resulting in disomy again (only visible as translocation in metaphase FISH), and (iii) loss of chromosome 22.
317. DISSECTING GLIOMA SYSTEMS BY GENOMICS, PROTEOMICS, AND MODELING
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Cancer systems are overwhelmingly complex. They, like healthy cells, are regulated by numerous genes and proteins in a highly coordinated fashion. The main difference is that in cancer cells, the rules of regulation have been altered by events that cause the underlying complex dynamical system governing cellular activity to behave in an aberrant manner. The complex nature of a cancer system, with its many unrevealed components and levels of complexity, calls for a more systematic measurement of gene and proteins and the development of suitable representational models that can ultimately be used to guide cancer researchers in their pursuit of understanding cancer and finding effective treatment. Using microarray technology, we have profiled gene expression of different grades of gliomas. In addition to some highly interesting individual markers such as insulin-like growth factor binding protein 2 (IGFBP2), we have developed a mathematical model called Probabilistic Boolean Network (PBN) to construct a network based on expression of 600 genes that are functionally well characterized. The network has revealed novel relationships among genes, some of which have subsequently been confirmed by experiments. For example, the PBN model revealed a relationship between IGFBP2 and NFKappaB, which was experimentally confirmed. To extend our understanding, we have recently constructed a reverse-phase protein lysate array with 90 different glioma tissues and assayed for the expression of 50 signaling proteins. To gain mathematical analyses have revealed key protein expression and posttranslational modification events during glioma progression. For example, akt phosphorylation is most dominant in glioblastomas. In summary, we are beginning to be able to dissect the glioma systems with genomics, proteomics, and bioinformatics.

318. TELOMERASE AND ESTROGEN RECEPTOR ROLES IN GLIOMAS
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The most common primary brain tumors are glioma. Glioma cells that respond to chemotherapy have shown a decline in the activity of a specific enzyme called telomerase. This enzyme is usually only active in undifferentiated cells. However, it has been detected in 94% of neuroblastoma and 100% of oligodendroglioma. Numerous studies have concentrated on the induction level of hTERT, the major subunit of telomerase in cancer tissue, but few workers have correlated the level of telomerase with hsp90 and p23, which is a chaperone for the telomerase. It is proposed here that if there is high constitutive expression level of hTERT, then there may also be a high expression level of hsp90 and p23, since this is a cancer suppressor. Furthermore, a high brain concentration of telomerase protein may offer an alternative and direct indicator of malignancy. In this study, the level of telomerase in cell samples has been quantitatively measured by several methods. Results indicate an elevated constitutive expression of cell samples is serially diluted and in triplicate on the array. The main difference is that in cancer cells, the rules of regulation have been altered by events that cause the underlying complex dynamical system governing cellular activity to behave in an aberrant manner. In addition to some highly interesting individual markers such as insulin-like growth factor binding protein 2 (IGFBP2), we have developed a mathematical model called Probabilistic Boolean Network (PBN) to construct a network based on expression of 600 genes that are functionally well characterized. In summary, we are beginning to be able to dissect the glioma systems with genomics, proteomics, and bioinformatics.

319. HYPERDIPLOIDITY DEFINES A DISTINCT CYTOSTATIC ENTITY OF AGGRESSIVE MENINGIOMAS
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The most common chromosomal aberration found in meningiomas of all grades is monosomy 22. Progression and recurrence of meningiomas is usually associated with stronger hyperdiploidy, i.e., monosomy of further autosomes and, most frequently, heterozygous loss of chromosome 1p. Rarely, however, hyperdiploid karyotypes occur; the objective of this study was to explore the cytogenetic and histopathologic patterns as well as the clinical significance of hyperdiploidy in meningiomas. A consecutive series of 460 meningiomas were cultured in vitro and cytogenetically characterized by using standard banding techniques and, in one structurally aberrant case, spectral karyotyping (SKY). In patients with hyperdiploid meningiomas, clinical and histomorphological data as well as results of long-term postoperative follow-up were compared with data from patients with cytogenetically typical meningiomas. We identified a subgroup comprising about 4% of all meningiomas that do not display the common chromosome losses but instead a strikingly uniform pattern of hyperdiploidy. Mostly in the absence of structural chromosome rearrangements, these meningiomas each have between 49 and 56 chromosomes, with trisomy 12 (14/16 cases), trisomy 20 (13/16 cases), trisomy 5 (12/16 cases), and trisomy 17 (10/16 cases), along with variable trisomies of all other autosomes except #1, #2, and #21. Chromosome losses are rare, affecting #22 in 2/16, and #7 and #18 in only 1/16 cases. Histomorphologically, the hyperdiploid meningiomas show intermediate differentiation with patternless growth and/or microscopically degenerative. However, a loss of chromosome 22 may be correlated with a persistent fibrous growth pattern. The proliferative potential in terms of increased mitotic activity and Ki-67 labeling index is significantly elevated; all investigated hyperdiploid meningiomas were assigned to WHO grade II. Fourteen patients in whom tumor resections were determined to be Simpson grade I or II and 2 patients with Simpson grade III could be followed up after tumor extirpation. In 2 patients, recurrences were documented and 3 patients died during the period of observation. We conclude that hyperdiploidy constitutes a small but clinically relevant entity of biologically aggressive, histopathologically atypical meningiomas (WHO grade II), which are cytogenetically distinguishable from the majority of common-type meningiomas.

320. PHARMACOLOGICAL REVERSAL OF GENE SILENCING IN MALIGNANT GLIOMA: A WHOLE GENOME MICROARRAY ANALYSIS
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Promoter methylation and histone modification play a critical role in transcriptional regulation. We explored the role of histone deacetylase (HDAC) inhibitors in malignant gliomas and found that the histone deacetylase inhibitor, trichostatin A (TSA), a specific inhibitor of histone deacetylase activity, successfully reversed silencing of genes in malignant glioma cell lines. Whole genome microarray analysis identified specific genes differentially expressed in malignant astrocytes in response to treatment with Trichostatin A (TSA), a specific inhibitor of histone deacetylase activity. ChIP analysis of histone modifications identified specific promoter region alterations in selected differentially regulated genes. Microarray studies were performed on 11 primary glioblastoma cell lines (UI series), the T98 and U87 cell lines, and normal human astrocytes. Three experimental paradigms were used: (1) a time-course analysis of T98 cells treated with 1 μM TSA for 6, 12, 24, 36, and 48 h, (2) a dose-response analysis of T98 cells treated with increasing concentrations of TSA (300 nM to 5 μM) for 24 h, and (3) the silencing response in 8 of 11 primary lines and U87 cells treated with 1 μM TSA for 24 h. Biological replicates were performed independently in triplicate for each condition. A cohort of TSA regulated genes was selected as an intersection of the three experimental paradigms. Statistical analysis using Significance of Microarrays (SAM) and ANOVA-Q-Value (Storey) with maximum stringency (q < 0.001) identified specific promoter region alterations in selected genes. Whole genome microarray analysis has identified specific HDAC inhibitor, TSA, reverses gene silencing in malignant gliomas and provides insight into the mechanisms of aberrant transcriptional regulation during malignant astrocyte transformation. The identification of epigenetic silenced genes represents a potential entry point for biomarker development and therapeutic intervention.
321. MOLECULAR CLASSIFICATION OF GLIOMAS BY COMPARATIVE GENOMIC HYBRIDIZATION ARRAY IS CORRELATED WITH PROGNOSIS
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The purpose of our study was to screen gliomas for genomic imbalances by the comparative genomic hybridization array technique (CGH). DNA from 100 unselected diffuse gliomas were investigated by a genome-wide array-based CGH technique (a total of 1 Mb mapped and amplified BAC DNA were spotted in an array format on glass, providing an average resolution of 1 Mb across the human genome). The genomic profiles of all tumors were classified by using clustering software. The results were correlated with the outcome of the patients. The most frequent alterations were loss of 1p, 19q, 9p (P16/CDKN2A locus), and 10q; gain of chromosome 7, and amplification of PDGFRa. In addition, CGH detected less well-documented recurrent abnormalities (loss of 4, gain of amplification of PDGFRa, MDM4, MDM2 and CDK4 locus). The results were concordant with those of a previously published work.

322. ANALYSIS OF “MEDIUM THROUGHPUT” QUANTITATIVE LOH IN ANAPLASTIC OLIGODENDROGLIOMA
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Allelic losses on chromosomes 1p and 19q are known to occur frequently in anaplastic oligodendrogliomas. Current assessment of LOH in oligodendrogliomas is based on 3-4 distally located CA-repeat polymorphism markers. A call of LOH is made if LOH is detected at all informative markers. We recently used capillary electrophoresis for assessing LOH at 15 markers on 1p and 4 markers on 19q in 93 tumors. We derived meaningful thresholds for the quantitative LOH through the use of a latent mixture model that leveraged the measurements from normal specimens to gain information about the non-LOH distribution. We applied latent class analysis to cluster subjects according to patterns of LOH and found three distinct LOH profiles among our samples: a group with LOH at all markers, a group with moderate LOH, and a group with low LOH. The survival outcomes of the high-LOH group were significantly more favorable than those of the other groups, which were not different from each other. We used the latent class model to multiply impute values for the noninformative LOH outcomes and thereby increased the power of the survival analyses. Last, we developed constrained estimation techniques to enable complete joint modeling of all 19 markers while avoiding overfitting the data. These techniques can be applied more generally to the analysis of other genomic assays, including array comparative genomic hybridization (aCGH).

323. IDENTIFICATION OF NOVEL GliOBlastoma Tumor Suppressor Gene Candidates on CHROMOSOME 10q24-25
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Allelic losses on chromosome 10 are common genetic alterations in glioblastomas that are found in approximately 60% to 90% of the cases. Molecular genetic analyses suggested the presence of up to 3 distinct tumor suppressor gene loci at 10p, 10q25, and 10q24-35, respectively. The PTEN tumor suppressor gene has been identified at 10q23. The KIF6 gene at 10p15 has been suggested as another glioblastoma suppressor gene, but does not appear to carry frequent alterations in these tumors. Thus, the target genes on 10p and 10q24-35 are not known yet. We performed loss of heterozygosity, gene expression analysis of 17 genes located on 10q24-35 in a series of 34 primary glioblastomas and 12 secondary glioblastomas to identify novel candidate tumor suppressor genes that are downregulated in glioblastomas. The genes were either selected from the NCBI Human Genome Browser (www.ncbi.nlm.nih.gov) or from microarray expression profiling data of glioma cell lines treated with the demethylating agent 5-aza-deoxycytidine and the histone deacetylase inhibitor trichostatin A. So far, we identified 3 genes (ADD3, EMA2, and OAT) that show markedly reduced mRNA levels relative to non-neoplastic brain tissue in substantial fractions of glioblastomas. Furthermore, treatment of glioma cell lines with 5-aza-deoxycytidine and trichostatin A resulted in increased expression of these genes, suggesting that their transcriptional downregulation may be promoted by hypermethylation. These three genes represent interesting novel tumor suppressor candidates that are presently further investigated for promoter hypermethylation and coding region mutations.

324. GENES THAT PROMOTE CELL GROWTH ARE ABERBRANLY EXPRESSED IN PILOCYTIC ASTROCYTOMA
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Astrocytoma are the most common brain tumors occurring in children. The majority of these are low-grade pilocytic (grade I) and diffuse (grade II) astrocytoma comprising 80% of cases. Little is known about the genomic processes underlying the development of pilocytic astrocytoma. Previous cytogenetic studies have revealed that the majority of low-grade pediatric astrocytoma appear to be karyotypically normal. It is therefore possible that abnormal gene expression rather than chromosomal abnormalities are involved in the development of these tumors. The oligonucleotide Affymetrix Human Genome U133 Array representing 33,000 genes was used to generate expression profiles of 14 grade I pediatric astrocytoma biopsy samples and three normal brain controls. Genespring version 6.1 was used for the data analysis including the completion of 1-way ANOVA statistical tests. In total 1340 genes were differentially expressed in tumor samples compared to normal brain controls; 1063 were upregulated and 277 were downregulated. These genes were clustered into components of the Wnt signaling pathway, Wnt5A, PDZK1, PKC, CK1a, KRP6, Cyclin D1, E, and TCF, as well as genes associated with the Notch and TGF-beta signaling pathways, Bmi-1, Jag1, Bmp2, Hey-1, Id1, and Bcl2. Growth promoters PDGFRa, PDGFRb, and FGF2 were also upregulated. The downregulation of SMAD7 and SMURF1 may increase TGF-beta pathway signalling, and the downregulation of tumor suppressor genes p57, p19, and MTUS1 may also contribute to tumor growth. In contrast, the tumor suppressor gene p53 was significantly upregulated. Differential gene expression in pilocytic astrocytoma indicates increased activity of pathways involved in cell growth.

325. GENES EXPRESSION PROFILING OF GLIOMAS
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We analyzed the expression level of 3456 genes in 109 glioma using adapter-tagged competitive PCR (ATAC-PCR), a high-throughput reverse transcription-PCR technique. The purpose of this study is to investigate the molecular features of glioma through a large-scale gene expression profiling. A total of 109 gliomas specimens including 80 glioblastomas, 10 anaplastic astrocytomas, 12 diffuse astrocytomas, and 7 anaplastic oligodendrogliomas were obtained from surgical resection. We firstly surveyed the genes actually expressed in glioma using expressed sequence tag (EST) sequencing. A 3rd cDNA library was constructed by using a mixture of RNA from 12 gliomas, and we randomly selected 3036 genes from this EST collection. We then developed PCR primers for amplifying 420 genes known to be expressed in glioma from previous literature. The expression level of these genes in sample RNAs derived from 109 gliomas was assayed by the ATAC-PCR technique. ATAC-PCR is an advanced version of quantitative competitive PCR, characterized by the addition of unique adaptors for different cDNAs, measuring the relative expression of samples against the control. In this assay, using seven adaptors, 4 samples and 3 known amounts of controls were processed in a single reaction. The PCR gene has been performed using an adaptor primer, the sequence of which was from the common part of adaptors, and a primer specific to the gene of interest, the sequence of which was included in the 3’ end fragment. Amplified fragments were separated by denaturing polyacrylamide-gel electrophoresis, and the amount of fragments was measured by an automated sequencer. We analyzed the expression levels of 3456 genes in 109 gliomas. The unsupervised hierarchical cluster analysis revealed that different types and grades of glioma had a unique gene expression pattern of a specific group of genes. In addition, glioblastomas were divided into...
molecular groups, each of which possessed a distinct gene expression signature. Our gene expression study showed that each histological type and grade of glioma has a distinct gene expression signature. Our results may lead to molecular classification of gliomas.

326. NAVIGATING GLOBAL TRANSCRIPTOMICS TO THERAPEUTIC TARGETS ON INVASIVE GLIOMA CELLS


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Gliomas are the most common primary brain tumors, they exhibit highly invasive behavior, which is a major cause of the morbidity and prompt lethality of the disease. A better understanding of the determinants of glioma migration and invasion may explain the ubiquitous recurrence of glioblastoma and may reveal novel therapeutic targets aimed at invasive cells. Motile and stationary cell populations from seven human glioma cell lines and three primary glioblastoma cultures were isolated from a monolayer radial migration assay for gene expression analysis using oligonucleotide microarrays. Differentially expressed genes between these two populations were identified. As glioblastoma cells were identified using a pattern recognition approach that integrates a priori knowledge in conjunction with expression data as implemented in GABRIEL (Genetic Analysis by Rules Incorporating Expert Logic), a Web-based application designed for rule-based analysis (Proc. Natl. Acad. Sci. USA 99, 2118, 2002). Principal component analysis (PCA) is a method for reducing high dimensional data into a few components that represent the majority of variation within the data. PCA of the differential expression data revealed two discriminating patterns, a glioblastoma motile cell profile and a glioblastoma stationary cell profile. Two genes (AK098354 and Cyr61 [cystein rich 61]) following these profiles were used in GABRIEL’s proband rule-based function to find subsets of genes with similar expression patterns. A differential expression of eight candidates (four from each subset) was validated by QRT-PCR. Of these eight, Cyr61 and CTGF (connective tissue growth factor) are secreted extracellular proteins in the CCN family of growth factors, (Cyr61, CTGF, and Novo) whose properties include tumorigenesis and cell migration. Immunofluorescence confirmed increased protein expression in motile and stationary cells in a migration assay. Immunohistochemistry on glioma invasion tissue microarrays revealed expression of Cyr61 and CTGF in association with invasion. siRNA knockdown of Cyr61 showed a reduction in mRNA expression. Its effect on motility will be examined in a migration assay. These findings provide strong evidence that Cyr61 and CTGF play important roles in glioma invasion potentially leading to new anti-invasion therapies.

327. THE TRANSCRIPTION FACTOR FOXL1 IN GLIOMAS

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Foxl1 is a winged helix/forkhead transcription factor previously called brain factor 1 (BF-1). It is critical for the normal development of the brain as demonstrated by the lack of cerebral hemispheres in knockout mice. In addition, the chicken ortholog (Qin) is an oncogene that was originally isolated from avian sarcoma virus 31; it both transforms cultured chicken embryonic fibroblasts and induces fibrosarcomas in birds. Evidence also suggests that Foxg1 contributes to deregulated proliferation by repressing transcription of the tumor suppressor p21CIP. The project described was made possible in part by grant number CA009512 from the National Cancer Institute and by the America Brain Tumor Association.

Abstracts from the World Federation of Neuro-Oncology Meeting

Experiments to evaluate the impact of Ras, Akt, and PDGF on Foxg1 in modeled gliomas in vivo are ongoing as are experiments to determine the effect of Foxg1 to cause gliomas. The forkhead transcription factor Foxg1 is a strong candidate for normal brain development and is overexpressed in both low- and high-grade human gliomas. By modeling the pathways driving human glioma growth, we demonstrated that Ras signaling may be particularly important for the expression of Foxg1 in glia. We are validating these results in vivo with mouse glioma models and are also testing the glioma-genic capacity of Foxg1. The project described was made possible in part by grant number CA009512 from the National Cancer Institute and by the America Brain Tumor Association.

328. IDENTIFICATION OF PROTEIN MARKERS FOR BRAIN CANCER USING EXPRESSION PROTEOMICS

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Gliomas comprise nearly one-half of primary brain tumors and one-fifth of all primary spinal cord tumors. Combined, grade III and IV gliomas represent about 40% of all primary brain tumors in patients aged 40 to 49 years, and cell lines were collected from patients older than 60 years. In most clinical series, grade III gliomas comprise approximately 10% and grade IV 90% of the total number of high-grade, malignant primary brain tumors. We have carried out DIGE (Difference gel electrophoresis) analysis on glioma biopsy samples. The DIGE technology enables identification of different samples to be run on the same gel by pre-labeling the samples with three different cyanine dyes. Each sample is covalently labeled with a different dye from mass and charge-matched set of fluorescent CyDyes, cyanine 2 (Cy2), cyanine 3 (Cy3), and cyanine 5 (Cy5). Effectively, gels can be standardized by using one sample as an internal standard (pool sample). The pool sample is prepared by mixing equal amounts of protein from each individual homogenate. Proteomics promises the discovery of biomarkers and tumor markers for early detection and diagnosis, novel protein-based drug targets for anticancer therapy, and new end points for the assessment of therapeutic efficacy and toxicity. The focus is using the DIGE 2D system to enable large-scale analyses of samples to generate data sets that can be used to differentiate between the various brain cancer stages and types. Fifty human brain tissues were run in duplicate, together with an internal pool sample on each gel. Protein was extracted with buffer 1 (2% ASB-16, 8 M urea, 5 mM magnesium acetate, 20 mM Tris-base) and rehydrated in buffer II (4% CHAPS, 7 M urea, 2 M thiourea, 5 mM magnesium acetate, 1.2 mM Destrack, 1% I2 buffer, 20 mM Tris-base pH 8.5). The standard sample is ideally a pool comprising equal amounts of each of the 50 brain tissues being compared. The 150 images were acquired, and then spot detection and alignment was carried out using the Decyder software. In addition, the gels for picking protein spots were run. The spot cut list was generated covering proteins whose expression levels were changing, as well as set of marker proteins that were constant throughout the DIGE experiment. The proteins of interest were automatically picked, in-gel digested, and then digitally fingerprinted by MALDI-TOF. Reproducible DIGE spot maps of brain tumor proteins were obtained. The protein spots were occasionally distributed over the whole gel but more concentrated in the pH range 4–7. The protein analyses gave identification confidences for 118 spots cut from the preparative gel. One main protein that was clearly dramatically upregulated was astrocyte glial fibrillary acidic protein. This marker is used by histologists to determine whether a tumor is a glioma or not. A low-sulfate fragment of the chondroitin sulfate proteoglycan called brevican was also identified which known to be excreted by tumor cells of patients with brain tumors. A series of proteins were identified in this preliminary analysis that include known generic tumor markers such as 78 kDa glucose-regulated protein, annexin I, and several carbonic anhydrase isofoms. Other proteins that were thought to be tissue-specific markers were found, and a number of interesting findings will be presented and further discussed. The day will come when people will be screened for hundreds of diseases through a simple blood test if our vision is fulfilled. Through “proteomics,” conditions like human cancer will be diagnosed at a stage early enough so that there will be a good chance they can be treated and cured.
329. IDENTIFICATION OF A NOVEL HOMOZYGOUS DELETION REGION AT 6Q23.1 IN MEDULLOBLASTOMAS USING HIGH-RESOLUTION ARRAY CGH ANALYSIS
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We used a high-resolution array comparative genomic hybridization analysis for whole-genome analysis of medulloblastomas. Although there have been many genomic surveys of medulloblastomas in the literature, such an approach has not been performed before. We identified several consistent chromosomal aberrations. These included consistent chromosomal gains of 2q23-p25 (5.6% (57.39%), 9q34 (47.37%), as well as losses of 3q25.33-q26.32 (57.9%), 4q31-33 (42.11%), 6q22-27 (57.9%), 8p21.3-23 (78.95%), 10q22-26 (57.9%), 16q22-q24 (63.16%), and 17p13 (31.6%). This information will lead to a better understanding of medulloblastoma tumorigenesis and identify new molecular markers for therapeutic intervention and drug discovery. One of the most notable findings is homozygous deletion on chromosome 6q23. Homozygous deletion of this region was found on cell line DAOY, whereas copy loss was detected on 30% primary medulloblastomas. Further study using 30 pairs of STS markers confined a 0.887 Mb minimal region of homozygous deletion at 6q23.1 which was flanked by markers SHGC-14149 (6q22.33) and SHGC-110551 (6q23.1). Quantitative RT-PCR analysis showed complete loss of expression of 2 genes located at 6q23.1, AK091351 (hypothetical protein FLJ34032) and KIAA1913, on cell line DAOY. mRNA levels of these genes was reduced in cell lines D283 and D384, as well as 50% (AK091351) and 70% (KIAA1913) of 10 primary tumors. Frequent detection of reduced expression of AK091351 and KIAA1913 implicated that these 2 genes may play a critical role in medulloblastoma tumorigenesis.

330. MOLECULAR CLASSIFICATION OF HUMAN GLIOMAS USING MATRIX-BASED COMPARATIVE GENOMIC HYBRIDIZATION
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Gliomas are morphologically, biologically, and clinically heterogeneous brain tumors whose classification is based on histological features. However, evidence is increasing that the different glioma types are associated with distinct genetic aberrations, which may provide useful information for tumor classification as well as prediction of prognosis. To facilitate the molecular classification of gliomas, we established a microarray that consists of genomic clones representing tumor suppressor genes, proto-oncogenes and chromosomal regions frequently gained or lost in gliomas, as well as reference clones distributed evenly throughout the genome in approximately 15 Mbp intervals. These microarrays were used for matrix-based comparative genomic hybridization (matrix CGH) analysis of 70 gliomas. Matrix CGH results were validated by molecular genetic analyses of candidate genes, loss of heterozygosity studies, and chromosomal CGH. Our results indicate that matrix CGH allows for the sensitive and specific detection of gene amplifications, as well as low-level copy number gains and losses in gliomas. Furthermore, molecular classification based on matrix CGH data closely paralleled histological classification, e.g. ependymoma, clinically important differential diagnoses, such as diffuse astrocytoma versus oligodendroglioma, anaplastic astrocytoma versus anaplastic oligodendroglia, and oligodendroglia versus glioblastoma, as well as primary versus secondary glioblastoma. Thus, matrix CGH is a powerful technique that allows for an automated genomic profiling of gliomas and represents a promising tool for their molecular classification.

331. DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF TRANSFORMING GROWTH FACTOR-BETA (TGF-BETA) SIGNALING AND THE IMPACT OF A SPECIFIC LOW-MOLECULAR-WEIGHT TGF-BETA INHIBITOR IN HUMAN MALIGNANT GLIOMA CELL LINES
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Malignant gliomas remain among the most lethal forms of cancer, with resistance to radiation and chemotherapy. The identification and targeting of pathways critical to the phenotype of cancers offer new hopes in the treat-
333. MICRO-GENOMICS OF SCHWANN CELLS IN PERIPHERAL NERVE SHEATH TUMORS (PNST)
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Neurofibroma (nfb) subtypes have varying biology with dermal nfb remaining benign, plexiform nfb having an ~0% risk of becoming malignant termed PNST. Although consisting of many cell types, Schwann cells are the primary transformed cells in all subtypes of nfb. Schwann cell transformation is a result of a common bi-allelic inactivation of NEU and neurofibromin expression, in addition to alterations at the genomic level which likely confer their varying biological properties. Limited conventional CGH studies on nfb have not yet led to an understanding of these additional genetic alterations in the Schwann cells of these nfb subtypes. Schwann and endothelial (control) cells were isolated using laser capture microdissection to create a high-resolution genetic alteration map using array-CGH analysis on BAC and cDNA arrays. In total, 8 plexiform nfb and 8 PNSTs have been analyzed. Genetic losses were more prevalent in the plexiform nfb, compared to PNSTs, where gains were more common. Losses of regions on chromosomes 1, 2, 10, 13, and 17 were common in both benign and malignant tumors. Gains on chromosome arm 4q, 5p, 6q, 8q, 10q, 11q, 13q, and 17q were commonly found in PNSTs. Genes located in regions lost in both plexiform nfb and PNSTs are those potentially involved in initiating nfb. Similarly, regions of gain found in PNSTs are those with a maximum expression in primary tumors. These array-CGH results will be confirmed using FISH and/or real-time PCR. FISH analysis using BAC probes for the Nf1 gene corroborated the findings of the array analysis. Currently, we are utilizing the available NCBI databases to screen for potential novel oncogenes and tumor suppressor genes that would be present in these chromosomal regions. These candidate genes will be further utilized to screen for the frequency of the alteration in a larger cohort of tumors. Analysis of dermal nfb is ongoing.

334. REAL-TIME QUANTITATIVE PCR REVEALS LOSS AT 22q12.3-13.3 IN TWO THIRDS OF PEDIATRIC EPENDYMOMA AND DEFINES A MINIMUM DELETION OF 3.4Mb
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Ependymomas are glial cell-derived tumors which arise from the ependymal lining of the ventricular system of the central nervous system. They manifest preferentially in childhood, where they account for up to 10% of intracranial tumors. At present, the genetic events that contribute to the progression of pediatric malignant ependymoma remain essentially unknown, with up to 50% of tumors appearing karyotypically normal. One of the most common chromosomal aberrations reported in intracranial ependymoma is monosomy of 22q. To investigate genetic alterations that may be implicated in phosphorylation-dependent ubiquitination, the RNA binding protein RBM9 (22q12.3), MFNG (22q31.1), which may be involved in the Notch pathway, the -catenin interacting protein coding gene C22orf2 (22q13), the chromobox homolog CBX7 (22q13.1), and the SET binding factor SFBF (22q13.1). Overall, we observed loss of 22q in 65% of cases, higher than previously reported in pediatric ependymoma using other methodologies. The most common region of loss maps between loci RBM9 and CBX7, spanning 3.4 Mb of chromosome 22. Mutation analysis of two candidate genes mapping to this region (C22orf2 and CBX7) did not reveal any mutations. Further analysis of the promoter region of CBX7 in tumors showing underexpression of the CBX7 transcript but no allele loss revealed that this promoter is not methylated and therefore other mechanisms, such as histone deacetylation, may be responsible for the silencing of the CBX7 gene in our cohort. Our results provide further evidence that one or more tumor suppressor genes critical for ependymoma development are located at 22q12.3-13.1. Further analysis of this region is necessary to identify candidate transcripts.

335. WHOLE GENOME CDNA MICROARRAY ANALYSIS OF GLIOMAS: A SUBSET OF GENES DIFFERENTIATES INVASIVE FROM LESS OR NON-INVASIVE LOW-GRADE LESIONS
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Diffuse gliomas WHO grade II-IV are generally not amenable to cure. Their ability to invade the surrounding normal parenchyma diffusely and eventually regress the tumor bulk is unique among cancer types and is probably the main obstacle for therapeutic intervention. The invasive potential of these tumors is proportional to the tumor grade. However, magnetic resonance imaging (MRI) and surgery can reveal striking differences in the invasion of the brain in tumors sharing the same histopathological characteristics and grade, e.g., in WHO grade II gliomas. We have used a high-throughput microarray approach to analyze RNA from individual gliomas and immediately transferred into RNA later for storage. Tumors were grade for their invasive behavior according to MRI (T2-weighted and FLAIR-sequence). Total RNA was isolated from the samples and processed according to standard protocols for use in the GeneChip Human Genome U133 Plus 2.0 Array from Affymetrix which allows analysis of the expression level of over 47,000 transcripts and variants. The data were analyzed by hierarchical and two-way clustering algorithms. Five-hundred eight genes were identified by a permutation test and a P value <0.01 after a permutation test were identified and selected. These probesets allowed clustering of the samples according to histological diagnosis and WHO grade of malignancy. Invasive and non-or less-invasive low-grade gliomas could be differentiated by hierarchical and coupled two-way clustering. Whole-genome expression analysis of low-grade gliomas identifies subsets of genes differentially expressed in highly and non- or less-invasive tumors.

336. FUNCTIONAL ASSESSMENT OF GBM-DERIVED COMPOSITE BAC CLONES IDENTIFIED USING END SEQUENCE PROFILING
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End sequence profiling (ESP) is a novel technique that reveals structural alterations in tumor genomes such as translocations, inversions, deletions, and amplifications. To accomplish this, both ends of a number of BAC clones from a tumor genome-derived BAC library are sequenced and mapped onto the assembled normal human genome sequence. Recent ESP studies on breast, brain, and prostate tumors and cell lines have identified a number of structurally aberrant genomic clones. While the functional significance of these clones remains to be determined, one provocative hypothesis is that they encode novel composite transcripts important for tumor development, akin to the BCR-ABL fusion gene. Indeed, fusion transcripts have been identified by transcript ESP in breast cancer cell line MCF7. To test whether glioblastoma (GBM)-derived composite BACs are functionally significant for GBM formation, we are testing their effects on proliferation, apoptosis, and motility when transfected into immortalized human astrocytes and brain tumor cell lines. Phenotypic alterations in response to transfection are being measured by using a combination of flow cytometry and high-content automated imaging microscopy. A total of 5 BACs are currently under investigation, each of which has been draft sequenced and fingerprinted in respective collaborations with the Joint Genome Institute and the Genome Sciences Centre at UBC to confirm their composite nature. Results from these studies will be presented at the conference. These studies take an important first step toward discovery of novel tumorigenic mechanisms in brain cancer and potential development of tumor specific markers and therapeutics.

337. DNA HYPERMETHYLATION OF MULTIPLE TUMOR SUPPRESSOR GENES IN DIFFERENT TYPES OF MENINGIOMAS
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Aberrant methylation of CpG islands in promoter of human genes is known as an alternative mechanism of gene silencing that contributes to tumorigenesis in various human tumors. We have examined methylation status of 5 tumor suppressor genes in 81 human meningiomas (61 benign, 12 atypical, and 8 malignant meningiomas) by methylation-specific polymerase chain reaction to determine roles for DNA methylation during
transformation to meningioma. Five tumor suppressor genes including p16, p14, RB, RASSF1A, and E-cadherin showed different profile of methyla-
lylation status on CpG islands in their promoter regions depending on stages of meningioma. Methylation frequencies of tumor suppressor genes in benign, atypical and malignant meningiomas were 42.6%, 66.7%, and 50.0% for p16; 16.4%, 50.0%, and 25.0% for p14; 42.6%, 47.1%, and 37.5% for RB; 1.0%, 8.0%, and 12.5% for RASSF1A; 41.0%, 66.7%, and 12.5% for E-cadherin, respectively. Except RASSF1A, other tumor suppressor genes showed relatively high methylation status in meningioma. Forty-six of six-
ty-one (75.4%) benign meningiomas, 7 out of 8 (87.5%) malignant meningio-
mas, and all 12 cases (100%) of atypical meningiomas showed hypermethy-
lation, as did 32.5% of the test genes. Supernumerary methylation was more frequently in atypical meningiomas than in any other tumor stages: 66.7% for p16, 50% for p14, 41.7% for RB, and 66.7% for E-cadherin. Our results suggest that DNA methylation is a frequent and an early event in tumorigenesis of meningiomas, and it appears to be a major molecular mechanism for meningiomas, especially for atypical type.

338. THE P15INK4B/P16INK4A/RB1 PATHWAY IS
FREQUENTLY Deregulated IN HUMAN PITUITARY ADENOMAS
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Pituitary adenomas are common benign intracranial neoplasms. How-
ever, their tumorgenesis is not yet clearly defined. Inactivation of genes involved in the negative cell cycle regulatory p15INK4b-p16INK4a-cyclin D/CDK4-RB1-mediated pathway (RB1 pathway) is one of the most common and important mechanisms in the growth advantage of tumor cells. Recently, much attention has been focused on the importance of alternative mechanisms of gene inactivation, particularly promoter hypermethylation in the transcriptional silencing of such tumor suppressor genes. Based on the rare occurrence of inactivation by gene mutations and deletions of the RB1 pathway in pituitary adenomas, we investigated the deregulation of the RB1 pathway in 42 sporadic human pituitary adenomas, especially focusing on the methylation status of this pathway as determined by a methylation-
specific PCR assay. Homozygous deletion of the p15INK4b or p16INK4A gene was detected in one adenoma each. Amplification of the CDK4R gene was not apparent in any of the pituitary adenomas presently examined. Promoter hypermethylation of the p15INK4b, p16INK4A, and RB1 genes was detected in 15 (35.7%), 30 (71.4%), and 12 (28.6%) of the adenomas, respectively. Promoter hypermethylation of the p15INK4b gene coincided with p16INK4A alteration and/or RB1 methylation, whereas p16INK4A and RB1 methyla-
tions tended to be mutually exclusive (I = 0.019). Thus, the vast majority of the adenomas (38/42, 90.5%) displayed alterations of the RB1 pathway. None of the clinicopathological features including the proliferation cell index was significantly correlated with any particular methylation status. Our results suggest that inactivation of the RB1 pathway may play a causal role in pituitary tumorgenesis, with hypermethylation of the p16INK4A gene being the most common deregulation, and further that RB1 and p16INK4A methyla-
tions tend to be mutually exclusive but occasionally coincide with p15INK4b methylation.

339. COMPREHENSIVE GENE EXPRESSION ANALYSIS
FOR NEWLY DIAGNOSED PATIENTS (PTS) WITH PRIMARY
CNS LYMPHOMA (PCNSL)
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High-dose methotrexate (MTX)-based chemotherapy in combina-
tion with WBRT for pts with newly diagnosed PCNSL has been shown to improve disease control and survival. However, disease recurrence is common, and treatment-related neurotoxicity is an increasingly recog-
nized complication. The addition of rituximab has improved survival and disease control in systemic non-Hodgkin’s lymphoma. In a phase 2 trial we added rituximab to MTX, procarbazine, and vincristine (R-MPV) and decreased the dose of WBRT in pts who achieved a complete response (CR) after 2 cycles of chemotherapy. Sixteen twelve (12) pts were enrolled from August 2002 to October 2004. Three pts received rituximab as a single agent, 100 mg/m2 on day 1 and MTX 3.5 g/m2 with vincristine 1.4 mg/m2 on day 2. Procarbazine 100 mg/m2 a day was given for seven days during odd-numbered cycles. Pts achieving a CR after 5 cycles received dose-reduced WBRT (2340 cGy) while pts with less than a CR received 2 more cycles, after which they received standard WBRT if a CR was still not achieved. All pts received two cycles of Ara-C 3 g/m2 after WBRT. Sixteen pts were followed with prospective neuropsychological evaluations. CSF + serum levels of rituximab were assayed in pts with an Ommaya reservoir. Fifteen of the 23 treated pts have been assessed for response after R-MPV (five pts are still on study, two pts were taken off study for toxicity including grade 3 nephrotoxicity and death from neutropenic sepsis in one pt each, and data on one pt is still being gathered). Eleven of the fifteen (73%) had a CR, two (13%) a partial response (PR) for an overall response rate of 93% and (two) 13% progressed. The median number of cycles of R-MPV received was five, with four patients needing seven cycles to achieve a CR. The eleven pts with a CR received 4500 cGy and one received 3640 cGy. The most common grade 3 or 4 toxicities were granulocytopenia (34%), hypotension (24%), and hyperglycemia (19%) followed by hepatotoxicity, thrombocytopenia, coagulopathy, hypophosphatemia, hypokalemia, and anemia (10%–15% each). No grade 3 or greater neurotoxicity has been reported to date. The safety and efficacy of R-MPV is comparable to MPV. Further investigation of this regimen in PCNSL is warranted.

340. COMBINED IMMUNOCHEMOTHERAPY WITH REDUCED DOSE WHOLE BRAIN RADIOTHERAPY (WBRT) FOR NEWLY DIAGNOSED PATIENTS (PTS) WITH PRIMARY
ATYPICAL Meningioma (ATM), and it appears to be a major molecular me-
chanism for meningiomas, especially for atypical type.

We were able to identify the gene cluster which varied with malignancy grade by analyzing a gene expression profile with cDNA microarray. This study revealed that the building of a therapeutic strategy against grade IV astrocytomas, which is very difficult to treat, is enabled on the basis of this gene information.

341. VACCINATION OF RECURRENT GLIOMA PATIENTS WITH TUMOR LYSEATE-PULSED DENDRITIC CELLS ELICITS IMMUNE RESPONSES: RESULTS OF A CLINICAL PHASE 1/2 TRIAL
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The objective of this study was to investigate the safety and immuno-
logical responses of dendritic cells therapy pulsed with autologous tumor lysisate in patients with malignant glioma and to examine whether tumor lysisate-pulsed dendritic cells were able to eradicate recurrent malignant glioma (16 and 6 patients with grade 4 and grade 3 gliomas, respec-
tively) entered in the phase 1/2 clinical study of dendritic cell vaccination. All patients were recurrent malignant glioma patients who were resistant to the conventional medical treatment. Twenty-two patients participated in GM-CSF (1000 IU/ML) and IL-4 (300 IU/ML). RPMI-1640 supplemented with 1% autologous serum, pulsed with autologous tumor cell lysate and activated with OK 432 (0.1KE/ml). The mean numbers of vaccinations of these patients were 6.2 times intradermally close to a cervical lymph node and 4.7 times intratumorally via an Ommaya reservoir. Clinical responses were 1 partial response, 2 minor responses, and 8 cases of no change evaluated by radiological findings. Dendritic cell vaccination

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elicited T-cell-mediated antitumor activity, as evaluated by ELISPOT assay after vaccination in 6 of 14 tested patients. This protocol was generally well tolerated. This study demonstrated the safety and antitumor effects of autologous tumor lysate-pulsed dendritic cell therapy for patients with malignant glioma.

Bevacizumab (Avastin) is a monoclonal antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF). CPT-11 is a semisynthetic camptothecin that binds to and inhibits DNA topoisomerase I, an enzyme necessary for DNA replication. This combination of agents has shown clinical benefit in a clinical trial of metastatic colorectal cancer, while bevacizumab alone was associated with inferior survival in the same population. In phase 2 clinical trials of CPT-11 in patients with recurrent glioma, 4/39 pts demonstrated tumor regression. Some malignant gliomas overexpress VEGF, suggesting that bevacizumab may have clinical activity in this population, possibly increasing the response rate over CPT-11 alone. Between March and December 2004, 21 patients with malignant glioma treated with this combination (BEV/CPT-11) were evaluated for treatment response and toxicity. A 6-week course consisted of bevacizumab, 5 mg/kg, every other week × 2, and CPT-11, 125 mg/m² every week × 4, followed by a 2-week rest. MRI scans were obtained after each cycle of treatment. Bevacizumab was not dose-reduced, but CPT-11 doses were reduced for grade 3 or 4 myelosuppression. Of the 21 pts, 11 were GBM and 10 were other high-grade gliomas; median age was 42 (30–73) and median number of prior treatments was 3 (2–10). Toxicities included neutropenia, diarrhea, epistaxis, emesis, and asthenia. One pheo exhibited a transient partial hemorrhage and 2 patients required gastrointestional perforation; both are previously reported toxicities of bevacizumab. Four additional pts died of non-treatment-related complications. Of the 21 pts who could be evaluated for response, there were 1 CR, 8 PRs, and 11 SD. Thirteen pts remain on treatment, 5 after more than 4 cycles. In all pts, radiographic responses were accompanied by reduction in both peritumoral edema and contrast enhancement; most patients who did not meet the criteria for partial response did show clinical and/or radiographic improvement. These early results suggest an improvement in response rate and duration of response over treatment with CPT-11 alone and a possible role for bevacizumab in primary therapy for high-grade glioma.

343. THE FAS/FAS LIGAND PATHWAY FAILS TO INDUCE APOPTOSIS IN EXPERIMENTAL GLIOMA CELLS AND COULD BE RESPONSIBLE FOR THE APOPTOSIS OF T-CELL SURROUNDING MALIGNANT GLIOMA IN VIVO

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Human and experimental gliomas have been shown to express the Fas-receptor (FasR). Activation of this receptor by direct agonists could trigger caspase-3/8-mediated apoptosis. FasR agonist-mediated apoptosis of tumor invading lymphocytes has also been demonstrated. Therefore, the role of Fas-ligand (Fas-L) as an anti-tumor pathway remains controversial. In this study we tested the ability of recombinant-FasL and of a FasR agonist-antibody (Ab) to induce apoptosis in 9L cells, and we explain the tumor immune-evasion in the 9L-gliosarcoma model. Fas expression was determined in 9L, F98, U251, and U373 cells by Western blot. Cytoxicity of recombinant-FasL and FasR agonist-Ab was evaluated in vitro against 9L. Cells were treated with 50, 100, 250, and 500 ng/ml of FasL and FasR agonist-Ab. Percentage of cell viability was established by using the MTT assay. Statistical analysis was done using the Student t-test (P < 0.05 was considered significant). For the in vivo studies, 33 Fischer 344 rats were intracranially implanted with 9L and perfused/fixed on day 10. Tumor samples were processed for immunohistochemical staining and confocal microscopy analysis. Monoclonal antibodies were used against CD3 (Mouse monoclonal, Serotec), FasL (Rabbit polyclonal, Santa Cruz Biotechnology), GFAP (Mouse monoclonal, NeoMarkers; Rabbit polyclonal, Dako), and caspase-3 (Rabbit polyclonal) to identify lymphocytes, FasL, astrocytes, and apoptotic lymphocytes. A robust band at 48 kDa confirmed strong FasR expression in all cell lines tested. Treatment with recombinant-FasL at 50 ng/ml and 500 ng/ml decreased cell viability to 98% ± 0.03% and 95% ± 0.03%, respectively. Similarly, treatment with FasR agonist-Ab at 50 ng/ml and 500 ng/ml decreased cell viability to 95% ± 5% and 96% ± 1%, respectively. Percentage of growth was statistically insignificant for the tested concentrations. Compared to controls, animals intracranially challenged with 9L exhibited significantly higher expression of FasL, caspase 3, and CD3+ cells. Apoptotic CD3+ cells were identified by co-localization of CD3 and caspase 3. CD3+ cells were identified peri- and intra-tumorally by distinctive clustering patterns in the center of the tumor. Although FasR was strongly expressed in all cell lines tested, the treatment with FasL and FasR agonist-Ab failed to induce apoptosis. Under these experimental conditions, both FasR agonist-Ab and recombinant-FasL are ineffective in treating brain tumors. Rats intracranially implanted with 9L gliosarcoma exhibit marked upregulation of Fas-ligand in GFAP + tumor cells. The presence of apoptotic CD3+ cells suggests that a Fas-ligand-mediated immune-evasion mechanism is responsible for T-lymphocyte apoptosis in the 9L gliosarcoma model.

344. GPNMB: A NEW TARGET FOR HUMAN HIGH-GRADE GLIOMAS (HGL) IMMUNOTHERAPY

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Targeting neoplastic HGL cells for treatment with monoclonal antibody (Mab) constructs requires makers expressed on the cell surface of gliomas but not normal brain. We here identified promising new HGL tumor targets, human transmembrane glycoprotein nmb (GPNMBwt), and a splice variant form (GPNMBsv) leading to an in-frame insertion of 12 amino acids in the extracellular domain (ecd). We have performed genetic and immunohistochemical (IHC) evaluation of human gliomas to determine incidence, distribution, and pattern of localization of GPNMB antigens in brain tumors as well as survival analyses. We have generated anti-GPNMB antibodies by immunizing rabbits and mice with plasmid DNA coding the ecd of GPNMB, followed by boost with corresponding recombinant proteins. To determine the GPNMB mRNA transcript and protein expression levels in glioma samples, we have performed quantitative RT-PCR (49 cases) and IHC (140 cases) in HGL patient cases and glioma GPNMB transfected cell lines. We also characterized five monoclonal antibodies using Western blotting, BIAcore, Scatchard, and FACS analyses. Survival analyses were also conducted based upon IHC information from 80 newly diagnosed patients (63 glioblastoma multiforme [GBM] and 17 anaplastic astrocytomas [AA]) and the RNA expression data from 29 newly diagnosed patients (26 GBM and 3 AA). The real-time PCR results from 49 HGL biopsies indicated 31/36 (86%) GBM, 3/6 (50%) AA, and 3/7 (43%) anaplastic oligodendrogliomas (AO) were positive for gpnmRNA transcripts (gpnmB６, plus gpnmBsv). gpnmBsv was detected in 16/36 (44%) GBM and 4/6 (67%) AA. In normal brain samples, little or no expression was noted for GPNMBw, and GPNMBsv mRNA. We have obtained and characterized anti-GPNMB rabbit polyclonal rabbit antisera #2640 as well as two IgG Mabs, G11 and A3, and three IgG1 Mabs, U2, U4, and U5. The binding affinity constant Kd of Mabs to GPNMB was 2.7 to 96 × 10^9 M^-1 and 7.6 to 47 × 10^9 M^-1 by BIAcore and Scatchard analysis, respectively. A larger HGL study (n = 140) revealed detectable GPNMB by IHC with antisera #2640 and MAb G11 in a membranous and cytoplasmatic pattern, with occasional focal perivascular reactivity in 21/32 (66%) AA and 75/108 (70%) GBM. Quantitative flow cytometric analysis of 11 GPNMB positive GBM fresh biopsy specimens revealed GPNMB cell surface molecular density at 1.1 to 7.8 × 10^10 molecules/cell, levels sufficient for Mab targeting. The survival analysis demonstrated newly diagnosed AA/GBM patients over the age of 45 have a higher risk of death (hazard ratio of 2.5). Significantly, in this population (AA/GBM or GBM alone) univariate analyses show that patients with relative mRNA transcript levels greater than 3-fold over normal brain also have a higher risk of death (hazard ratio of 5 to 7). Increased mRNA levels correlated with higher survival risk and elevated protein expression in HGL biopsy samples, combined with the detection of surface membrane proteins in glioma cells to indicates that GPNMB is a valuable tumor-associated antigen in immunotherapeutic approaches for malignant gliomas.
345. IMMUNIZATION WITH AUTOLOGOUS GLIOMA CELLS TRANSFECTED WITH IFN-G GENE SIGNIFICANTLY PROLONGS SURVIVAL IN GBM-PATIENTS OLDER THAN 50 YEARS. L. Salford,1,2 E. Ask,1 P. Sjesö,1,2 G. Skagerberg,1,2 C. Bärués-Koch,2,4 C. Blennow,1,2 A. Darabi,1,2 C. Efregren,1 E. Englund,1 S. Janelidze,1 E.-M. Larsson,1 Å. Lilja,1 B.R.R. Persson,1 A. Rydelius,1,3 S. Strömblad,1,12 F. Visse,1,2 and B. Widgren1,2,1 Department of Neurosurgery, 2The Rausing Laboratory, and Departments of 1Neurology; 3Medical Radiation Physics, 4Neuropsychology, 5Neuropathology, 6Neuroimmunology, and 7Tumor Immunology, Lund University, Lund, Sweden

Based upon earlier experimental work by our group, we have completed the first part of a human immunogen therapy study of patients with GBM. The main goal of the study is to determine whether immunization for a glioblastoma tumor cell line, designated to express antigenic markers, can be of benefit to glioblastoma patients. A glioblastoma cell line was derived from a patient who died after 23 months and one is still alive after 26.5 months and one is still alive after 23 months with a minor tumor resection. We have performed 10 immunizations, and 1 had eight and 1 more than six immunizations. The immunization takes place in the dermis of the upper arm. Seven days after each immunization, a skin biopsy is taken from the centre of the injection sites. The composition of the cellular infiltration in the skin is studied by markers for T lymphocytes (CD3); helper, subset of T cells (CD4); cytotoxic T-cells, subset of T cells (CD8); natural killer cells (CD16); and B lymphocytes, B cells (CD20). Also, the number of functional T cells is studied: IL-2, IL-4, IL-10, IL-12, IL-18, TNF-a, TNF-b, IFN-g and TGF-b1, 2, and 3. Peripheral blood is sampled both before and after each immunization after each immunization event. Co-culture of lymphocytes from immunized patients with glioma cells both in vitro and in vivo, data, of D2C7 by glioma-bearing Fischer rats intravenously or into the carotid artery. After 24 h, the rats were sacrificed and fresh frozen sections of the brains searched for fluorescent macrophages. Sphingosin were infiltrated regularly by monocytes in vitro and tumor tissue. The monocytes expressed MR8, MAC387 (MRP14), and 258 IgM. In the rat glioma model, accumulation of the marked monocytes was observed 24 h after injection into the peripheral blood. The experimental model presented here allow us to analyze interactions between monocytes and glioma cells both in vitro and in vivo. A better understanding of these interactions may help to overcome the inactivation of the monocytes' tumor response by glioma cells.

346. PRE-CLINICAL EVALUATION OF D2C7, A MONOCLONAL ANTIBODY REACTIVE FOR BOTH THE WILD TYPE AND VARIANT III MUTANT EPIDERMAL GROWTH FACTOR RECEPTOR, FOR RADIOTHERAPY OF MALIGNANT GLIOMAS. A. Bokovský, C. Pegram, K. Peixoto, M.R. Zalutsky, D.D. Bigner1, Departments of 1Pathology and 2Radiology, Duke University Medical Center, Durham North Carolina, USA

The epidermal growth factor receptor (EGFR) is expressed by normal epithelial cells in tissues but overexpressed in 70%–94% and 60%–90% of anaplastic astrocytomas and glioblastomas (GBM), respectively. In GBM, 71% of GBM also expressed EGFR variant III (EGFRvIII), which is not found on normal tissues. Monoclonal antibodies (mAbs) targeting either the wild-type EGFR (wt) or EGFRvIII have been developed, and one of them (Mab-425) has been introduced in radioimmunotherapy trials for patients with GBM. EGFRvIII is a preferred target because of its higher tumor specificity, some glioma cells may express only wt. Herein, we evaluated the biologic properties of D2C7, a novel mAb specific for both wt and EGFRvIII, with the hypothesis that it could serve as a more generally applicable radiodendal drug carrier for the targeted radiotherapy of patients with malignant gliomas. D2C7 was generated by using hybridomas reacting with the extracellular domain (ecd) of wt or EGFRvIII. The affinity for wt and EGFRvIII was determined by using a plasmid resonance experiments compared the tissue distribution of 125I-labeled D2C7 and 131I-labeled anti-wt MAb EGFR1 (Exp. 1), 131I-labeled control MAb P588 (Exp. 2 and 4), or 131I-labeled anti-EGFRvIII MAb L8A4 (Exp. 3) in a subcutaneous xenograft of the human glioma cell line H157. EGFRvIII was normalized to the expression obtained from an RNA pool of 4 normal brain tissues. Ratios (sample RNA level/normal brain RNA level) above 2 were considered as positive. Frequencies of expression were observed as follows: ALK, 8%; NA-17A, 8%; MAGE-A3, 12%; TRP-2, 38%; IL13Rα, 8%; IL13Rβ, 8%; NA-17A, and MAGE-A3, by Q-PCR (quantitative-polymerase chain reaction). All these antigens have previously been identified in brain tumor, often in small series of various histologies. In addition, they all contain HLA-A2-restricted CD8+ T-cell epitopes in their sequence. All GBMs were considered as positive. Frequencies of expression were observed as follows: ALK, 8%; NA-17A, 8%; MAGE-A3, 12%; TRP-2, 38%; IL13Rα, 8%; IL13Rβ, 8%; NA-17A, and MAGE-A3, by Q-PCR (quantitative-polymerase chain reaction). All these antigens have previously been identified in brain tumor, often in small series of various histologies.
As effective antigen-presenting cells, dendritic cell (DC)-based hybrid vaccine has shown much promise in cancer therapy. But there have been few approaches in human glioma treatment, and it is an area of research to be addressed in our country. In this study, DC-glioma fusion cells (FC) were prepared by the fusion of human glioma cells and the DC derived from peripheral blood mononuclear cells of the same patients. In vitro anti-glioma activity induced by FC was studied. The FC were prepared in the presence of polyethylene glycol, the proliferation capacity of a patient’s T cells stimulated with autologous glioma lysate showed a significant enhancement of cytotoxic reaction, and the cytotoxicity against autologous glioma cells by the patient’s T-cells primed with FC was also assessed by lactate dehydrogenase (LDH) assay. The FC preparation method was constructed. The patient’s T cells primed with irradiated (1.5 Gy) FC induced a more remarkable killing than the T cells primed with DC or autologous glioma cells (P < 0.01), and the cytolytic effects were up from 28% to 90% as the effector-target ratio increased. But irradiated FC primed T cells only induced about 0.01%, and the cytolytic effects were up from 28% to almost 100%. The general tendency is for malignant cells to be present in cultures in large numbers initially but rather for normal cells to outnumber the malignant cells quickly. Later on, the normal cells cease to grow, and the tumor cells become dominant. The time elapsing between biopsy and development of a cell culture in which the majority of the cells are malignant varies from a few weeks to more than six months. The low frequency of tumor cells in cultures at certain stages makes the cultures unsuitable at such points for immunogene therapy with use of autologous tumor cells. We also demonstrate efficient transfection results with the use of adenovirus expressing human interferon gamma and green fluorescent protein.

In order to improve survival of patients with a GBM, new therapeutic strategies must be developed. The use of a death-inducing ligand such as TRAIL (TNF-related apoptosis-inducing ligand) seems a promising innovative therapy. The aim of this study was to quantify the expression of the death regulating receptors TRAIL-R1 and TRAIL-R2, and the TRAIL ligand on primary GBM tumor specimens and to correlate this expression with survival. Sixty-two tumor specimens were taken from patients who had had a craniotomy with debulking of a primary GBM. Expression of TRAIL and TRAIL receptors was assessed by immunohistochemistry, both quantitatively (% of positive tumor cells) and semi-quantitatively (staining intensity) within both perinecrotic and intermediate tumor zones. RT-PCR of GBM tumor tissue and controls (other astrocytic tumors and normal brain) was performed to show expression of TRAIL receptor mRNA. Immunohistochemistry showed a slight diffuse intracytoplasmic and a stronger membranous staining for TRAIL and TRAIL receptors in tumor cells. Semi-quantitative expression of TRAIL showed a significantly higher expression of TRAIL in the perinecrotic area than in the intermediate zone of the tumor (P < 0.0001). A mean of 13% TRAIL positive tumor cells was found. A positive correlation was found between TRAIL expression (semi-quantitative) and survival (P = 0.008). Mean tumor cell positivity for TRAIL-R1 was 19%. A positive correlation was found between survival and the percent of TRAIL-R1 positive tumor cells (P = 0.049). Mean TRAIL-R2 expression was 27%. TRAIL-R2 expression was significantly higher expressed than TRAIL-R1 (P = 0.005). A positive correlation was found between TRAIL-R1 and TRAIL-R2 expression (P = 0.002). RT-PCR analysis detected a negative relation between the amount of TRAIL-R1 mRNA and the WHO grade of astrocytic tumors (P = 0.005). Immunohistochemistry and RT-PCR show a positive correlation between TRAIL-R1 expression and survival, and therefore TRAIL-R1 expression seems to be a prognostic indicator. TRAIL-R2 is significantly more expressed within tumor tissue than TRAIL-R1. Therefore TRAIL-R2 is of more importance as a target for future TRAIL therapy than TRAIL-R1. However, the way the death-inducing signal through the TRAIL-R2 receptor is regulated differs from TRAIL-R1 signaling. This has to be taken into account when developing new therapeutic strategies using TRAIL.
Investigated the immunogenicity of an exogenous gene product in a murine K. Reilly
or RelA, and stably transfected clones were established and confirmed by
Future therapeutic strategies will need to address pervasive immunosup-
change the expression of any cytokines in normal brain, but induced a
mRNA levels, they also demonstrated an increase in TGF-β2 expression
an increase in IL-10 (363-fold), IFN-γ (48-fold) and IL-1β (only 8-fold)
by semi-quantitative real-time PCR 24 h after injections with CpG DNA,
immune stimulation, normal tissue and RG2 tumor tissue were analyzed
sue. To investigate changes in baseline cytokine expression following
interferon-gamma (IFN-γ), or IFN-γ with lipopolysaccharide (IFN/LPS). CpG-
by trypan blue and TUNEL. The expression of Bcl-2, Bfl-1, and RelA overexpression is an effective means of protecting T lymphocytes from apoptosis mediated by GBM. Furthermore, the anti-
dexferoozymes may be useful in vivo as an adjunct drug in GBM immunotherapy for promoting T-cell survival and function.

353. CYTOKINE EXPRESSION IN RG2 GLIOMAS FOLLOWING TREATMENT WITH CPG DNA, INTERFERON-GAMMA, AND INTERFERON WITH LPS
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The efficacy of immunotherapies against malignant gliomas is likely limited by the immunosuppressive tumor environment. Our lab has previ-
ously shown that intracranial RG2 gliomas demonstrate high expression of both immuno-stimulatory (IL-1β and IFN-γ) and immunosuppressive cytokines (IL-10 and TGF-β1) mRNA as compared to normal brain tissue. To investigate changes in baseline cytokine expression following immune stimulation, normal tissue and RG2 tumor tissue were analyzed by semi-quantitative real-time PCR 24 h after injections with CpG DNA, interferon-g (IFN-g), or IFN-g with lipopolysaccharide (IFN/LPS). CpG-
jected normal brain tissue demonstrated a 21-fold increase in IFN-γ and IL-1β and an 1160-fold increase in IL-10 mRNA as compared to sham-
treated normal brain. TGF-β1 or TGF-β2 expression did not change in these samples. Although CpG-treated RG2 tumors also demonstrated an increase in IL-10 (363-fold), IFN-γ (48-fold) and IL-1β (only 8-fold) mRNA levels, they also demonstrated an increase in TGF-β2 expression (10-fold). Treatment of normal brain or RG2 tumors with IFN-γ did not change the expression of any cytokines in normal brain, but induced a
5.6-fold increase in TGF-β2 mRNA in RG2 tumors. IFN/LPS treatment increased the expression of IFN-γ, IL-1β, and IL-10 in normal brain (13.9-, 34-, and 15-fold increases over sham-treated normal brain). However, these effects were limited in RG2 tumors, with no change in IFN-γ expression and only a 6-fold increase in IL-1β expression compared to sham-treated tumors.

In a clinical study glioma patients were immunized intraurally in the right arm with autologous glioma cells secreting IFN-γ, peripheral blood leucocytes (PBL) and immune infiltration at the immunization site were studied to monitor the patients’ immune response. Before and during the 18- to 30-week immunization schedule, blood samples were collected, and PBL were restimulated with tumor cells in vitro for 5 days. The restimulated cells were assayed for lymphocyte subset distribution by flow cytometry, cytokine production by flow cytometry, and ELISPOT and proliferation by thymidine incorporation. Immunohistochemically stained tissue sections from the immunization site were analyzed for lymphocyte infiltration. Blood samples from 5 patients were analyzed and compared with survival data, immune infiltration, and DTH reactions at the vaccination site. No correlation or discernable pat-
tern could be observed from proliferation and lymphocyte subset analyses.
Increased IFN-γ mRNA levels measured by ELISpot were seen in 2/5 patients with a prolonged survival. A decrease in IFN-γ mRNA production was observed during tumor recurrence. Detectable levels of IL-10 were observed displaying a weak association with shorter survival. Lymphocyte cytokine production did not correlate with lymphocyte infiltration or DTH reac-
tions at the immunization site. Compared to control patients, immunized patients had a mean prolonged survival of 170 days. No clear correlation between immune cell influx at the immunization sites and cell lysis activity or prolonged survival could be found. The mean survival time of patients immunized with IFN-γ-secreting tumor cells is prolonged by
3.5 months. The two patients with the longest survival time also had an increased number of IFN-γ-secreting producing lymphocytes.

354. IMMUNE REJECTION OF GLIOMA IN A MURINE MODEL
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Viral vectors encoding therapeutic genes are being investigated as novel agents for glioma therapy. These candidate therapeutic genes may modulate the immunogenicity of viral vectors and affect vector efficacy to ensure tumor growth independent of viral activities. We investigated the immunogenicity of an exogenous gene product in a murine model using ovalbumin (OVA) to study whether an exogenous antigen presented in conjunction with an immuno-modulated lytic lesion of glioma cells lines was established by transfection and cloning of KR-158 glioma cells (H-2b); OVA expression was confirmed by immunofluorescence and West-
ern blotting. The tumorigenicity of wild-type, low- and high-expressing OVA-transfected glioma cells to induce subcutaneous tumors in syngenic C3H/B10 mice was compared. Although as few as 104 wild-type KR-158 glioma cells generated ultimately lethal bulky fl ank tumors. Unlikely flask of GM gangliosides. The H-2k T cell line was transfected with plasmids expressing the gene for Bcl-2, Bfl-1, or RelA, and stably transfected clones were established and confirmed by quantitative PCR and Western blot analysis. Five GBM cell lines were co-cultured with wild-type Jurkat cells and Jurkat cells over-
expressing the various transgenes. Jurk cells apoptosis was measured by trypan blue and TUNEL. The expression of Bcl-2, Bfl-1, and RelA was measured by quantitative PCR and Western blot in wild-type Jurk cells after co-culture with the apopritic GBM cell lines. Expression levels in GBM cell lines induced high apoptosis of wild-type Jurkat cells and resting or activated T cells in co-culture experiments. The expression levels of Bcl-2, Bfl-1, and RelA were subsequently reduced in the wild-type Jurk cells following co-culture with the apoptritic GBM cell lines. Overexpression of Bcl-2, Bfl-1, and RelA, however, conferred protection by at least 60% against the four apoptritic GBM cell lines. As far as drug protection, all three achieved some protection. However, dexferoozymes may be useful in vivo as an adjunct drug in GBM immunotherapy for promoting T-cell survival and function.

355. INCREASED NUMBER OF LEUKOCYTES SECRETING IFN-GAMMA IN PATIENTS IMMUNIZED WITH AUTOLOGOUS IFN-GAMMA SECRETING GLIOMA CELLS CORRELATE WITH PROLONGED SURVIVAL
In a clinical study glioma patients were immunized intraurally in the right arm with autologous glioma cells secreting IFN-γ, peripheral blood leucocytes (PBL) and immune infiltration at the immunization site were studied to monitor the patients’ immune response. Before and during the 18- to 30-week immunization schedule, blood samples were collected, and PBL were restimulated with tumor cells in vitro for 5 days. The restimulated cells were assayed for lymphocyte subset distribution by flow cytometry, cytokine production by flow cytometry, and ELISPOT and proliferation by thymidine incorporation. Immunohistochemically stained tissue sections from the immunization site were analyzed for lymphocyte infiltration. Blood samples from 5 patients were analyzed and compared with survival data, immune infiltration, and DTH reactions at the vaccination site. No correlation or discernable pat-
tern could be observed from proliferation and lymphocyte subset analyses.
Increased IFN-γ mRNA levels measured by ELISpot were seen in 2/5 patients with a prolonged survival. A decrease in IFN-γ mRNA production was observed during tumor recurrence. Detectable levels of IL-10 were observed displaying a weak association with shorter survival. Lymphocyte cytokine production did not correlate with lymphocyte infiltration or DTH reac-
tions at the immunization site. Compared to control patients, immunized patients had a mean prolonged survival of 170 days. No clear correlation between immune cell influx at the immunization sites and cell lysis activity or prolonged survival could be found. The mean survival time of patients immunized with IFN-γ-secreting tumor cells is prolonged by
3.5 months. The two patients with the longest survival time also had an increased number of IFN-γ-secreting producing lymphocytes.

356. IL-13 RECEPTOR ALPHA 2 PEPTIDE ANALOGUES INDUCE HIGHER LEVELS OF IL-13 RECEPTOR ALPHA 2 (345-353) SPECIFIC CD8+ TLYMPHOCYTE RECOGNITION OF GLIOMA PATIENT-DERIVED CD8+ CELLS AND HLA-A2 TRANSGENIC MICE
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Restricted and high-level expression of interleukin-13 receptor alpha 2 (IL-13Rα2) in a majority of human malignant gliomas makes this pro-
tein an attractive vaccine target. We have previously described identification of IL-13Rα2 (345-353) peptide as an HLA-A2-restricted cytotoxic T lymphocyte (CTL) epitope. However, as it remains unclear how efficiently peptide-based vaccines can activate specific CTLs in HLA-A2+ patients with malignant gliomas, we have examined whether peptide-analogues can be used for optimal expansion and activation of IL-13Rα2 specific CTL. We synthesized three IL-13Rα2 (345-353) analogues. OVA-expressed analogues of the carboxyl terminal isocline (I) for valine (V) and the amino terminal tryptophan (W) for either alanine (A), glutamic acid (E) or non-substituted
that showed statistical significance was serum IL-12 levels where there was 0.0 pg/ml), and 33% of metastatic tumors at 0.0 pg/ml. The only factor these findings indicate that highly antigenic IL-13R

D. ALTERATION IN THE TH1 AND TH2 CYTOKINE D. ALTERATION IN THE TH1 AND TH2 CYTOKINE D. ALTERATION IN THE TH1 AND TH2 CYTOKINE D. ALTERATION IN THE TH1 AND TH2 CYTOKINE

357. ENHANCED ANTI-TUMOR IMMUNE RESPONSE AFTER SELECTIVE INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN RATS CARRYING INTRACEREBRAL GLIOMA CELLS IMMUNIZED WITH INFN-GAMMA SECRETING GLIOMA CELLS


In this study we aimed to clarify whether IFNg could enhance anti-tumor immune responses. We investigated both an iNOS selective, L-N^3-(aminoethyl)benzyltetrahydroisoquinoline (L-NNAME), and a non-selective, L-N^3-(aminoethyl)methyl ester (L-NNAME), inhibitor of NOS. Both L-NIL and L-NNAME were able to inhibit NO production and enhanced the proliferation and production of IFNg from polyclonally activated spleen cells in vivo. However, L-NIL had a broader window of efficacy and a lower minimal effective dose. In correlation to in vivo results, only spleen cells from rats treated with L-NIL, and NOT L-NNAME, had an enhanced proliferation as well as IFNg production in response to tumor cells. Furthermore, L-NIL was able to prolong the survival of rats with intracerebral tumors after therapeutic immunizations with tumor cells secreting IFNg. These results imply that selective inhibition of iNOS can enhance anti-tumor immune responses evoked by IFNg treatment.

358. ALTERATION IN THE TH1 AND TH2 CYTOKINE PROFILE IN PATIENTS WITH BRAIN TUMOURS

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The objective of this study was to evaluate the TH1 and TH2 cytokine profile in patients with brain tumors. IL-12 is a key cytokine mediating a TH1-type response, and IL-10 is a TH2-type pleiotropic cytokine. These two cytokines act in an antagonistic fashion, with a balance point that varies between the physiological and pathological state (O’Hara et al., Clin. Cancer Res. 4, 1943, 1998). The study was designed as a prospective, non-randomized study; we analyzed the cytokine profile in patients with brain tumors to compare changes in serum IL-10 and IL-12 levels in patients with newly diagnosed brain tumors. An initial cohort of 65 patients aged 24 to 78 years were recruited between December 2002 and December 2003 following radiological diagnosis of a space-occupying lesion. Serum samples were obtained prior to surgery. Age and sex matched control patients (n = 36) were also recruited. Serum IL-10 and IL-12 were measured by quantitative ELISA (BioSource International). There were 12 benign, 5 low-grade, 10 high-grade, and 18 metastatic tumors. IL-12 was detected in only 47% of the controls (median [m] 0.0 pg/ml). IL-10 was detected in 92% of patients with benign tumors (m = 2.4 pg/ml), 60% of low-grade tumors (m = 0.0 pg/ml), 67% of high-grade tumors (m = 0.1 pg/ml), and 78% of metastatic tumors (m = 0.3 pg/ml). Conversely, serum IL-12 was detected in all patients in the control group (m = 52.2 pg/ml). However, IL-12 was only detected in 67% of benign tumors (m = 12.1 pg/ml), 80% of low-grade tumors (m = 41.1 pg/ml), 85% of high-grade tumors (m = 0.0 pg/ml), and 33% of metastatic tumors at 0.0 pg/ml. The only factor that showed statistical significance was serum IL-12 levels where there was reduction in the high grade and metastatic group compared with controls (P < 0.001). The TH1-like cytokine IL-12 decreased significantly as tumor stage progressed; however no increase in the antagonistic TH2-like cytokine IL-10 was observed.


We have previously demonstrated that injection of murine central nervous system (CNS) tumors with dendritic cells (DC) secreting interferon (IFN)-α (DC-IFNα) remarkably enhanced the therapeutic effect of peripheral vaccinations with ovalbumin (OVA)-specific T cell epitopes. The injected DC-IFNα migrated from the CNS tumor site to the draining cervical lymph nodes (CLNs), where they cross-primed tumor antigen-specific CTLs (Okada et al., 2004). To further determine the effect of this "prime and boost" vaccine-strategy on the effector cell function, we employed adoptive transfer of OVA-specific type I CD8 effector T (Tc1) cells derived from OVA-specific T cell receptor transgenic OT-1 mice. In addition, to further understand molecular events induced by the IFN-α delivery in the CNS tumors, we analyzed the gene-expression profiles within the tumor microenvironment using Codelink Mouse 20K microarray system. Syngeneic C57BL/6 mice bearing day 5 OVA-transfected B16 melanoma (M05) in the CNS received intratumoral (i.t.) delivery of DCs ex vivo transfected with adenoviral murine IFN-α or control vector Δ3. On day 6, these mice also received intravenous injections with 5 × 10^6 Tc1 cells. DC-IFNα induced expression of IFN-inducible protein (IP)-10, which is a potent chemokine for type I immune effector cells, from M05 cells. Tc1 cells also expressed CXCR3, which is a primary receptor for IP-10 and a high level of CD44 activation marker. As expected from these observations, mice treated with i.t. DC-IFNα and i.v. Tc1 injections resulted in prolonged survival of the host animals and accumulation of higher numbers of OVA-tetramer + CD8 + cells in the cervical lymph nodes in comparison to the control treatment mice that received i.t. DC-5 and i.v. Tc1. Microarray analyses of the CNS tumors indicated that local IFN-α expression upregulated a variety of immune-regulatory molecules such as major histocompatibility complex (MHC) class I chains, a DC maturation factor 2, 5 oligoadenylate synthetase and T cell activation marker CD69, suggesting that local IFN-α expression enhances antigen presentation processes and activation of effector T cells within the tumor microenvironment. In addition, interleukin (IL)-23 has been recognized as a critical mediator of chronic inflammatory responses in the CNS, we evaluated the effect DC-mediated delivery of IL-23 to the CNS tumors. Although IL-23 did not induce IP-10 from the tumor cells or IFN-γ from the Tc1 cells, tumor cells from IL-23 and Tc1 treated mice demonstrated a remarkably increased number of OVA-tetramer + CD44 + cells in comparison to the control mouse and IFN-α-treated animals, suggesting that IL-23 has a unique ability to attract antigen-specific effector cells to the tumor via distinct mechanisms than IFN-α. These results warrant further evaluation of a combined approach with IFN-α and IL-23 delivery to CNS tumors.

360. PILOMYXOID ASTROCYTOMA: A RARE BUT CLINICALLY SIGNIFICANT SUBGROUP OF OPTIC PATHWAY GLIOMA

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Optic pathway/hypothalamic gliomas occur mostly in children, belong to the histological subgroup of pilocytic astrocytomas (PA), and take highly variable clinical courses during adolescence and later in adulthood. It has been stated that the pilomyxoid variant (pPAx) is more aggressive and more malignant. We review a series of 59 cases of recent pediatric and adult cases including three pPAx and specifically focus on one particular case of pPAx and its remarkable clinical and neuroradiological course. Between 2000 and 2004, 37 children and 22 adults were operated with intracranial PAs of which 12 were optic pathway PAs (20.1%). Of the 8 pediatric cases, 3 were limited to the optic nerves, chiasm, and retrochiasmatic “extrachiasmatic” area of the optic tract. Two cases expanded into the hypothalamus/third ventricle, and three were localized to the optic tract. In the adult population, only two cases were related to the optic system, one being optochiasmatic with extension into the third ven
tricle and one originating from the optic tract with mainly temporal extension. As for the neuroradiological growth pattern, three groups of tumors can be distinguished in our group: (1) solid, non-cystic, mostly extrace- rebral polymorphous neoplasms; (2) cystic intraparenchymal tumors; and (3) solid, non-cystic inhomogeneously enhancing tumors. A case of pmPA was found among the latter group, diffusely spreading from throughout the hemisphere and the brainstem. Within this tumor, strongly enhancing nodules with rapid growth appeared at different times and places requiring transylvian and transventricular decompression, respectively, for neuro- logical symptoms. These nodules both turned out to be of the pilomyxoid subtype and also during surgery were clearly distinct, being of a greenish jelly-like texture. The complete removal of the local control while new nodules rapidly occurred. The pilomyxoid variant of PA seems to harbor cell clones that rapidly expand despite absence of any signs of anaplasia. They appear to have limited invasive capacity because even within the diffuse tumor, they can be localized after a few years. The highly diverse behavior of the different compartments of this tumor allow recommendation of aggressive treatment either by microsurgery or interstitial radiosurgery for these spherical nodules which determine the poorer outcome of these cases.

361. DUMBELL TRIGEMINAL SCHWANNOMA IN A CHILD: COMPLETE REMOVAL BY A ONE-STEP PTERIONAL SURGICAL APPROACH

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The objective of our study was to describe a rare case of a trigeminal schwannoma in a child and the surgical procedure performed for therapy. A six-year-old girl presented with tiredness, pain in the neck region, gait disturbances, and dysarthric speech. She did not have any skin manifestations like café au lait spots or subcutaneous tumors. Family history did not suggest familial neurofibromatosis. Imaging studies revealed a unilateral dumbbell-shaped tumor, both extending in the middle and posterior fossa, centered over Meckel’s cave. A one-stage surgery was performed by pterional craniotomy. The tumor was first debulked in the middle fossa, then peeled from the wall of the cavernous sinus, followed by extirpation of the tumor from the posterior fossa. The tumor extended to the caudal cranial nerves, and was completely removed. Trigeminal fascicles were distributed throughout the tumor. Histopathological examination revealed a schwannoma. Trigeminal schwannoma is a rarely occurring tumor in childhood, only nine cases having been reported in the literature thus far. Furthermore, although several often multi-staged surgical strategies for dumbbell tumors are described, in this patient the tumor was eradicated by a one-stage pterional approach.

362. ATYPICAL TERATOID/RHABDOID TUMORS: A NEW MALIGNANT PEDIATRIC BRAIN TUMOR TO BE DISTINGUISHED FROM MEDULLOBLASTOMA/PNET

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The clinicopathological features of atypical teratoid/rhabdoid tumor (AT/RT), a new entity among malignant pediatric brain tumors, and the differential diagnosis from primitive neuroectodermal tumor (PNET)/medulloblastoma are described. The clinicopathological features of 140 AT/RTs including 7 Japanese cases, 70 cases of Mayo Clinic and American Pediatric Oncology Group, and 63 published cases subject to analysis included patient age and sex at presentation, symptoms, neurologically signs, tumor location, histopathological features and outcome. The patients with AT/RT aged from 22 days to 14.9 years. There was a 1.5:1 male predominance. The clinical symptoms were related to tumor location and usually manifested at an early stage. Of the 140 AT/RTs, 61% were located in the posterior fossa, 20% in the cerebral hemispheres, 5% in the suprasellar and/or third ventricular regions, 5% in the pineal region. Histologically, AT/RT is defined as a polymorphous neoplasm often featuring rhabdoid, PNET, epithelial, and mesenchymal components. AT/RTs usually include PNET, Nilson's, and MIB-1 revealed a correlation with the survival. Our data indicate that despite optimal treatment with surgery, radiation therapy, and adjuvant chemotherapy, the prognosis for patients with medulloblastoma remains dismal. Further clinical studies will need to address the exploration of more aggressive therapeutic strategies in combination with conventional craniospinal irradiation and intensive platinum-based chemotherapy.

363. THERAPEUTIC EFFICACY AND PROGNOSTIC FACTORS IN MEDULLOBLASTOMAS

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Medulloblastomas are the most common central nervous system malignancies in children. Combined modality treatment with radiation and che- motherapy has substantially improved disease control and extended patient survival. Nevertheless, medulloblastoma remains a treatment challenge, and considerably subjective controversy exists about the best therapeutic management for patients with such tumors. In the present study, we retrospectively analyzed a series of 21 patients with newly diagnosed medulloblastomas treated by surgery, radiation, and adjuvant chemotherapy. For the entire study popula- tion, the median overall survival (OS) was 56 months, with 5-year OS rate of 47%, and the median disease-free survival (DFS) was 41 months, with 5-year DFS rate of 44%. Radical surgery, low-stage according to the Chang Staging System, and platinum chemotherapy were significantly associated with longer DFS and/or OS. Patients who were less than 3 years of age exhibited a tendency toward a shorter survival. None of the immunohis- tochemical markers including GFAP, S-100, NSE, synaptophysin, TrkA, TrkB, and MB-1 revealed a correlation with the survival. Our data indi- cate that despite optimal treatment with surgery, radiation therapy, and adjuvant chemotherapy, the prognosis for patients with medulloblastoma remains dismal. Further clinical studies will need to address the exploration of more aggressive therapeutic strategies in combination with conventional craniospinal irradiation and intensive platinum-based chemotherapy.

364. PEDIATRIC OLGODENDROGLIOMA IN THE MOTOR STER

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We present the case of an eight-year-old boy who presented with focal seizures. MRI showed a mass (diameter 4 cm) in the left motor cortical area with slight contrast enhancement on the floor of left sulsus centralis and no contrast enhancement in the motor strip. Subtotal surgical resection was guided by intraoperative ultrasound and intraoperative CT. Direct bipolar cortical electrophysiological mapping informed the neurosurgeon about the motor functionality of the exposed structures, which were infiltrated by tumor tissue. Finally, the contrast medium enhanced tumor part could be resected due to combined use of intraoperative imaging and intraoperative electrophysiology. Tumor within the motor strip was spared to avoid func- tional impairment. Although Ki-67 staining showed elevated proliferative activity, a low-grade oligodendroglioma (WHO grade II) was diagnosed after histomorphological examination. Loss of heterozygosity (LOH) anal- ysis was carried out using microsatellite markers and PCR based assay. No allodic losses on chromosome arms 1p and 19q were shown. Post-surgery the boy presented with paresthesia and mild motorc function disability of the right hand, which diminished after a few days. The focal seizures of the right arm persisted after surgery. Postoperative MRI (after 3 and 11 months) demonstrated a stable, remaining T2 extension in the motor strip. There were no signs of tumor progression. As a consequence, the patient received no further adjuvant chemotherapy.

365. PEDIATRIC NEURO-ONCOLOGY FROM 2500 BC TO NOW: A HISTORICAL PERSPECTIVE

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Pediatric neuro-oncology has evolved as an international subspecialty since the establishment of the International Symposium of Paediatric Neuro-oncology in 1985. Essentially, the scientific basis which forms the basis for modern practice can be traced to 2500 BC. Studying the evolution of this specialty identifies development of scientific discover- ies, technical inventions, and combined modality working from the late nineteenth century than that of Paediatric Neuro-oncology. Consultation of a wide variety of texts, Web sites, experienced colleagues, and other historical sources helped create a historical timeline, depicting events, people, institutions, journals, scientific advances, awards and political events that have shaped the current state of knowledge and practice. This information will be used to stimulate
Further contributions of important historical events, nationally and internationally, by a request to delegates to identify additional events as part of an ongoing chronology of events that will evolve. The first draft has already been processed by several Brain & Spinal Tumor Editors. D.A. Walker, J.A.G. Punt, R. Taylor, and G. Perlongo (Arnold). A summary of the timeline will be presented of the evolution of neuroscience, neurosurgical practice, institutions, diagnostic imaging, radiotherapy, chemotherapy, cancer science and epidemiology that have been identified so far as making major contributions to the clinical and scientific practice of neuro-oncology. Further collection and subsequent dissemination of information from this timeline will stimulate international debate of the national and international mandates for brain tumor treatment. Clinical developments, effective clinical services for children with brain and spinal tumors in our respective national health systems.

366. MALIGNANT CENTRAL NERVOUS SYSTEM TUMORS IN CHILDREN UNDER 3 YEARS OF AGE: SINGLE INSTITUTIONAL EXPERIENCE
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It is perceived that the prognosis is poor in central nervous system (CNS) tumors diagnosed in children less than 3 years of age. Surgery and chemotherapy are the mainstays of treatment. Radiotherapy is rarely used as a modality of treatment, primarily due to concerns about its effects on brain growth and neurodevelopmental outcome. The purpose of this study was to review the types of tumors, mode of presentation, and management strategies used, as well as to assess outcome.

Patients under the age of 3 years diagnosed with CNS tumors between 1 September 1994 and 31 August 2004 were identified retrospectively from the database in a UK/C BG pediatric oncology centre. Analysis of management and outcome was performed using patient records. Thirty-four patients were identified, of which 18 were male and 16 female. The median age at diagnosis was 20.8 months (interquartile range, 12.4–28.5 months). The pathological diagnosis was available in 32 patients: primitive neuroectodermal tumor (PNET) (12 patients), astrocytoma (8 patients), optic glioma (2 patients), ependymoma (2 patients), gangliocytoma (1 patient), and pineoblastoma (1 patient). Two additional patients with CNS tumors had no pathological diagnosis. Six patients were treated with surgery alone, 5 patients with chemotherapy alone, and 7 patients with both modalities. Surgery, chemotherapy, and radiotherapy were used to treat 1 patient with optic glioma and 1 patient with ependymoma. The patient with brain stem glioma was treated with radiotherapy alone. Five patients, including 1 with PNET, received no active treatment and remained alive. Six patients with PNET and the 2 patients without pathological diagnoses died, having not received active treatment. At the end of the 10-year period, 19 patients overall were still alive. Of the children who had not survived, 11 had PNET, 1 had astrocytoma, and 2 did not have a pathological diagnosis. There was no apparent treatment impact on survival in the last 5 years of patients diagnosed with a CNS tumor below the age of 3 years, 19 out of 34 patients (55%) are alive at the time of the study. The prognosis remains poor, but this appears to be largely attributable to the high mortality associated with PNET, the most common CNS tumor in this series.

367. CHRONIC LOW-DOSE CHEMOTHERAPY FOR REFRACTORY OR RECURRENT BRAIN TUMORS OF CHILDHOOD
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Prolonged low-dose chemotherapy, which exploits the schedule dependency of the agents, has sometimes demonstrated increased activity in both hematological and solid refractory malignancies of childhood compared with bolus administration. This makes it a reasonable choice for palliative treatment in children who are otherwise incurable. A prospective phase 2 study has been conducted in our institute to evaluate the efficacy and toxicity of prolonged schedule oral etoposide in children with refractory or relapsed tumors in a palliative treatment setting. Patients were treated with oral etoposide (50 mg/m² per day given daily for 21 consecu- tive days every 4 to 5 weeks) after failing in medical, radiological, and/or surgical treatments. End point for follow-up was disease progression. Four patients with refractory tumors were entered. Patient 1 was a 6-year-old boy with suprasellar mature teratoma with malignant component. Two years after the initial treatment with repeated surgeries, radiotherapy, and chemotherapy, he had local relapse. Failing in ICE and BEP regimen chemotherapy and gamma-knife treatment, oral etoposide was initiated for progressive tumor. Stable disease duration was 28 months with oral etoposide. Patient 2 was a 4-year-old girl with suprasellar immature teratoma. Six months after high-dose chemotherapy with thiopeta and topotecan regimens and PBCST for the first local relapse, tumors were entered. Oral etoposide was initiated for the recurrent tumor after failing in ICE regimen chemotherapy. Progression-free survival was 11 months. Patient 3 was a 1-year-old girl with pontine low-grade astrocytoma. Oral etoposide was begun after failing in initial chemotherapy with vincristine and carboplatin. She has been alive at home for 31 months without any signs of tumor progression. Patient 4 was a 13-year-old boy with brain stem tumor. Failing in initial radiotherapy (54 Gy), oral etoposide was begun. Four months after the treatment, MRI revealed tumor progression and whole brain radiation therapy (36 Gy) was initiated. He has been at home without tumor progression for 8 months. Chronic oral etoposide and weekly vinblinide appeared to be well tolerated, had modest toxicity, and showed excellent palliative effect in the child with refractory and progressive brain tumors. The present phase II dose scale investigation seems justified to determine the indication and the optimal use of these treatments.

368. THERAPEUTIC STRATEGY FOR ATYPICAL TERATOID/RHABDOID TUMOR: A REPORT OF TWO CASES AND REVIEW OF THE LITERATURE
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Atypical teratoid/rhabdoid tumor (AT/RT) of the central nervous system is a highly malignant neoplasm that primarily affects children less than 5 years of age. Despite aggressive multimodality therapy including surgical resection, chemotherapy, and irradiation, prognosis is dismal. Here we report two young AT/RT patients with extended survival. The first patient was a male, 13 months of age at presentation. The tumor was located in the right cerebellar hemisphere without radiographic metastatic disease. Initial therapy consisted of gross total surgical resection followed by 7 cycles of mild chemotherapy (cisplatin and etoposide). No relapse occurred within 33 months of initial treatment. The second patient was a female, 24 months of age at presentation. The tumor was located in the fourth ventricle without radiographic metastatic disease. Initial therapy consisted of gross total surgical resection followed by 3 cycles of mild chemotherapy (cisplatin and etoposide). Relapse occurred within 3 months of initial treatment. Post-recurrence treatment consisted of surgical resection and extended local irradiation to a dose of 3600 Gy. After 17 months of continued remission, cerebrospinal dissemination occurred. Following extended chemotherapy (cisplatin/etoposide and ifosfamide), the size of the tumor was reduced. After 4 months of continued remission, dissemination around the brainstem recurred, and the patient died at 27 months of initial surgery. It is important to consider the therapeutic strategy best suited to each individual AT/RT patient. For children less than 3 years of age, we hesitate to use radiotherapy because it impairs physical and mental development; therefore, extended surgical resection and chemotherapy should be selected as initial therapies. Some cases of AT/RT may not be sensitive to chemotherapy and once we have determined that chemotherapy is ineffective, we should not hesitate to induce radiotherapy even if the child is less than 3 years old. Previous reports have indicated that most AT/RT are radiosensitive. In the event of recurrence, we recommend surgical resection and intensive chemotherapy.

369. RESIDUAL DISEASE IN INTRACRANIAL MALIGNANT GERM CELL TUMORS (GCTs): FINDINGS GENERATED IN SIOP CNS GCT 96 AND THEIR PROGNOSTIC IMPLICATION
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Residual disease is a predominant risk factor in most of the malignant brain tumors. Therefore, the impact of residual tumor at the end of treatment has been analyzed in patients with intracranial malignant GCTs who are registered in the SIOP CNS GCT 96 trial. Residual disease is defined as any abnormal contrast enhancement persisting in the former tumor region. Residual disease was registered in 48% of patients at the end of therapy. The patients under study are considered to be either germinoma patients or non-germinoma patients. For the germinoma patients, after diagnosis, treatment consists either of craniospinal irradiation 24 Gy (ICE) or boost (option A) or a chemotherapy with carboplatin/VP16/ifosfamide fol-
370. MTG AND p53 STATUS PREDICT TEMOZOLOMIDE SENSITIVITY IN HUMAN MALIGNANT GLIOMA CELLS M. Hermisson, A. Klumpp, W. Wick, J. Klughaus, G. Nagel, W. Roos, B. Kaina, and M. Weller; 1General Neurology, University of Tuebingen, Tuebingen; 2Toxicology, University of Mainz, Mainz; 3Neuro-oncology, University of Nottingham, Nottingham, UK

Temozolomide (TMZ) is an alkylating agent which prolongs survival when administered during and after radiotherapy in the first-line treatment of glioblastoma and which also has significant activity in recurrent disease. O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme attributed a role in cancer cell resistance to O6-alkylating agent-based chemotherapy. Using a panel of human glioma cell lines, we first show here that the levels of MGMT expression are a major predictor of TMZ sensitivity in human glioma cells. The MGMT inhibitor, O6-benzylguanine (O6-BG), sensitzes MGMT-positive glioma cells to TMZ, whereas MGMT gene transfer into MGMT-negative cells confers protection. The anti-apoptotic BCL-XL protein attenuates TMZ cytotoxicity in MGMT-negative cells and is not modified by O6-BG treatment. Together, these results suggest that the determination of MGMT expression and p53 status will help to identify glioma patients who will or will not respond to TMZ.

371. THE COMPARABLE EFFECT OF EXO- AND ENDOGENOUS CANNABINOIDS ON APOPTOSIS IN HUMAN MALIGNANT GLIOMA CELLS AND RAT GliOMA CELLS L. Storer, A. Luo, Kalutostage, D. Walker, and T. Parker; 1Department of Child Health; 2School of Biomedical Sciences, University of Nottingham, Nottingham, UK

Synthetic cannabinoids act by mimicking endogenous substances (endocannabinoids) which activate specific cannabinoid receptors (Guzman, 2003). Cannabinoids have been shown to induce apoptosis, decrease tumor growth, and inhibit tumor angiogenesis in a number of different transformed cell lines and primary cells (Blazquez et al., FASEB J. 17, 529, 2003; Guzman, Nature Rev. 3, 745, 2003; Sanchez et al., FEBBS Lett. 472, 39, 1998). In this study the effect of cannabinoids and endocannabinoids with and without irradiation on the human medulloblastoma cells. Cells were seeded in 6-well plates at a density of 50,000 cells/ml. The apoptotic effect of cannabinoids and endocannabinoids was studied by replacing the media after 24 h with either anandamide, 2-arachidonyl glycerol (AG), anandamide analogues (CB, agonist), Methandamide (CB, agonist) or AM281 (CB, antagonist). Dose concentrations were used on 10^{-4} M, 5 x 10^{-8} M, and 10^{-10} M. Positive controls were incubated with 10 IM M132, a known apoptotic inducer. In the presence of DMOSO or ethanol (carriers) provided the baseline level of apoptosis. Cells were incubated for 3 and 7 days. Cells were harvested and incubated with Annexin V and propidium iodide. The number of live and the number of apoptotic cells were counted by flow cytometry. The results showed that at least one drug dependent apoptosis was induced in all cases. Dayo cells incubated with WIN-55,212-2 at 5 x 10^{-5} M. However, there is little drug-dependent apoptosis following 3 days incubation of Dayo and C6 cells with all other drugs tested. Trends show that after 7 days of incubation the levels of apoptosis increased dramatically with all agonists. Treatment with AM281 alone did not result in apoptosis at all concentrations. Control experiments showed the carriers had no significant effect on apoptosis at concentrations lower than 5 x 10^{-4} M on both cell lines. These results provide preliminary evidence to justify further evaluation of Action of cannabinoids as therapeutic agents in the treatment of medulloblastoma and glioma brain tumors.

372. LEVITIRACETAM (LEV) MONOTHERAPY IN PATIENTS WITH PRIMARY BRAIN TUMORS (PBTs): Efficacy, Side Effects, and CSF Plasma Pharmacokinetics M. Preventi, M. Chamberlaine, S. Kesari, P. Wen, K. Edwards, A. Van Horn, and M. Glantz; 1Neuro-oncology, University of Massachusetts, Worcester, Massachusetts; 2Neurology, University of Massachusetts Memorial Medical Center, Worcester, Massachusetts; 3Neuro-oncology, University of Southern California, Los Angeles, California; 4Neuro-oncology, Massachusetts General Hospital, Boston, Massachusetts, USA

LEV is a broad spectrum antiepileptic drug (AED) with a novel mechanism of action, excellent tolerability, no induction of the hepatic P-450 system, and a half-life permitting twice daily dosing. These features suggest that LEV would be an attractive AED for patients with PBTs, in whom seizures are common and often poorly controlled, and in whom P-450 interactions are a critical issue. Fourteen patients with PBTs and intraventricular reservoirs for administration of intra-CSF chemotherapy received a single oral loading dose of LEV at doses between 500 and 3000 mg. Plasma and CSF LEV concentrations were measured serially between 0 and 48 h after LEV administration. Data including epidemiologic features, seizure characteristics, tumor type and therapy, concurrent medications, prior AEDs, and dose, side-effects, and efficacy of LEV and any other AEDs was also prospectively collected on 150 consecutive PBT patients receiving LEV either as initial or replacement monotherapy for tumor-related seizures. Loading doses of LEV of up to 3000 mg were well tolerated. The mean T_{1/2} of LEV in plasma was 5.2 h (range, 1–7), and in CSF 7.3 h (range, 3–15). The mean % of LEV in serum was 13.3 h (14–20), and in CSF 24 h (13–40). No patients discontinued LEV secondary to adverse events. The most common adverse events were mood disturbance (8%) and sedation/fatigue (7%). Seventy-five percent of patients had complete or good (≤1 seizure/month) seizure control with LEV, 83% of patients were receiving at least one p-450 metabolized drug, and 76% of patients were treated with at least one p-450 metabolized chemotherapeutic agent. Peak levels of LEV are achieved rapidly in serum and CSF after a single oral loading dose. The long half life of LEV in the CSF suggests possible duration of action and potential for daily dosing. Efficacy and tolerability are excellent, and P-450 interactions are absent. LEV should be considered as first- and second-line therapy in patients with PBTs.

373. CHIMERIC PEPTIDES AS TUMOR-SELECTIVE DELIVERY SYSTEMS S. Jones and J. Howl; Molecular Pharmacology Group, School of Applied Sciences, University of Wolverhampton, Wolverhampton, UK

The cell-type-specific targeting of cytotoxic agents and other functional moieties can be achieved by using peptide address motifs that selectively bind to high density at the tumor site. Numerous studies have confirmed the utility of ligands for G protein-coupled receptors as components of heterofunctional peptide chimeras that are selective biological probes. Our current efforts are directed toward the further development of chimeric peptide constructs that employ sequences derived from GPCR ligands or cell penetrant motifs to affect the selective delivery of cytotoxins and signal transduction modulators to tumor cells. We have designed and synthesized a range of hybrid constructs consisting of cytotoxic (peptide and non-peptide) covalently linked to an address peptide derived from the C-terminal of gastrin (G7; H-AGYGMDF-NH2). The G7 homing motif targets a novel binding site expressed by U373MG astrocytic tumor cells that is distinct from classical CCK/CCK receptors. Moreover, biological responses following activation of this novel membrane-bound protein may offer additional therapeutic advantages. For example, G7 receptor activation is reported to inhibit the mobility of malignant astrocytoma in vivo and to avoid the growth-promoting effects of gastrin (Pannequin et al., J. Pharmacol. Exp. Ther. 302, 274, 2002). We
evaluated the cytotoxicity of our chimeric peptides by comparing changes in cellular viability using MTT conversion assays. Our data indicate that chimeric peptides dose-dependently and rapidly (<8 h) reduced the viability of U87MG/C-cell. Moreover, as a chimeric amino-terminal extension, the G7 address motif enhanced the cytotoxicity of both mastoparan (H-INLKLAALAKKL-NH2) and G (KLAKLAK) peptides reported to stimulate necrosis and/or apoptosis of eukaryotic cells. In conclusion, hybrid G7 dimerizes to enhance the efficacy of cytotoxic agents and may be valuable probes to investigate and manipulate additional aspects of astrocytoma cell biology. This work was supported by The Wellcome Trust.

374. INTRATUMORAL PHARMACOKINETICS DETERMINED WITH MICRODIALYSIS IN A PATIENT WITH GLIOBLASTOMA MULTIFORME FOLLOWING SYSTEMIC ADMINISTRATION OF HIGH DOSE METHOTREXATE F. Ali-Osman, T. S. Phuphanich, E. McKenzie, and S. Grossman; NAVBTS CNS Consortium, Johns Hopkins Hospital, Baltimore, Maryland, USA

Delivering potentially therapeutic concentrations of chemotherapeutic agents to brain tumors remains a major concern in the design and interpretation of clinical trials in neuro-oncology. This is the first report of the use of microdialysis in a human to monitor local concentrations of a systemically administered anticancer drug within a brain tumor. This study was conducted under a research protocol approved by the National Cancer Institute and Institutional Review Board at each participating site. Patients with recurrent and refractory glioblastoma multiforme (GBM) where a surgical biopsy to confirm tumor progression after prior surgery, radiation, Gliadel, and temozolomide were eligible. A microdialysis catheter was placed within residual contrast-enhancing tumor at the time of surgery. Modified Ringer’s solution was delivered to the catheter at 1 ml/min. On the following day, 12 g/m2 of MTX (adjusted for renal function) were administered as a 4-h intravenous infusion. Plasma and microdialysate were collected at 30-min intervals from one hour before dosing to 24 h after completing the infusion. MTX was assayed using high-performance liquid chromatography with mass spectrometric detection, with a 0.5 ng/ml lower limit of quantitation. The first patient to be evaluated was a 51 year old who had a biopsy to confirm tumor progression after prior surgery, radiation, Gliadel, and temozolomide. The plasma pharmacokinetics of MTX behaved as expected. Renal function remained unchanged, and the leuokovin rescue was administered as per protocol. MTX concentrations in extracellular fluid within the GBM rose rapidly to a maximum of 57 mM and subsequently decayed in a monoeponential manner with a half-life of 12.3 h. As this was considerably slower than the loss of MTX from plasma, MTX concentrations in the dialysate actually exceeded the plasma levels beginning at 16 h after the MTX infusion ended. The patient subsequently developed urosepsis with neutropenia, but no mucositis or diarrhea, and died 3 weeks later after care was withdrawn. Detailed review by NABTT, CTEP, and the study site found no association with the microdialysis catheter and an uncertain relationship to MTX. This initial experience has demonstrated the utility of microdialysis to monitor intratumoral concentrations of a chemotherapeutic agent in a patient with a brain tumor using microdialysis techniques. This approach could significantly impact the selection of drugs, dosing schedules, and routes of administration in clinical neuro-oncology trials.

375. A NOVEL MECHANISM OF PKA- AND PKC- DEPENDENT DRUG RESISTANCE IN HUMAN GLIOMAS INVOLVING PHOSPHORYLATION AND METABOLIC ACTIVATION OF GLUTATHIONE S-TRANSFERASE P1 (GSTP1) F. Ali-Osman,1 H. Lo,2 G. Antoun, A. Friedman,1 H. Friedman, and D. Bigner;1 Surgery, Duke University Medical Center, Durham, North Carolina; 2Neuro-Oncology, M.D. Anderson Cancer Center, Houston, Texas, USA

The human GSTP1 protein, involved in phase II metabolism of many anticancer agents used in brain tumor therapy and in the regulation of cell signaling in response to stress, is highly expressed in a significant proportion of human malignant gliomas. The overexpression and nuclear localization of GSTP1 has been associated with drug resistance and poor outcome in gliomas and glioblastomas. We report here a novel mechanism of glioma resistance to chemotherapy that involves crosstalk between the GSTP1 protein and two Ser/Thr protein kinases, PKA and PKC, frequently activated in human gliomas, resulting in phosphorylation and significant enhancement of GSTP1 metabolic activity. Using cell-free systems and MGR3 human glioblastoma cells, we showed both PKA and PKC to phosphorylate GSTP1-GSH-dependent, with stoichiometry of 0.4 ± 0.03 and 0.53 ± 0.02 mole phosphate per mole GSTP1 protein. In the presence of GSH, eight different PKC isoforms (α, βII, δ, ε, γ, θ, and η), belonging to the three major PKC subclasses phosphorylated the GSTP1 protein, albeit with varying efficiencies. Enzyme kinetic studies with GSTP1 proteins mutated at candidate amino acid residues established Ser42 and Ser184 as putative phospho-acceptor residues for both kinases in the GSTP1 protein. The catalytic efficiency, kcat/Km, of the phosphorylated GSTP1 was more than double that of the unphosphorylated protein. In glioblastoma cells, activation of PKA and PKC signaling resulted in almost a 3-fold increase in GSTP1 metabolic activity. We showed that expression of the GSTP1 protein in glioblastoma cells to BNCU, cisplatin, and 4-hydroxyroxophamide. These findings demonstrate PKA- and PKC-dependent phosphorylation of GSTP1 as a significant post-translational regulation of GSTP1 function. The GSH-dependence of the phosphorylation and enhanced metabolic activity of GSTP1 under high intracellular GSH conditions, such as present in most drug-resistant tumors, the GSTP1 protein will exist in a hyperphosphorylated and metabolically more active state. This increased resistance of glioblastoma cells toward activation of PKA and PKC and the association of GSTP1 metabolic activity indicate the crosstalk between the Ser/Thr kinases and GSTP1 as a novel mechanism of drug resistance in glioma cells. The findings also explain, in part, the high resistance of PKC-overexpressing cells to chemotherapy and suggest that PKC inhibitors have a potential to overcome GSTP1-associated drug resistance in gliomas. This work was supported by grants ROI CA91438, ROI CA79644, and P50-CA108786 from the NIH (USA).

376. ICOVIR-2, AN OLD FRIEND WITH A NEW FACE: E2F-1 MEDIATED GLIOMA SELECTIVITY FOR DELTA24-RGD M. Alonzo,1 R. Alemany,2 C. Gomez-Manzano,2 W.K.A. Yung,1 H. Jiang,1 and J. Fueyo1; 1Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; 2Terapia Genica, Instituto Catalan de Oncologia, Barcelona, Spain

Oncolytic adenoviruses are promising therapies for the treatment of gliomas. However, the major hurdles that these treatments encounter are selectivity and efficacy. Efficacy can be enhanced by broadening the tropism of the adenovirus. In this regard, we increased the infectivity of the D24 adenovirus inserting and integrin-binding motif (RGD) into the adenoviral fiber. Specificity can be achieved by means of regulatory elements that provide both cell-specific gene expression and viral replication in cancer cells. In this study, we developed a system in which E2F-1 promoter drives the expression of the mutant E1A, in the context of D24-RGD construct (Icovir-2). We hypothesized that E2F1 promoter will increase the selectivity of Icovir-2 in gliomas due to the absence of a functional RB pathway in these tumors and the low activity of E2F1 in normal brain. In order to characterize the therapeutic potential of Icovir-2 we carried out in vitro studies using two different glioma cell lines, U-251MG and U-87MG, expressing high and low CAR, respectively. Icovir-2 showed cytopathic effect and replication capacity in both cell lines. Interestingly, restoration of the RB-pathway by means of transfer of pRB or p21 completely abrogated the oncolytic effect of Icovir-2. Importantly, restoration of the RB-pathway in cells infected with D24-RGD showed partial rescue (30%) from the cytopathic effect. These findings suggest that Icovir-2 is a more selective agent for Icovir-2 for glioma cells in comparison to D24-RGD. Assessment of E1A levels by Western blot revealed also reduced expression levels of the protein in cells infected with Icovir-2 versus D24-RGD. Moreover, fiber expression was decreased in D24-RGD but not in Icovir-2 infected cells. Importantly, Icovir-2 was unable to replicate in arrested NHA, and the MTT analysis showed a significantly reduced cytotoxicity in comparison to D24-RGD. Expression levels of E1A in NHA were substantially reduced in comparison to NHA infected with D24-RGD. ChIP analysis showed that the cellular E2F1 was able to bind and activate the Icovir-2 promoter and that transfer of pRB resulted in transcriptional repression of E2F1 at the promoter location by pRB. Notably, in vivo studies involving tail vein injection (5×10⁶ pfu) followed by monitoring the levels of GPT (IU/L) showed that liver toxicity induced by Icovir-2 was significantly lower than toxicity induced by D24-RGD. Our data showed that Icovir-2 presents improved therapeutic index in human cell lines versus D24-RGD. We conclude that E2F1 promoter specific oncolytic adenovirus against cancer cells.

377. EFFECT OF DEXAMETHASONE ON THE CYTOTOXIC EFFECT OF CLOMIPRAMINE IN HUMAN ASTROCYTIC CELLS IN VITRO S. Amar, K. Parker, R. Lisle, and G.J. Pilkington; 1Neuro-Oncology Group, School of Pharmacy & Biomedical Sciences, University of Portsmouth, White Swan Road, Portsmouth, UK

Dexamethasone is a steroid frequently prescribed to patients with primary high-grade brain tumors in order to control cerebral edema. Our laboratories have already demonstrated that a tricyclic antidepressant, clo-
mipramine, selectively induces apoptosis in cultured brain tumor cells by compromising the mitochondrial respiratory chain via complex III and consequently activating a caspase pathway to cell death. It has been previously shown that dexamethasone inhibits and promotes apoptosis in vitro. Clomipramine and dexamethasone are known to be interacting medicaments. Additive, synergistic, or inhibitory effects of dexamethasone on the apoptotic effect of clomipramine need therefore to be considered. In a series of experiments IPSB-18 (passage 46), cultured anaplastic astrocytoma cells, and UPESC (passage 11), a non-neoplastic astrocyte-rich population of cells derived from the temporal lobe of an epileptic patient were exposed to clomipramine (10 μM) or dexamethasone (50–50 μM) for 24 h. They were then exposed to clomipramine (0–30 μM) or dexamethasone (0–50 μM) for 72 h (i.e., alternative clomipramine/dexamethasone and dexamethasone/clomipramine treatment schedules). MTT assays were performed to assess cell viability after drug exposure. We have also shown that dexamethasone pretreatment of non-neoplastic astrocytes modulates oxygen utilization using a Clarke Oxygen electrode assay and promotes apoptosis (Annexin V assay). Pretreatment with clomipramine and subsequently with dexamethasone showed no treatment-related effect in both cell lines. However, in tumor cells, pretreatment with dexamethasone followed by clomipramine treatment resulted in shift from cell death at <10 μM (dexamethasone followed by dexamethasone) to cell death at concentration ~2 μM. This effect was not observed in non-neoplastic cells. Studies are currently in progress in order to elucidate the molecular mechanisms underlying these effects. This research is supported by the Samantha Dickson Research Trust.

378. CYTOKINES REGULATE INTERLEUKIN 13 RECEPTOR ALPHA2 EXPRESSION IN GLIOMA CELLS

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We documented that a vast majority of patients with high-grade gliomas (HGG) overexpress IL13Ra2, a restricted receptor for interleukin 13 (IL13) and that this receptor is a very attractive target for anti-HGG molecularly targeted therapies. We have suggested that epigenetic regulation of IL13Ra2 expression may play a critical role. We found that supra-physiologic concentrations of EGF and TNFα, and less than the levels of IL4, could further increase the levels of the receptor in HGG cells. We found that increased levels of the receptor were correlated with cell line passage. We had suggested that epigenetic mechanisms involving DNA methylation and, therefore, decreased expression of IL13Ra2 in HGG, and recent studies revealed that the promoter region of the receptor’s gene possesses AP-1 and also STAT-6 binding sites. We next examined whether an already elevated IL13Ra2 can be further upregulated, and thus we conducted series of experiments in which HGG cells were treated with either AP-1 or STAT-6 stimulatory cytokines, individually or in combination, and the levels of the receptor were monitored. The IL13Ra2 protein levels were examined by Western blot and immunohistochemistry (IH) in a variety of HGG cells and normal cells. Thus, the cells were treated with epidermal growth factor (EGF) or IL4 or tumor necrosis factor alpha (TNFa), AP-1, and STAT-6 stimulants, respectively. We have found that serum-starved HGG cells, such as A-172 MG, U-251 MG, G48a, SNB-19, and U-87 MG, had the levels of immunoreactive IL13Ra2 decreased significantly as detected by Western blotting and IH. This is suggestive that IL13Ra2 is overexpressed in HGG in a constitutive manner. The addition of EGF or TNFa or IL4 to cells increased prominently the levels of IL13Ra2 protein, usually by three- to tenfold, the extent of which was cytokine-cell line- and time-dependent. For example, EGF upregulated the receptor most potently after 24-h treatment in the U-251 MG cells and after 36-h treatment in G48a cells, while IL4 alone had little effect. We next examined whether an already elevated IL13Ra2 can be further upregulated by the studied cytokines in the presence of serum in HGG cells. We found that supra-physiologic concentrations of EGF and TNFa, and less of IL4, could further increase the levels of the receptor in HGG cells. The same experiments were performed on transformed normal glial cells (SVGp12), human endothelial cells (HUVEC), and keratinocytes (HaCat). In general, the background levels of immunoreactive IL13Ra2 were very low in those cells when compared with HGG cells. EGF, TNFa, or IL4 produced an increase in the receptor levels, but the levels of IL13Ra2 were still much lower than in HGG cells. IL13Ra2 is currently utilized preclinically and clinically as a target for a variety of therapeutic approaches, such as targeted recombinant cytotoxins, viruses, cytotoxic T cells, and vaccines. In this work, we demonstrate that a short-term pretreatment of HGG cells with AP-1 and/or STAT-6 stimulating cytokines should provide further therapeutic advantage by upregulating the levels of IL13Ra2.

379. NATURAL SESQUITERPEN ALCOHOL ALPHA-BISABOLOL, A NONTOXIC COMPOUND, STRONGLY INDUCES APOTOPSIS IN GLIOMA CELL LINES

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Among human tumors, glioblastoma is one of the most malignant ones, and despite aggressive surgical resection and radiotherapy, the median survival in these patients does not normally exceed 1 year (De Angelis, N. Engl. J. Med. 344, 114, 2001; Surawicz et al., J. Neurooncol. 40, 151, 1998). The use of systemic chemotherapeutic agents may improve the efficacy of treatment, but its use is associated with significant toxicity, and the overall prognosis remains poor (Parker et al., CA Cancer J. Clin. 46, 5, 1996). Carmustine (BCNU) is, at concentration corresponding to LD10 (13 mg/kg), not able to completely kill glioma cells in vivo (Rosenblum et al., J. Neurosur. 58, 177, 1983). Numerous natural compounds have been reported to be potential anti-glioma agents, although major parts of these sank into oblivion. A-bisabolol is a small, odly sesquiterpene alcohol with molecular mass of 222.37 Da, isolated from the essential oil of a variety of plants, shrubs, and trees. Its toxicity in animals is very low (LD50 = 13–14 g/kg, Merck Index). In the present study, we report the cytotoxic effect and the type of death induced by a-bisabolol in glialoma cells. We examined, as a human glioma cell model, T67 and U87 cell lines and, as an animal model, a human glioma cell line C6. At 2.5–3.5 mM, the viability of these cells was reduced to 50% with respect to untreated cells in 24 h. Furthermore, the same concentrations failed to affect the viability of normal rat astroglial cells, in line with its reported non-toxicity in rats (Hernandez-Ceruelos et al., Toxicol Lett. 135, 103, 2002; Villegas et al., J. Nat. Prod. 64, 1357, 2001). At higher concentrations (10 mM) a-bisabolol killed completely the cells. Judging from caspase 3 activation, poly(ADP-ribose) polymerase cleavage, DNA ladder formation, and hyp-G1 accumulation, the cytotoxicity triggered by a-bisabolol results from the induction of apoptosis. Two major routes, extrinsic and intrinsic, have been identified through which cytotoxic drugs induce apoptosis. The first one is mediated by death receptors. In the second pathway, mitochondria play essential roles. The dissipation of mitochondrial inner transmembrane potential and the release of cytochrome c from mitochondria indicate that apoptosis occurs, in our experiments, through the intrinsic pathway. Taken together, these results point out that a-bisabolol may be considered a novel compound able to inhibit glioma cell growth and survival.

380. ENHANCED EFFICACY AND MR IMAGING OF CONVECTION-ENHANCED DRUG DELIVERY

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Convection-enhanced delivery (CED) is a promising technique for distribution of drugs into brain and tumor tissue, currently being tested in several phase 2/3 clinical trials. We have previously presented the clinical application of diffusion-weighted MRI (DWMRI) for monitoring CED of Taxol in recurrent GBM patients. CED is known to depend on several physical and physiological parameters. After accounting for these variables, initial clinical experience shows significant variability in the extent of convection among patients and among drugs. Therefore, increasing tumor response to CED is essential, as well as real-time monitoring of the extent of convection and its early effects on the tissue. Solutions containing combinations of Cremaphore, Taxol, carboplatin, ethanol, sucrose, and human serum albumin in different concentrations were mixed with Gd-DTPA and infused into normal rat striatum or intratumorally in rats bearing large 9L tumors. T1 MRIs were acquired immediately post-treatment to assess the extent of convection, and DWMRIs were acquired 24 h later to assess tissue response. Some rats were monitored by MRI for an additional 4 days to demonstrate subsequent formation of necrosis. The extent of convection was reflected by the T1 MRIs. Limited convection was characterized by signal backflow along the catheter and into the ventricles, while efficient convection presented significant spread into the striatum. CED with cytotoxic infusates was followed by significant changes in subsequent DWMRIs. The extent of these changes correlated significantly (Taxol, r2 = 0.75, P < 0.01; T1, r2 = 0.71, P < 0.01) with the extent of CED. When signal backflow was not observed, including with toxic infusates, no changes were detected on DWMRIs. The efficacy and extent of convection were found to correlate significantly with infusate viscosity (r2 = 0.73, P < 0.003). While low-viscosity infusates tend to backflow, high-viscosity infusates tend to
form efficient convection. Increasing the viscosity of a carboplatin solution with sucrose led to a significant increase of infusate distribution within the stratum, depicted by the immediate T1 MRIs ($P < 0.008$). This effect was also observed when corresponding increased in tumor tissue changes was depicted by the later DWMRIs ($P < 0.007$). In all 9L tumor-bearing rats, DWMRI revealed no tumor response, consistent with the immediate T1 MRIs, which showed that the Taxol leaked out of the tumor and accumulated in normal surrounding tissue. Our data suggest that DWMRI can be used for real-time assessment of CED efficiency and early assessment of tissue response. In addition, increasing the viscosity of solvents may be a simple way to significantly enhance the efficacy of CED treatments, thus increasing their antitumor effect.

### 381. TARGETING MALIGNANT GLIOMAS WITH A LOW-MOLECULAR-WEIGHT RAF/VASCULAR ENDOTHelial GROWTH FACTOR RECEPTOR INHIBITOR

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Low-molecular-weight tyrosine kinase inhibitors against several growth factor receptors have shown promise in preclinical malignant glioma studies, but more modest activity in clinical trials. We are currently investigating novel intracellular kinases that may be frequently active in gliomas. Raf is a subunit of the RAS/RAF mitogenic signaling inhibitor that regulates downstream effector molecules. Several studies have suggested that that Raf plays an important role in tumor growth or that Raf activating mutations are found in a variety of malignancies, including breast, lung, and colorectal carcinomas. Raf is a critical intracellular mitogenic signaling mediator downstream from the G-protein Ras family members that is occasionally mutated in gliomas. Additionally, RAF and RAS activities are increased in most malignant gliomas through activation of growth factor receptor pathways. As multiple growth factor receptors converge onto Raf, disrupting Raf function may offer broad activity against tumors dependent on this pathway. AAL881 is a novel, orally administered, small-molecule inhibitor of kinase activity associated with Raf and vascular endothelial growth factor (VEGF) receptors.

We have now shown that AAL881 treatment of a highly resistant human glioma cell line, D54MG, inhibits phosphorylation of downstream signaling effectors, colony formation, VEGF secretion, and invasion through an artificial matrix. In addition, AAL881 inhibits cellular proliferation by producing cell-cycle G(0) arrest without inducing significant apoptosis. In vivo studies of athymic mice, AAL881 treatment was well tolerated without significant weight loss. A short-term (two-week) course of AAL881 treatment in athymic nude mice with established subcutaneous D54MG xenografts not only significantly delayed tumor growth in 5/10 mice, but also cured 5/10 mice in repeated trials. Tumors less responsive to AAL881 treatment displayed decreased proliferation relative to control tumors. AAL881 therapy in athymic mice with intracranial D54MG xenografts more than doubled median survival compared to the control group. Of note, D54MG xenografts display minimal sensitivity to VEGFR tyrosine kinase inhibitors suggesting that either Raf plays an important role in tumor growth or that Raf activating mutations are highly active against this tumor. Based on these results, Raf may represent a useful therapeutic target in gliomas. AAL881, a novel Raf/VEGF kinase inhibitor, may offer a promising therapy against malignant gliomas. This work was supported by grants from Accelerate Brain Cancer Cure and the Pediatric Brain Tumor Foundation.

### 382. COMBINATION THERAPY WITH PERIFOSINE AND TEOmozolomide FOR GLIOMA TREATMENT: PRECLINICAL TRIAL USING A MOUSE GLIOMA MODEL

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Glioblastoma multiforme (GBM) is an incurable glial tumor even with intensive therapy consisting of surgery followed by radio-chemotherapy. Many newer forms of treatment have been tried for GBM, but these have not been efficacious. Presenting, the best chemotherapeutic drugs for GBM are alkylating agents such as carmustine (BCNU) and temozolomide. Alkylating agents are in the less-sensitive class of antitumor drugs, but are first oral alkylphospholipid with a marked cytotoxic effect and fewer side effects. Unlike most chemotherapeutic drugs that target the nuclear DNA, perifosine interferes with the cell membrane and blocks signal transduction pathways. Recent studies have suggested the molecular mechanism of perifosine action has the capacity to enhance radiation or other anticancer drugs. However, the exact mechanisms of perifosine’s effect on gliomas are still unclear. To develop a new paradigm for glioma therapy, we used mouse glioma cell lines to investigate the mechanism of action of perifosine alone and in combination with temozolomide. We also addressed this in vivo by using a mouse glioma model. Mouse gliial cells transformed with PDGF-B, Kras, Akt, Kras-Akt, and LacZ were used as an in vivo model. These cells were treated with temozolomide and 45 μg perifosine and analyzed with cell proliferation assay, Western blot, and cell cycle analysis. Gliomas were induced in mice by PDGF gene transfer to the nestin-expressing neural progenitor cells. Tumor-bearing mice were detected by bioluminescence imaging, and image-positive mice were treated with Cetuximab and temozolomide administration of 100 mg/kg temozolomide and oral administration of 30 mg/kg perifosine. Mice were then sacrificed and tumor histology was examined. Perifosine and temozolomide inhibited mouse glioma cell growth in a dose-dependent manner, and these drugs had a synergistic effect in cell culture. Cell cycle analysis showed a stronger G1 arrest with this combination rather than with each drug alone. In vivo, the mouse glioma model also demonstrated reduction in tumor size by imaging and minimal staining for proliferation markers by immunohistochemistry. Combination therapy of perifosine and temozolomide is effective in a mouse glioma model and could be a new candidate in treatment of human gliomas.

### 383. THE LUMINESCENT ATP ASSAY OR THE COLORIMETRIC MTS ASSAY—WHICH IS BETTER FOR CHEMOSENSITIVITY TESTING IN MALIGNANT GLIOMAS?


Chemoresistivity testing has been used clinically most successfully in leukemia. Non-neurological malignancies provide large amounts of tumor tissue for drug testing, while very little tissue is available in malignant astrocytomas due to limited surgery. There is a need for an assay methodology sensitive enough to deal with small numbers of glial cells. Toward this end we have developed a cell death assay by measuring ATP levels following exposure of primary glial cells to different chemotherapeutic agents which were compared with a tetrazolium dye reduction assay (MTS). Also, we have investigated the role of apoptotic cell death by determining the level of caspase enzyme activation within the cells. Astrocytic tumor tissue obtained at surgery was disaggregated and plated in culture flasks. At confluence the cells were trypsinized and transferred to 96-well tissue culture plates at 70% confluence, five concentrations of each drug (cisplatin, BCNU, paclitaxel, and etoposide) were added to the wells. After a period of 72 h, cell proliferation was measured by using the one-step MTS and ATP assays (Promega). A separate luminescent microtiter plate was used to measure apoptosis activity by measuring caspase levels by using the one-step caspase assay (Promega). All tumors were either WHO grade III or IV astrocytomas. Assays were performed at the first passage, although the rate of cell growth was low with some cultures limiting the plating density on the microtiter plates. Toward this end we have observed a high correlation ($R^2 > 0.9$) was observed between the ATP and MTS assays when plating density per well was high ($>1000$). However, the correlation was poor ($R^2 < 0.5$) with low plating densities. There was variability in the sensitivity of different primary cultures to the drugs used, with only cisplatin showing a consistently toxic effect in the assays used. High levels of caspase activation were only observed with paclitaxel and cisplatin at the concentrations used, despite cell death being seen at lower concentrations. The luminescent ATP assay has a greater sensitivity to predict drug response in glial cells, with lower cell numbers than the colorimetric MTS assay, making it a potential tool for testing small biopsies. Estimation of caspase levels show that at lower concentrations cisplatin and paclitaxel may cause cell death through non-apoptotic mechanisms.

### 384. TISSUE INHIBITOR OF METALLOPROTEINASE-3 EXPRESSION IN THE CONTEXT OF AN ONCOLYTIC ADENOVIRUS INFECTED GLIOMA: IN VIVO METALLOPROTEINASE ACTIVITY IN VIVO BUT DOES NOT ENHANCE ANTI-TUMOR EFFICACY IN MALIGNANT GLIOMA

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A promising new approach to treatment of solid tumors is the use of oncolytic viruses designed to replicate specifically in malignant cells. We hypothesized that insertion of a transgene encoding a secreted protein...
which exerts effects on angiogenesis, apoptosis, and tumor cell infiltration would improve the anti-tumor activity of these agents. Tissue inhibitor of metalloproteinases 3 (TIMP-3) constitutes an interesting transgene candidate for intratumoral use in combination with other inhibitors of TIMP-3 activity and current therapies. To assess the effects of TIMP-3 gene transfer to glioma cells, we first employed a replication-defective adenovirus encoding TIMP-3 (Ad.TIMP-3). Infected U-87MG, U-87EGFR, and U-251MG glioma cells with Ad.TIMP-3 in vivo showed a reduction in tumor cell proliferation up to 86%. Peptide-specific labeling of Ad.TIMP-3 infection of a panel of primary glioma cell cultures obtained from patients material decreased the viability of these cells and induced morphological changes characteristic for apoptosis. On the basis of these findings, we proceeded to construct a conditionally replicating adenovirus encoding TIMP-3. The TIMP-3 expression cassette was inserted into the E3 region of the adenoviral backbone containing a 24 bp deletion in E1A. This novel oncolytic adenovirus, Ad24TIMP-3, demonstrated enhanced oncolytic activity in a panel of glioma cell cultures compared to the control oncolytic virus Ad24Luc. To confirm inhibition of in vivo MMP activity by Ad24TIMP-3, nude mice bearing subcutaneous glioma xenografts received intratumoral injections of Ad24TIMP-3 or Ad24Luc. The functional activity of TIMP-3 was imaged noninvasively by using a near-infrared fluorescent MMP-2-activated probe. Tumoral MMP-2 activity was significantly reduced by 58.3% in the Ad24TIMP-3 treated tumors 24 h after injection. A study into the therapeutic effects of combined oncolytic and antiproteolytic therapy was performed in both a subcutaneous and an intracranial model for malignant glioma comparing Ad24TIMP-3 to Ad24Luc. Treatment of subcutaneous (U-87MG) or intracranial (U-87EGFR) tumors with Ad24TIMP-3 and Ad24Loc both significantly inhibited tumor growth and survival compared to PBS-treated controls. However, no significant difference in anti-tumor activity between these oncolytic viruses was found. TIMP-3 was produced by glioma cells infected with Ad24TIMP-3 in vivo and in vitro. The biological effect resulting from an oncolytic adenovirus expressing TIMP-3 can be imaged in vivo non-invasively demonstrating the functional expression of the gene of interest. The anti-tumor effects of Ad24D2 could not be improved by insertion of the TIMP-3 gene when injected during tumor development in two murine models for malignant glioma.

385. OPTIMAL BIOLOGICAL DOSE AND SCHEDULE OF INTERFERON ALPHA TO REDUCE THE GROWTH OF HUMAN GliOBlastOma IMPLANTED ORTHOTOPICALLY IN NUDE MICE
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The purpose of this study was to optimize the antitumor activities of pegylated IFN-alpha (PEG-IFN-alpha) against U87MG human glioblastoma cells growing orthotopically in nude mice. Twenty days after the intracranial inoculation of tumor cells, groups of mice (n = 5) were injected with different doses of PEG-IFN-alpha (1,000, 5,000, 10,000, 25,000, and 125,000 units) per week subcutaneously. PEG-IFN-alpha at 10,000 units decreased the expression of basic fibroblast growth factor and matrix metalloproteinase-2 most effectively. More than 25,000 unit injection did not show the reduced expression of proangiogenic molecules by interferon. Administration at the optimal biological dose (10,000 units, twice a week) decreased tumor uptake (control: 6/6; PEG-IFN-alpha: 2/6) and progressive growth of human glioblastoma cells. Mice that received chronic treatment (4 weeks) reduced in tumor uptake and size compared with the short-term treatment (2 weeks). With the immunohistochemical study, there was significant inhibition in the expression of proangiogenic molecules with decreased microvessel density by PEG-IFN-alpha. The data suggest that determination of optimal biological dose for PEG-IFN-alpha is important in the clinical trial for glioblastoma patients.

386. PRECLINICAL FEASIBILITY AND SAFETY STUDY OF A NOVEL ULTRASONIC DELIVERY SYSTEM FOR INTRA-PARENCHYMAL DRUG DELIVERY IN BRAIN TUMORS
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We have developed a novel ultrasonic dispersion device (UDT) to treat infiltrative primary brain tumors by intratumoral delivery of therapeutic molecules. The UDD consists of a dispersion wire within a guide tube. The transmitter tip can be coated with a variety of particles of different size and character. Ultrasonic pulses of different profiles induce a high frequency vibration (sonication) of the wire tip, which disperse particles from the coated tip into the adjacent tissue. We conducted initial studies in a rat brain model to investigate the local and remote effects of UDT on normal brain tissue, and subsequently the feasibility and characteristics of in situ compound dispersion of various compounds. The tested microparticles were purple D502/6290 (30 nm, Bangs Lab.), blue Fe3O4 (100 nm), and blue Polystyrene (180 nm). The UDT probe was introduced stereotactically into the right frontal lobe of male Fischer rats. The sonication was performed with two different power profiles as a single treatment cycle. Profile I lasted for 24 s with a source power of 1 Watt with pulses of 2 s. Profile II lasted for 120 s, source power 2 Watt with pulses of 2 s. After the procedure the probe was removed and the brains were harvested after 30 min, 24 h, 96 h, and 10 days. Macroscopic and microscopic evaluation was performed on H&E stained serial histological brain sections by a neuropathologist. Summary of the research results: A total of 24 rats received a single cycle of treatment. The stratification of the study groups was performed according to the two different sonication treatment profiles with 3 rats per each profile (2) and time points (4). A significant dispersion of the primary mass (solid and suspension) within brain tissue was observed in all rats. Longer sonication periods resulted in more extended distribution. During the post-treatment observation period, no abnormal behavior of the study animals was evident. Macroscopic and microscopic examinations of the brain tissue specimens were negative for any evidence of tissue damage, cyst formation, or necrosis. We have demonstrated the feasibility and safety of the UDD in a rat brain model. Further efficacy studies in tumor-bearing rats are ongoing.

387. INCREASED ONCOLYTIC POTENCY OF THE CONDITIONALLY REPLICATIVE ADENOVIRUS AD24-P53 WHEN COMBINED WITH RADIOTHERAPY IN VIVO
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Conditionally replicative adenoviruses (CRAds) represent a promising class of agents used against therapy-resistant brain tumors such as glioblastoma multiformes (GBM). The adenovirus Ad24-p53 has a 24 bp deletion in the E1a region limiting replication to Rb mutant cells and encodes the p53 tumor suppressor protein. It has shown superior oncolytic activity in glioma compared to the parental control Ad24. As both replication competent adenoviruses and exogenous p53 expression have been shown to enhance radiosensitivity, the current experiments were performed to assess the effect of Ad24-p53 and Ad24 in combination with radiotherapy in GBM monolayer cultures, multicellular spheroids, and mouse xenografts. U-87 and U-251 glioma cells growing in monolayers were irradiated with 3, 6, and 9 Gy. After 24 h, cells were infected at a multiplicity of infection (MOI) of 0.1 or 0.01 with Ad24-p53 or the control Ad24. Eleven days after treatment, viability was assessed by using a quantitative crystal violet assay. U-87 spheroids were irradiated at 4 and 9 Gy and infected with 5 × 104 plaque-forming units (PFU) of Ad24-p53 or Ad24. Viability was measured 12 days after infection using the tetrazolium salt-based WST-1 assay. U-87 subcutaneous mouse xenografts of approximately 170 mm3 in size were locally irradiated with 2 × 10 Gy on days 0 and 4 and received 4 × 106 PFU of Ad24-p53 on days 0, 2, and 4. Tumor size was measured 3 times weekly. The combination of Ad24-p53 and irradiation in U-87 or U-251 cells synergistically increased cell kill with maximally 72% compared to single treatments (Ad24-p53: 48% and 42% and irradiation: 48% and 35%; 9 Gy significantly reduced viability from 76% (infection alone) to 50% (Ad24: 80% vs. 75%, respectively). Combination treatment in U-87 mouse xenografts increased tumor growth delay from 2 days (Ad24-p53) and 23 days (irradiation) to 32 days, with 80% complete regression and 5/10 long-term survivors compared to 0/10 (irradiation) and 2/9 (Ad24-p53). Combining the radiosensitizing effects of a conditionally replicative adenovirus and p53 expression has the potential to greatly enhance the effect of radiotherapy in GBM. Here we show that combining the CRAd Ad24-p53 with irradiation improves the potency of both single treatments in vivo and in vitro. These results show great promise for a future clinical trial combining radiotherapy with Ad24-p53.

388. NEWCASTLE DISEASE VIRUS INDUCES APOPTOSIS IN GliOBlastoma CELLS
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Malignant gliomas remain incurable brain tumors despite aggressive treatment with surgery, irradiation, and chemotherapy. As a new approach the therapy with different kind of virus, for instance genetically engineered, achieved more and more interest. Some naturally occurring strains of Newcastle disease virus (NDV) may have an oncolytic potential against tumor cells. Therefore we asked whether NDV may induce cell death in glioblas-
Gem treatment (Gem/IR) at the IC50 dose (0.0175 mM). The analysis of cell necrosis. For these experiments, we have chosen 10 Gy in combination with cells treated with 15 Gy, the inhibition of cell growth was mainly due to an elevated amount of apoptosis (29%) at 48 h. Instead, in signifi cant inhibition in cell growth (67%), already at 24 h after treatment was observed. Moreover, 10-Gy IR treatment determined a tumor response in vivo testing using an animal model as the next step to therapeutic application.

389. GEMCITABINE SENSITIZES ASTROCYTOMA CELL LINE TO RADIATION EXPOSURE INCREASING APOPTOSIS

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Gemcitabine (dFdCyd, Gem) is a deoxycytidine analogue, recently indicated as a radiosensitizing agent in different types of tumor cell lines. In this study, we investigated the ability of Gem to enhance the radiosensitivity of human astrocytoma cell line (ADF). The effects of ionizing radiation (IR) and/or Gem, on cell growth, cell cycle distribution, and apoptosis were assessed. Cell viability and cell cycle distribution were determined by trypan blue exclusion test. Cell cycle perturbation and apoptosis were analyzed by flow cytometry. Escalating doses of 5, 10, and 15 Gy IR exposure caused a dose-dependent inhibition on the cell proliferation. A 5 Gy IR exposure produced a moderate antiproliferative effect, within 120 h from treatment, with an inhibitory effect less than 30%. Conversely, 10-Gy IR treatment determined significant inhibition in cell growth (67%), already at 24 h after treatment associated with an elevated amount of apoptosis (29%) at 48 h. Instead, in cells treated with 15 Gy, the inhibition of cell growth was mainly due to necrosis. For these experiments, we have chosen 10 Gy in combination with Gem treatment (Gem/IR) at the IC50 dose (0.0175 mM). The analysis of cell growth showed that Gem/IR combined treatment enhances the lethal effect induced by IR alone. Gem exposure, determining an accumulation in G1 phase (about 64%) influences cell sensitivity to IR-induced apoptosis. The IR alone treatment induced a remarkable apoptotic cell death (40%) that was increased by the Gem/IR exposure (68%). Gem sensitizes astrocytoma cell line to the radiation exposure rendering these cells more prone to radiation-induced apoptosis.

390. PRO-DRUG CONVERTING NEURAL STEM CELLS FOR THE LOCAL INTRACEREBRAL CHEMOTHERAPY OF HUMAN GIOBLASTOMA XENOGRAFTS

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Previous reports have demonstrated that neural stem cells (NSCs) distribute throughout experimental intracranial gliomas and are able to “track” invading tumor cells when implanted in the adult rodent brain. Based on this extensive tumor tropism NSCs are attractive candidates as a potential delivery system for therapeutic gene products in the treatment of invasive gliomas. Here, we investigated the therapeutic effectiveness of gloma-targeting NSCs expressing cytotoxic deaminase (CD) to convert systemically administered nontoxic pro-drug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which diffuses out of the NSCs and selectively kills dividing tumor cells. Murine NSCs C17-2 (lacZ/cytosine deaminase positive or negative) were stereotactically implanted (occipital) to well-established intracerebral U87 human glioblastoma xenografts in adult nude mice. Systemic treatment with 5-FC at 500 mg/kg started three days after NSC growth was assessed by magnetic resonance imaging and NSCs distribution by X-gal immunohistochemistry. Intracerebral implantation of 1.5 x 10^7 NSCs-CD followed by systemic administration of 5-FC inhibited the tumor growth as assessed by MRI 14 days after treatment start. Furthermore, the survival was significantly prolonged when compared to the animals of the control groups (no NSC implantation, implantation of NSC-mock plus 5-FC treatment or implantation of NSC-CD but no 5-FC). Histological analysis demonstrated a dramatic tumor reduction of NSCs although the cells were initially implanted distant from the main tumor mass. However, we were not able to detect any NSCs based on X-gal immunohistochemistry in the brains of nude mice analyzed as a control group two days after NSC injection. These results indicate that NSCs represent a potent new delivery system for the local intracerebral treatment of gliomas. However, NSCs may not survive within the tumor environment for a prolonged time, and therefore larger numbers of NSCs or modified NSCs may be necessary. Future studies should address the interaction of transplanted NSCs with the tumor environment.

391. THE EFFICACY OF ALGINATE ENCAPSULATED CHO-K1 SINGLE CHAIN TRAIL PRODUCER CELLS IN THE TREATMENT OF BRAIN CANCER

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Patients with astrocytic tumors in the central nervous system (CNS) have low survival rates despite surgery and radiotherapy. Innovative therapies and strategies must be developed to prolong survival of these patients. The alginate microencapsulation method, used to continuously release a certain cytotoxic drug in the vicinity of the tumor, is such a novel delivery strategy. Targeted TRAIL (TNF-related apoptosis-inducing ligand) is a death inducer that requires specific delivery for a tumor-associated cell receptor. We hypothesized that TRAIL seems promising as an anticancer drug. TRAIL used in this study was recombinantly coupled to a single chain variable fragment (scFv425) with specificity for the EGF receptor. The biological functionality of the TRAIL protein was determined in a transfected CHO-K1 cell line that was extensively characterized with regard to their biological functionality. The scFv425:scFv425 was released by treatment of CHO-K1 cells with a single chain anti-triple chain CAR-conjugated protein that was directed to the extracellular domain of the antigen. The CHO-K1 producer cells were encapsulated in an alginate capsule with a semipermeable membrane through which the scFcys could be released. Thus, scFv425:scFv425 was released in the left cerebral hemisphere of C57BL6 mice. In vitro studies showed that the scFv425:scFv425 was released as a stable polypeptide and could diffuse through the alginate capsule. The scFv425:scFv425 was released in the mouse without activating the endogenous immune system. Microencapsulation of CHO-K1–scFv425:scFv425 stTRAIL producer cells and subsequent their intracerebral implantation was technically feasible. This study justifies further in vivo experiments.

392. MTOR AS A THERAPEUTIC TARGET FOR RADIATION SENSITIZATION OF GLIOMA VASCULATURE

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It is known that radiation activates the PI3K/Akt pathway and that inhibition of PI3K or Akt sensitizes tumor vasculature to radiotherapy. mTOR is a downstream target of Akt, and we hypothesized that irradiation activates mTOR signaling in both glioma and endothelial cells. By inhibiting this activation, we hypothesized that we could increase radiosensitization of these cell lines. Two compounds which selectively inhibit mTOR, rapamycin and RAD001 (everolimus), were used in this study. Both compounds caused a significant increase in sensitization of vascular endothelial cells with only minor effects on glioma cell radiosensitivity as determined by clonogenic assay. Therefore, we specifically investigated the anti-angiogenic effects of mTOR inhibitors. Increased phospho-mTOR protein was detected in irradiated HUVEC cells with no detectable increase in total mTOR protein. Phospho-S6, a biomarker for mTOR signaling, was also found to be induced following irradiation, and this effect was inhibited by PI3K or mTOR inhibitors. Significant increase in cleaved caspase 3 was detected when Rad001 was combined with radiation. Endothelial tube formation was significantly diminished following treatment with rapamycin and 3 Gy. Power-weighted Doppler of glioma xenografts in mice showed a significant reduction in vasculature and blood flow compared with mice treated with 3 Gy or RAD001 alone. We conclude that irradiation activates mTOR signaling in vascular endothelium and that rapamycin and RAD001 increased apoptosis of endothelial cells in response to radiation. To the authors’ best knowledge, this is the first study that demonstrates that mTOR inhibitors may be a way to target the vasculature by radiosensitizing the vascular endothelium resulting in better tumor control, as seen in experiments demonstrating increased tumor growth delay in mice treated with rapamycin.
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392. NON-PATHOGENIC POLIOVIRUS RECOMBINANTS FOR THE TREATMENT OF MALIGNANT GLIOMA
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Histone deacetylase (HDAC) inhibitors represent a novel family of anticancer agents, several of which are being tested in clinical trials. Valproic acid (VPA), a widely utilized anticonvulsant with a well-established toxicity profile, has recently been shown to inhibit HDAC. Our previous studies demonstrated that VPA is capable of inducing apoptosis, cellular differentiation, and senescence in vivo in medulloblastoma (MB) cell lines. Additionally, VPA treatment also resulted in potent cell cycle arrest, proliferation inhibition, and tumorigenicity suppression. These anti-tumor activities were mediated through induction of histone (H3 and H4) acetylation and regulation of multiple gene expression. To evaluate VPA’s anti-tumor effects in a preclinical model that closely recapitulates the biology of human MBs, we injected a total of 10^6 cells from four MB cell lines (DAOY, D283, MHH-MED-1, and MEB-MED-8A) into the right cerebellum of Rag-2 SCID mice. Two weeks later, after formation of a tumor mass, treatment with VPA through subcutaneously implanted osmotic pump (Aztec, model 2001) was initiated. We were able to maintain serum VPA concentrations around 70 Î¼g/ml for 7 days, after which a new osmotic pump was implanted to complete treatment for 2 weeks. Our results demonstrated that VPA’s treatment prolonged the survival of tumor-bearing animals for 40–70 days and reduced tumor size by 50–70%. These observations demonstrated that VPA is capable of inducing apoptosis, cellular differentiation, and senescence in vivo in medulloblastoma (MB) cell lines. VPA is a potent HDAC inhibitor, and our results strongly support the use of VPA as a novel treatment modality for malignant glioma.

393. HISTONE DEACETYLASE INHIBITOR VALPROIC ACID EXTENDS SURVIVAL OF SCID MICE BEARING ORTHOTOPICALLY HETEROPLANTED HUMAN MEDULLOBLASTOMA CELLS THROUGH INDUCTION OF APOPTOSIS
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We have developed a novel treatment modality for malignant glioma based on genetically modified polioviruses. Susceptibility to poliovirus depends on the availability of its cellular receptor, the immunoglobulin superfamily molecule CD155. Selective expression of CD155 in spinal cord motor neurons is believed to uniquely render this compartment susceptible to poliovirus infection and ensuing destruction, producing the histopathological hallmarks of paralytic poliomyelitis. We have demonstrated that the CD155 gene is upregulated ectopically in CNS malignancies, providing a target for therapeutic intervention with poliovirus. However, the inherent neuropathogenic potential of poliovirus would prohibit any therapeutic application of this virus in a human CNS tumor model. To overcome this obstacle, we have designed a vehicle that can manipulate the translation of a viral regulatory element involved in translation control. Poliovirus (+) strand RNA, unlike cellular mRNA, is not equipped with a 5' terminal cap structure and hence uses an alternative mechanism for translation initiation. This novel approach for translation initiation may be exploited for the expression of a cognate protein in tumor cells. In a comprehensive study conducted at the FDA, injection of the recombinant virus into the spinal cords of Cynomolgus macaques failed to induce poliomyelitis. However, the chimera retained wild-type growth potential and cell-killing ability in malignant glioma cells. We have recently deciphered the molecular mechanisms that repress rhinovirus IRES function in neuronal cells and favor viral propagation in malignant glioma. Our observations demonstrate widespread perturbations of the translation initiation apparatus in glioma cells that favor alternative mechanisms of protein synthesis at the IRES and promote virus growth and cytokinesis. A prototype oncolytic poliovirus/rhinovirus recombinant has been produced for further safety evaluations at the FDA and is scheduled to enter clinical investigation against glioblastoma multiforme in the near future.

394. NON-PATHOGENIC POLIOVIRUS RECOMBINANTS FOR THE TREATMENT OF MALIGNANT GLIOMA
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The therapeutic efficacy of adjuvant chemotherapy for the treatment of glioblastomas remains poor in extending patient survival, in part due to cellular resistance to apoptosis. Peripheral benzodiazepine receptor (PBR) is a putative member of the mitochondrial permeability transition pore which plays a critical role in controlling cellular apoptosis. PBR expression is dramatically upregulated in brain tumors. We hypothesize that overexpression of PBR may play a role in apoptotic resistance. Our aims were to investigate the therapeutic effectiveness of inhibiting PBR expression using antisense (AS) oligonucleotides (ODNs) in combination with chemotherapy to enhance glioma cell death as well as to elucidate the functional role of PBR in apoptosis in non-glioma cells. Two in vivo systems were established, both of which involve altering PBR expression levels and comparing apoptotic susceptibility between control and mis-expressed cells. In the glioma cell model, five 18-mer phosphorothioate-modified AS ODNs were designed. The most potent ODNs were selected based on their ability to inhibit PBR protein expression in T98G human glioma cells using Western blot analysis. Combination treatment of AS ODNs and camptothecin was assessed for changes in apoptosis (caspase-dependent PARP cleavage, TUNEL), and viability (trypan blue exclusion assay). In the lymphoma cell model, an expression vector construct containing PBR cDNA was transfected into Jurkat cells, a PBR-null cell line. Presence of PBR mRNA and protein expression in stable transfectants was confirmed by using RT-PCR and Western blot analysis, respectively. Extent of apoptosis (Annexin V staining, TUNEL) was compared between PBR over-expressing clones and vector control clones. T98G cells did not undergo any significant extent of apoptosis under high-glycine/TMP treatment alone. Of the five AS ODNs, AS5 at 100 nM and 200 nM showed most potent inhibition (34% ± 5% and 45% ± 8%, P < 0.05) of PBR protein expression. Combination treatment of AS
ODNs and camptothecin did not sensitize T98G cells to undergo apoptotic death. However, enhanced cytotoxicity, in an AS ODN dose-dependent manner, was observed. In Jurkat cells overexpressing PBR, apoptotic response following camptothecin treatment was attenuated about 30% in comparison to vector control cells. PBR protein expression was successfully inhibited by AS ODNs. PBR downregulation via AS ODNs enhanced camptothecin-induced cytotoxicity, possibly through apoptosis-independent pathways in glioma cells. PBR overexpression (RBTC) to represent the drug partially conferred resistance to apoptotic death, suggesting that targeting PBR presents an attractive therapeutic strategy to potentiate chemotherapy-induced cytotoxicity in both gliomas and other types of cancer.

397. ALKYLGlycerol-MEDIATED INCREASE IN DRUG DELIVERY TO THE NORMAL BRAIN AND TO BRAIN TUMORS IN RATS: REGULATION OF DRUG TRANSFER AND COMPARISON WITH HYPERMANNITOL AND BRADYKININ

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Response of malignant brain tumors to chemotherapy is often poor or transient due to poor penetration of most of anticancer agents across the blood brain barrier (BBB). The intracarotid administration of short-chain alkylglycerols has been reported to be an effective and low toxic strategy to increase the transfer of various cytotoxic drugs to the brain. We have investigated the delivery of methotrexate (MTX) to the brain of normal and of glioma-bearing rats (C6 and RG2) after i.a. injection of alkylglycerols. Results were compared with those obtained after BB-OPENING using hypertonic mannitol or bradykinin. In tumor-free rats, alkylglycerols induced a concentration-dependent increase in the delivery of MTX to the brain. This delivery (1-4 mg/kg) of alkylglycerol (120 and 130 mM) and 2-O-hexylglycerol (75 and 100 mM), MTX-concentrations in the ipsilateral hemisphere were increased 4- to 20-fold and 2- to 4-fold, as compared to controls. Osmotic BBB disruption with 1.4 M mannitol resulted in a very strong accumulation of MTX in the ipsilateral normal brain (114-fold), whereas were increased 4- to 20-fold and 2- to 4-fold, as compared to controls. Variations in the concentration and chemi-

398. HYPOXIA-DRIVEN CD/5-FC TREATMENT SUCCESSFULLY INITIATES BYSTANDER AND RADIATIONSENSITIZATION EFFECTS IN A HYPOXIC GLOILOBLASTOMA CELL LINE

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Current therapy for glioblastoma relies on a multimodal approach. However, efficacy of treatment is limited by therapeutic ratio: Doses sufficient to control cell line have often prove toxic to normal cells as well. Therefore, it is especially important to develop therapies that are specifically cytotoxic to cancer cells, either directly or through bystander or sensitizing effects. Hypoxic cells would be ideal targets for such an approach since they are specific to diseased tissue and often comprise the most treatment-resistant subpopulation of a tumor. Cytosine deaminase (CD) has been widely studied as a form of suicide gene therapy, acting by deaminating the nontoxic pro-drug 5-fluorocytosine (5-FC) to form the highly cytotoxic 5-fluorouracil (5-FU). Previous studies have found that CD can induce a bystander effect and radio sensitization in cancer cells. However, none of these studies were conducted under hypoxic conditions, which are prevalent in solid tumors and are typically resistant to therapeutic efforts. Therefore, in this study, experiments were set to answer these critical questions for our gene therapy strategy. We used a previously made gene construct consisting of the SVA40 minimal promoter under the control of 9 copies of hypoxia responsive elements (HRE). Under hypoxia, hypoxia inducible factor-1 (HIF-1) becomes activated and binds to HRE sequences, facilitating transcription of the yeast CD gene downstream. We performed colony-forming efficiency assays to assess survival of clonogenic cells, and found that both a large bystander effect and radiosensitization occurred under hypoxic conditions. This study was supported by CA-85336 and NS-42927.

399. A NOVEL ULTRASONIC DEVICE FOR DISTRIBUTION OF THERAPEUTIC PARTICLES: AN MRI-BASED FEASIBILITY STUDY IN RABBIT BRAIN

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Ultrasonic Dispersion Device (UDD) is a novel tool for intrastitial delivery of various therapeutic molecules and particles of various sizes. It is composed of an ultrasound source that transmits ultrasound energy to a probe with a drug-containing tip that is located within the tissue. Activation of the ultrasound (sonication) activates the spring-shaped tip and by controlled vibration drives the particles into the tissue. The purpose of the current study was to optimize UDD design and sonication parameters to achieve a significant homogenous distribution of particles in the normal rat brain. Polyvalent particles distribution was measured immediately post-treatment and to follow up the particle concentration over time as well as detect cytotoxic effects. The UDD was inserted stereotactically into the rat brain under general anesthesia, with the active region located in the center of the striatum. The probe was loaded with iron oxide (IO) nanoparticles prior to and during treatment. Various UDD design parameters, such as active region size and location along the probe, transmitter and cover materials, and probe diameter and particle loading, as well as various ultrasonic transmission parameters, such as pulsation sequence and bandwidth, were tested. Treatment duration was 1 to 5 min. Each set of parameters was tested in 3 rats up to a total of 36 rats. Rats were scanned by gradient-echo and T1/T2-weighted MRI immediately post-treatment to assess the IO distribution in the brain. Rats with homogenous IO distribution were scanned periodically by MRI for up to 6 weeks to test the IO washout timescale and possible cytotoxic effects. IO distribution in the rat brain was obtained in 22 out of the 36 rats treated so far. The maximal diameter of distribution, as calculated from the gradient-echo MR images, was 8 mm, and the maximal cross section of distribution was 0.42 cm2. Three rats, in which a homogenous significant IO distribution was observed, were followed by MRI. In the first follow-up scan, 4 days post-treatment, a decrease (estimated 20%–30%) in IO concentration was detected. The remaining IO distribution was stable throughout the 6-week follow-up. No cytotoxic effects were detected on the MR images. The preliminary data demonstrates the unique advantages of the UDD as a minimal invasive tool to achieve homogenous distributions of highly concentrated large particles in brain tissue. Such distribution was achieved in extremely short treatment durations. The long periods in which the large particles remain in the tissue with no apparent toxic effects may enable distribution of large, slow-releasing therapeutic agents coating the IO particles for effective treatment of CNS pathologies.
confocal microscope. NPs were taken up by Daoy cells into lysosomes. The uptake of NPs by Daoy cells was concentration and time dependent. Intracellular fluorescence intensity increased sharply within 2 h, and two plateaus were established, one from 2 to 6 h and the other after 10 h of incubation time. In organotypic slices, NP uptake was cell type dependent, and few NPs were taken up by macrophages. Only a few NPs could be seen in the extracellular space. A study on drug release from NPs, a reduction of fluorescence was observed using FACS. A time-series experiment using fluorescence microscopy showed about 50% of the dye had diffused out of cells after 4 h. These experiments aid our understanding of drug delivery by nanoparticles. They show that few NPs were taken up by macrophages due to the lack of chemotaxis and that NPs were rapidly degraded intracellularly, and showed release of both drug and NPs into the culture medium in a few hours.

401. DEVELOPMENT OF TRAIL THERAPEUTIC STRATEGIES FOR MALIGNANT GLIOMAS
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Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) induces apoptosis in malignant glioma cells and thus offers a new potential therapeutic agent for malignant gliomas. Unfortunately, many human malignant glioma cells are resistant to TRAIL, posing a major obstacle in TRAIL resistance. In this study, we investigated inhibitory mechanisms for development of combination treatments that can overcome the resistance. First, we examined a large panel of 26 human malignant glioma cell lines for their sensitivity to TRAIL and grouped them into two phenotypes, TRAIL sensitive and resistant. The resistant cell lines were analyzed for the sensitivity to the combination treatment with TRAIL and chemotherapy drug cisplatin and etoposide and divided into two subtypes, chemotherapy-sensitized resistant and complete resistant. TRAIL induced apoptosis through binding of cell surface death receptor DR4/DR5, leading to the assembly of death-inducing signaling complex (DISC) in the sensitive cells. In the DISC, apoptosis-initiating caspase-8 was cleaved and thus initiated caspase cascade leading to programmed cell death (apoptosis). In contrast, cellular Fas-associate death domain-like, IL-1β-converting enzyme-inhibitory protein (c-FLIP) and phosphoprotein enriched in diabetes (PED) were recruited to TRAIL-induced DISC and inhibited caspase-8 cleavage in the chemotherapy-sensitized resistant cells. Targeting c-FLIP and PED with small interfering RNA (siRNA) and the chemotherapy drugs sensitized the cells to TRAIL-induced apoptosis. The complete resistant cell lines were analyzed by the combined comparative genomic hybridization (CGH), G-bandng/spectral karyotyping (SKY), and fluorescence in situ hybridization (FISH) analyses with chromosomal region specific probes used to identify aberration of chromosomal regions that harbor key TRAIL signaling gene DR4/DR5, caspase-8, caspase-3, caspase-7, caspase-9, Bid, Bax, Bak, Bcl-2 and Smad. Loss or structural changes in these regions harboring DR4/DR5, caspase-8, Bid and Smad was simultaneously detected in the complete resistant but not sensitive cell lines. In conclusion, this study provides new TRAIL combination therapeutic strategies that target chemotherapy resistance. In addition, this study identifies genetic markers of TRAIL to be able to predict the responsiveness of malignant glioma to the TRAIL-based therapies.

402. THE INHIBITION OF THROMBOXANE SYNTHASE ACTIVITY: A NOVEL TARGET FOR THE TREATMENT OF MALIGNANT GLIOMAS
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Thromboxane synthase (TXSα), an enzyme of the arachidonic acid metabolism, is upregulated in human glial tumors and involved in the regulation of cell invasion, which can be a limiting factor of current therapeutic strategies in this disease. Recent in vitro studies indicate that the inhibition of thromboxane synthase activity at non-cytotoxic concentrations blocks the invasive phenotype of glioma cells. This in turn increases a pro-apoptotic disposition and therefore the susceptibility to standard apoptosis-inducing chemotherapeutic compounds like BCNU, camptothecin, and etoposide. Here, we evaluated the therapeutic effects of furegrelate, a clinically tested TXSα inhibitor, in orthotopic U87 human glioblastoma xenografts by using local intra-tumoral microinfusions at 0.5 and 2 mg/kg/d. Local delivery of furegrelate by osmotic mini-pumps at 2 mg/kg/d for 21 days significantly inhibited the growth of well-established orthotopic gliomas in a nude mouse model (76.12%, P = 0.005). Furegrelate and BCNU displayed strong synergistic effects on the in vitro induction of U87 glioma cell apoptosis as measured by an ELISA for DNA fragmentation. Therefore, we assessed the effects of furegrelate alone and in combination with BCNU on the survival of human glioma bearing nude mice. While local delivery of furegrelate at 2 mg/kg/d for 21 days synergized with BCNU on survival only marginally, the combination with systemically administered BCNU (15 mg/kg/d) enhanced the survival more than the single compounds alone. Our results indicate that targeting of the increased TXSα activity in human gliomas inhibits tumor growth in vivo by inducing pro-apoptotic, anti-proliferative, and anti-angiogenic effects. Local treatment with a TXSα inhibitor has the potential to enhance conventional chemotherapeutic schemes for malignant gliomas. Future studies need to evaluate modern modulators of TXSα activity and their effects in relationship to other metabolites of the arachidonic acid pathway.

403. INHIBITION OF 90-kD HEAT SHOCK PROTEIN POTENTIATES THE CYTOTOXICITY OF DNA-ALKYLATING AGENTS IN HUMAN GLIOMA CELLS
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Since gliomas are not curable surgically, it is necessary to develop an effective regime of adjuvant therapies, such as radio- or chemotherapy. Several ways to potentiate the cytotoxicity of antitumor agents have been reported. However, the effectiveness of drug combination treatment is limited by the emergence of drug resistance. Thus, many pathways can be targeted in an effort to sensitize tumor cells to chemotherapeutic agents. Previous studies revealed that a molecular chaperone 90-kD heat shock protein (Hsp90) is expressed at higher levels in human neoplastic tissues, including gliomas, than in normal tissues. Hsp90 participates in the stability and functions of its client proteins, which are involved in cell cycle regulation (ex. Wee1, Plk1), cell survival (ex. Akt, survivin), and oncogenesis (ex. raf-1, src), and it is involved in a cytoprotective mechanism against cellular stresses, such as DNA damage. We hypothesized that Hsp90 inhibitors might act as antitumor agents against glioma and potentiate the cytotoxicity of DNA-damaging agents. In the present study, we found that at a low concentration (3 nM) the Hsp90 inhibitor geldanamycin (GA), an ansamycin derivative, reduced the clonogenicity of U87MG human glioma cells in a p53-independent manner, and that GA potentiated the cytotoxicity of DNA-alkylating agents temozolomide and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on human glioma cells at a lower concentration (<1 nM). This GA-induced potentiation of DNA-alkylating agent-induced cytotoxicity was not a consequence of G2 checkpoint abrogation or degradation of the anti-apoptosis proteins Akt or survivin, and exogeneous Akt overactivation did not overcome GA-induced sensitization of U87MG cells to DNA-alkylating agents. Experiments using another Hsp90 inhibitor, radicicol, a macroryclic antibiotic, showed the results similar to those mentioned above. Although the mechanism of the GA-induced enhancement of the cytotoxicity of DNA-alkylating agents is still unclear, since two different types of Hsp90 inhibitors showed potentiation of the cytotoxicity of DNA-alkylating agents, and since this effect of Hsp90 inhibitors was clearly recognized with very low concentration of compounds that can be toxic to normal cells at much higher concentration, we conclude that Hsp90-targeted therapy may provide an effective strategy for the chemosensitization of human gliomas.

404. ADULT HUMAN MESENCHYMAL STEM CELLS: VEGF-DRIVEN INTERACTION WITH GLIOMA CELLS IN VITRO
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Much effort has been put into establishing human multipotent cells as carriers for malignant glioma therapy. The aim of our study was (1) to characterize factors that influence active movement of stem cells in the environment of a tumor infiltrated brain and (2) to test human adult mesenchymal stem cells (MSCs), which are easily available through bone marrow biopsies, for their migration in brain and invasive behavior and their interaction with human gliomas. Human MSCs were isolated from bone marrow biopsies carried out for hematological indications. Migration of human adult MSC- and rodent embryonal NSC-spheroids (cell line C17.2) was studied on different matrix compositions (chitin, tenasin, and laminin) and on non-chemo- treated and on cells treated with vascular endothelial growth factor (VEGF) with dose ranging from 0.05 to 500 ng/ml. VEGF was added in order to evaluate the role of glioma derived factors. To assess invasion, confrontational co-cultures of glioma (U373 GFP, C6 GFP, U251 GFP, C6 VEGF sense, C6 VEGF antisense transfected) and stem cell spheroids (human MSC and rodent NSC, respectively) were investigated. Invasion...
405. IN VITRO SAFETY AND EFFICACY OF A NOVEL CB2-SELECTIVE CANNABINOID CHEMOTHERAPEUTIC AGENT, KM-233, FOR THE TREATMENT OF HIGH-GRADE GLIOMA

KM-233 is a classical cannabinoid with good blood-brain barrier penetration that possesses a 2.3- and 27-fold higher affinity for the CB-1 and CB-2 receptors, respectively, relative to THC. In vitro tissue culture cytotoxicity assays were used to measure the anti-tumor effects of KM-233 against human U87 glioma cells. KM-233 was found to have significant cytotoxic effects against U87 human glioma cells and reproducibly demonstrated a dose-dependent, time-dependent, and pH-dependent inhibition of cell growth. Similar assays were used to compare the cytotoxic efficacy of KM-233 to Δ⁶-tetrahydrocannabinol and BCNU. In these studies, KM-233 was as efficacious in its cytotoxicity as Δ⁹-tetrahydrocannabinol, and far superior to the commonly used anti-glioma chemotherapeutic agent BCNU. Kinetic studies of the onset of activity of KM-233 demonstrated that cytotoxic effects of KM-233 against human glioma cells in vitro occur as early as two hours after adenoviral infection. Furthermore, we found that the dosing of KM-233 can be cycled on a daily basis without compromising its cytotoxic efficacy, thereby limiting potential toxicity from excessive exposure in vivo. To test the safety and efficacy of KM-233 against healthy adult brain tissue, an organotypic brain slice coculture model of cortex, striatum, and substantia nigra was used for dose-escalation studies. We found that, while there is some minimal toxicity associated with continuous administration of KM-233 in an organotypic brain slice culture model, cycling of KM-233 at doses that are exquisitely cytotoxic to glioma cells were well tolerated by healthy cultured brain tissue. These studies provide in vitro evidence that KM-233 shows promising efficacy against cultured glioma cell lines, shows minimal toxicity to healthy cultured brain tissue, and should be considered for preclinical development in animal models of glioma.

406. CONVECTION-ENHANCED DELIVERY OF LIPOSOMAL DOXORUBICIN ERADICATES U251MG INTRACRANIAL BRAIN TUMORS IN RATS

Convection-enhanced delivery (CED) of liposomes into brain and brain tumor xenograft models resulted in robust tissue distribution and could be demonstrated by MRI. CED of liposomal doxorubicin demonstrated a uniform and extensive distribution of therapeutic liposomes for treatment of brain tumors is a lofty goal. The efficacy of well-characterized clinically available liposomal doxorubicin liposomal drug delivery by CED was evaluated in U251MG human glioblastoma intracranial xenografts. CED of liposomal doxorubicin at a dose safe to the normal brain (0.2 mg/ml doxorubicin) was significantly more effective than systemic administration at the maximum tolerable dose. When CED of liposomal doxorubicin was compared with free drug, liposomal drug delivery demonstrated a 10-fold increase in therapeutic index, resulting from improved efficacy and safety. When used at a nontoxic dose, liposomal doxorubicin demonstrated improved survival over free doxorubicin at the same dose. In addition, at a tenfold higher dose (2 mg/ml doxorubicin), liposomal doxorubicin was less toxic than free doxorubicin. A study of the tissue distribution following CED revealed liposomal doxorubicin distributed over larger regions in the brain parenchyma and resulted in longer tissue retention of the drug at the site of initial distribution. Free drug, when infused by CED, did not distribute as well as liposomal doxorubicin and induced early onset tissue damage, which led to increased tissue toxicity. CED of liposomal doxorubicin shows promise for treating brain tumors. The combination with imaging may provide an effective strategy for brain tumor therapy.

407. SYNERGISTIC INTERACTION BETWEEN 17-AAG AND PHOSPHATIDYLINOSITOL 3-KINASE INHIBITION IN HUMAN MALIGNANT GLIOMA CELLS

The PI3K/Akt pathway is often constitutively activated in malignant glioma cells, in many cases as a result of mutation of PTEN, an endogenous inhibitor of Akt, which renders tumor cells resistant to cytotoxic insults, including those related to anticancer drugs. Pharmacological inhibition of this pathway may potentially restore or augment the effectiveness of conventional chemotherapy or other signaling-targeted agents. Because the heat shock protein (HSP) is involved in the conformational maturation of a number of signaling proteins critical to the proliferation of malignant glioma cells, we hypothesized that the combination of the PI3K inhibitor LY294002 and the HSP90 inhibitor 17-AAG would promote glioma cytotoxicity by decreasing both the activation status and levels of Akt, as well as downregulating the levels of other relevant signaling effectors. We therefore examined the effects of the LY294002 and 17-AAG, alone and in combination, on signal transduction and apoptosis in a series of malignant glioma cell lines. Simultaneous exposure to these inhibitors significantly induced cell death and irreversibly inhibited proliferative activity and colony-forming ability of the glioma cell lines. Quantitative analysis revealed that enhancement of the cytotoxic effects of 17-AAG and LY294002 by LY294002 of 17-AAG-related cytotoxicity was synergistic, leading to a pronounced increase in active caspase-3 and PARP cleavage. No significant growth inhibition or caspase activation was seen in control cells. The enhanced cytotoxicity of this combination was associated with diminished Akt activation and a significant downregulation of HSP90 (IC50 = 1.42 μM). Similar assays were used to compare the cytotoxic efficacy of KM-233 to Δ⁶-tetrahydrocannabinol and BCNU. In these studies, KM-233 was as efficacious in its cytotoxicity as Δ⁹-tetrahydrocannabinol, and far superior to the commonly used anti-glioma chemotherapeutic agent BCNU. Kinetic studies of the onset of activity of KM-233 demonstrated that cytotoxic effects of KM-233 against human glioma cells in vitro occur as early as two hours after adenoviral infection. Furthermore, we found that the dosing of KM-233 can be cycled on a daily basis without compromising its cytotoxic efficacy, thereby limiting potential toxicity from excessive exposure in vivo. To test the safety and toxicity of KM-233 against healthy adult brain tissue, an organotypic brain slice coculture model of cortex, striatum, and substantia nigra was used for dose-escalation studies. We found that, while there is some minimal toxicity associated with continuous administration of KM-233 in an organotypic brain slice culture model, cycling of KM-233 at doses that are exquisitely cytotoxic to glioma cells were well tolerated by healthy cultured brain tissue. These studies provide in vitro evidence that KM-233 shows promising efficacy against cultured glioma cell lines, shows minimal toxicity to healthy cultured brain tissue, and should be considered for preclinical development in animal models of glioma.

408. DOWNDREGULATION OF E1A PROTEIN EXPRESSION AS A NOVEL STRATEGY TO DESIGN CANCER SELECTIVE ADENOVIRUSES

Oncolytic adenoviruses are being tested as potential therapies for human malignant tumors, including gliomas. Here we report for the first time that the deletion of a 48-60 aa region in the CR1 domain of E1A resulted in low levels of E1A protein, conditioning the replication of the mutant adenoviruses specifically to cancer cells. In this study, we compared the oncolytic potencies of three mutant adenoviruses encompassing deletions within the CR1 (Δ24) or CR2 (Δ24) regions or in both regions (Δ24/39) of E1A protein. Cells exposed to Δ24/39 and Δ24/29 displayed a significant reduction in cell cycle regulatory proteins, such as pRb, CDK4, CDK6, and cyclin D1. Taken together, these findings suggest that the PI3K/Akt pathway plays a critical role in regulating the apoptotic response to 17-AAG through targeting this pathway could provide a potent strategy to treat patients with malignant gliomas.
409. ENHANCED CELLULAR RETENTION OF AN INTERNALIZING ANTI-EGFRVIII MONOCYCLIAL ANTIBODY RADIOIODINATED USING LYSG-[125I]ODOBENZOYL GLY-L-MALEIMIDE GEEK ([125I]IB-MAL-D-GEEEK), A PROSTHETIC GROUP CONTAINING NEGATIVELY CHARGED D-GLUTAMATES
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Monoclonal antibodies (mAbs) reactive to EGFRVIII, a mutant form of the epidermal growth factor receptor expressed by gliomas but not by normal tissues, are rapidly internalized and degraded after binding to EGFR-VIII-expressing cells. If mAbs are radioiodinated directly on the tyrosine residues, the radiolabeled catalytic tyrosine is rapidly washed out of the tumor. Furthermore, if anti-EGFRVIII mAbs are to be useful in radioimmunotherapy, radioiodination methods with which enhanced tumor retention of radioactivity can be achieved are necessary. Earlier, we evaluated a radioiodinating agent [131I]KRYR containing a tyrosine for labeling, 3 arginines for positive charge, and a lysine for mAb coupling via a maleimide bifunctional agent. In this study, we evaluated a novel agent with 3 negatively charged D-glutamates for lysosomal trapping, an iodobenzoyl moiety for minimizing dehalogenation, and a maleimide group for conjugation to mAb. Maleimidoglycine was prepared as a precursor for Gly-3-maleimidoo-GEEK preparation by solid-phase peptide synthesis. The lysine side chain of Gly-3-maleimidoo-GEEK was derivatized with 3-iodobenzoyl and 3-(tri-n-butylstannyl) maleimide moieties by treatment with the respective imido esters. The iodinated mAbs (L8A4) were conjugated to iminothiolane-treated L8A4, an anti-EGFRVIII mAb, in 54.3 ± 5.8% radiochemical yields. This radioiodinated agent was conjugated to iminothiolane-treated L8A4, an anti-EGFRVIII mAb in 54.3 ± 17.7% conjugation yields. The protein-associated radioactivity (methanol precipitation) of the labeled mAb was 94.3 ± 5.8%, and the immunoreactive fraction was 82.3 ± 2.2% (Linbro method). In vitro assays with the EGFRVIII-positive human glioma cell line indicated that this class of agents is effective for the radioiodination of internalizing mAbs. We are currently evaluating L8A4 radioiodinated using this new method in EGFRVIII-expressing tumor xenograft models.

410. S-FARNESYLTHIOLSALICYLIC ACID TARGETS RS SIGNALING IN A MOUSE MODEL OF GLIOMA
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The Ras signal transduction cascade is commonly activated in high-grade gliomas. Evidence suggests that farnesylation is critical for recruitment of Ras to the membrane and its subsequent activation. However, treatment with several farnesyltransferase inhibitors (FTIs) has failed to achieve therapeutic benefit. This failure may result from resistance by evoking alternative prenylation mechanisms. By contrast, S-farnesylthiosalicylic acid (FTS) is a competitive inhibitor of Ras that likely acts by displacing farnesyl from Ras proteins to induce alternative prenylation. To explore the mechanism of action of FTS in vitro and the therapeutic efficacy of FTS on a mouse model of glioma in vivo, we transformed neural progenitor cells with activated forms of Kras, Akt, or both, and treated these transformed cells with various concentrations of FTS. We explored the mechanism of action of FTS in vitro and the therapeutic efficacy of FTS on a mouse model of glioma in vivo. We transformed neural progenitor cells with activated forms of Ras, Akt, Ras + Akt, or Ras + Akt + PCGF-B by somatic gene transfer. Transformed progenitors were exposed to increasing doses of FTS and analyzed for changes in morphology, induction of apoptotic cell death by flow cytometry, and signaling effects by Western blotting. Synergistic effects with other drugs were addressed by combined treatments with a MEK inhibitor, an mTOR inhibitor, or an Akt inhibitor. Subcellular localization of Kras was determined by immunoﬂuorescence microscopy. Finally, we assessed the potential therapeutic effects of FTS in vivo in a Kras-driven glioma model. FTS reduced cell viability in a dose-dependent manner. Specifically, at low concentrations of the drug, only the Ras transformed cells underwent apoptosis, whereas at high doses FTS exhibited a generalized toxicity toward all cell lines. This suggests speciﬁcity for FTS activity against Ras, as the inactive Akt was not affected. At high concentrations, active Akt was protected against FTS-induced apoptosis at lower doses, and combined treatment with an Akt inhibitor restored sensitivity. The rescue provided by Akt was mTOR independent because addition of an mTOR inhibitor had no effect. Experiments are underway to examine the effects of FTS in vivo. FTS induces apoptosis in cells that are reliant on Ras signaling to maintain their transformed characteristics in a dose-dependent manner. Activation of Akt provides a rescuing effect that is abolished by treatment with an Akt inhibitor. These results suggest that FTS may potentially be a novel therapeutic agent for treatment of tumors with increased Ras signaling. As activation of Akt is also common in high-grade gliomas, combined therapy with FTS and an Akt inhibitor may be clinically useful and more effective than with either drug alone.

411. SYNERGISTIC AUGMENTATION OF VINCristINE-INdUCED CYTOTOXICITY BY PHOSPHATIDYLINOSITOL 3-KINASE INHIBITOR IN HUMAN MALIGNANT GLIOMA CELLS; EVIDENCE FOR THE INVOLVEMENT OF PI3 AND ERK SIGNALING PATHWAY
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Microtubule-interfering agents (MIA), such as vincristine, are widely used for the treatment of cancer, and are included in many treatment regimens for childhood brain tumors. The anticancer properties of MIA have been attributed to interference with microtubule assembly, impairment of mitosis, and cytoskeletal changes, with additional effects on microtubule-associated protein kinase signaling and caspase activation. Because malignant gliomas generally have dysregulation of PI3K/Akt signaling, which can promote cell survival and potentially limit the activity of such agents, we questioned whether PI3K inhibition with LY294002 could potentiate the efficacy of vincristine in a panel of glioma cell lines versus normal astrocytes. We therefore examined the effects of the LY294002 and vincristine, alone and in combination, on cell survival, signal transduction, and apoptosis in a series of malignant glioma cell lines versus normal astrocytes. We therefore examined the effects of the LY294002 and vincristine, alone and in combination, on cell survival, signal transduction, and apoptosis in a series of malignant glioma cell lines versus normal astrocytes. These results suggest that LY294002 is a promising reagent for the radioiodination of internalizing mAbs. We are currently evaluating L8A4 radioiodinated using this new method in EGFRVIII-expressing tumor xenograft models.

412. COMBINATION OF AN ANGIOGENESIS INHIBITOR WITH RADIOTHERAPY FAILED TO SHOW SYNERGISM IN AN ORTHOTOPIC MURINE GBM MODEL
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Clinical effects of irradiation in GBM patients are likely the result of a direct anti-neoplastic effect, as well as radiation-induced damage to the tumor vasculature. We hypothesized that additional damage to the tumor vessels, by combining radiotherapy with an angiogenesis-inhibitor, will enhance radiotherapy effectiveness. An orthotopic murine GBM (U251-G2) model was used, which we modified to allow focal brain irradiation. The antibody against murine VEGF-R2 (DC101) was given in a dose of 40 mg/kg every 3 days, starting on the same day as the irradiation (2 mCi iodine seeds). Treatment started 7 days after stereotactic injection of the cells in the right frontal lobe. Both treatment modalities were controlled with sham treatment, resulting in 4 groups. Mice were sacrificed when losing >20% weight, showing neurological signs, or after 13 weeks after cell inoculation. The combination of either SB203580 or z-VAD FMK, selective inhibitors of p38 MAPK and caspase signaling, respectively, abrogated the apoptotic response to the combination of LY294002 and vincristine. Taken together, these findings demonstrate that PI3K/Akt inhibition can potentiate the effects of vincristine and that the combination of molecularly targeted therapies and conventional agents could provide a potent strategy to treat patients with malignant gliomas.
413. EFFICACY AND SAFETY OF A REPLICATION-RESTRICTED VSV FOR THE TREATMENT OF GLOBOMASTOMA USING ORGANTOPTYIC BRAIN SLICE AND RODENT MODELS OF INTRACRANIAL GLIOMA

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Vesicular stomatitis virus (VSV) is an enveloped negative strand RNA virus being evaluated for use in treatment a variety of tumors. A sensitive organotypic brain tissue slice-glioma coculture system was used to evaluate use of recombinant, wild-type VSV (wt-VSV) in the treatment of glioma. Previous work has shown that even when replication of wt-VSV was blocked by pretreatment of the slice culture with interferon-β, the integrity of neuronal tissues were significantly damaged following exposure to wt-VSV. This neurotoxicity would pose a major limitation for clinical use of wt-VSV. In this report, we describe the use of a recombinant, replication-restricted version of VSV (GS-VSV) to quantitate the efficacy and safety of this virus for glioma therapy. In contrast to the results observed with wt-VSV, we found that GTx-v401 exhibited minimal cytotoxicity in the organotypic slice culture while displaying high levels of oncolytic activity toward glioma cells growing within the slice. We also observed no virus-induced loss of neuronal integrity as measured by MAP-2 staining and no change in the electrophysiological properties of the slice culture. We further developed these studies with in vivo experiments designed to compare and contrast the safety and efficacy of replication competent recombinant wt-VSV and replication restricted GTx-v401 in reducing tumor volume in a rat model of glioma. In these studies, we found that the use of wt-VSV to treat intracranial glioma resulted in an overwhelming encephalopathy and caused unacceptable morbidity and mortality. In sharp contrast to these results, administration of GS-VSV directly to the intracranial tumor bed was well tolerated and effective at reducing the tumor load. In the highest plaque-forming unit dose of GS-VSV tested (107 pfu, 5 logs higher than the lowest dose of wt-VSV tested), there was little morbidity and no mortality associated with administration to the tumor site. A 71% reduction of tumor surface area was noted in the GS-VSV treated group versus controls. We also found no significant differences between treated and control animals with respect to weight loss, neurological changes, or neurohistopathological changes at all doses tested. GS-VSV appears to be safe and effective when used in vivo to treat intracranial glioma and warrants further development as an adjuvant therapy.

414. COMIATION OF IMATINIB MESYLYSTE (STI571, GLEVEC) AND TEMOZOLOMIDE (TEMODAR) DISPLAYS INCREASED ACTIVITY AGAINST GLIOMA XENOGRAFTS

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Temozolomide is an orally available methylator now widely used in glioma therapy. Although temozolomide has activity against malignant gliomas, development of resistance is common. We have sought to increase the activity of temozolomide in these cancers. Imatinib mesylate is a novel low-molecular-weight ATP-mimetic inhibitor of several tyrosine kinases, including platelet-derived growth factor receptors (PDGFRs). Although imatinib mesylate has been relatively inactive in monotherapy trials against gliomas, in vivo studies indicate that this compound may offer a synergy with temozolomide through increased tumor accumulation by decreasing interstitial fluid pressure and blocking retrograde blood-brain barrier transporters as well as disrupting tumor survival mechanisms. The combination of imatinib mesylate and temozolomide (24 mg/kg x 5 days starting two days before induced loss of neuronal integrity) was performed on imatinib-treated and control glioma cells. The malignant glioma cell line U87MG and LN1308 expressed PDGFRα and PDGFRβ receptors, but not c-KIT. T98G human malignant glioma cells expressed neither PDGFR receptors nor c-KIT. RG human malignant glioma cells expressed both PDGFR receptors and c-KIT. Treatment with temozolomide caused dose-dependent downregulation of PDGFR, in all expressing cell lines, while PDGFRα expression was less affected. Expression of c-KIT in RG cells was also downregulated by imatinib treatment in a dose-dependent manner. Phosphorylation specific multi-imunoblot (KineworksKPS) in U87MG cells treated with imatinib showed significant functional activation (up to 32% of control) of MAP kinases ERK1 and ERK2, compared with untreated control cells. Phosphorylation of other kinases in the MAPK signaling pathway, such as MEK1 and MEK2, and of kinases outside this pathway, was downregulated by imatinib. Quantitative RT-PCR in U87MG cells showed upregulation of the ERK phosphate kinase MKP-1 and downregulation of the ERK kinase MAP kinase phosphatase 3 treatment with imatinib. Finally, simultaneous specific inhibition of ERK and kinase in imatinib and combination treatment using the MAPK inhibitor PD098059 significantly increased the toxicity of imatinib in U87MG glioma cells. In conclusion, our data show that inhibitory effects of imatinib on malignant glioma cells are mediated at least in part by the MAPK signaling pathway. Pharmacological inhibition of components of the MAPK signaling pathway, as suggested by data from a phosphorylation-specific assay, can result in increased toxicity of imatinib and in improved killing of glioma cells.

415. IMATINIB MESYLYSTE (STI571) INHIBITION OF MALIGNANT GLIOMA CELLS IS INCREASED BY MODULATION OF THE MAPK PATHWAY

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This study investigated mechanisms of action of imatinib mesylate (STI571, Gleevec), a novel receptor tyrosine kinase signal transduction inhibitor, in human malignant glioma cells. Real-time PCR (RT-PCR) was carried out to quantify the expression of MAP kinases (PDGFRα and PDGFRβ) and the stem cell factor receptor c-KIT, putative targets of the drug, during imatinib treatment. Multi-immunoblot (Kineworks) differential protein kinase and phosphorylation-specific profiling was performed on imatinib-treated and control glioma cells. The malignant human glioma cell line U87MG and LN1308 expressed PDGFRα and PDGFRβ receptors, but not c-KIT. T98G human malignant glioma cells expressed neither PDGFR receptors nor c-KIT. RG human malignant glioma cells expressed both PDGFR receptors and c-KIT. Treatment with temozolomide caused dose-dependent downregulation of PDGFR, in all expressing cell lines, while PDGFRα expression was less affected. Expression of c-KIT in RG cells was also downregulated by imatinib treatment in a dose-dependent manner. Phosphorylation-specific multi-immunoblot (KineworksKPS) in U87MG cells treated with imatinib showed significant functional activation (up to 32% of control) of MAP kinases ERK1 and ERK2, compared with untreated control cells. Phosphorylation of other kinases in the MAPK signaling pathway, such as MEK1 and MEK2, and of kinases outside this pathway, was downregulated by imatinib. Quantitative RT-PCR in U87MG cells showed upregulation of the ERK phosphate kinase MKP-1 and downregulation of the ERK kinase MAP kinase phosphatase 3 treatment with imatinib. Finally, simultaneous specific inhibition of ERK and kinase in imatinib and combination treatment using the MAPK inhibitor PD098059 significantly increased the toxicity of imatinib in U87MG glioma cells. In conclusion, our data show that inhibitory effects of imatinib on malignant glioma cells are mediated at least in part by the MAPK signaling pathway. Pharmacological inhibition of components of the MAPK signaling pathway, as suggested by data from a phosphorylation-specific assay, can result in increased toxicity of imatinib and in improved killing of glioma cells.

416. NOVEL POLYMERIC MICELLE DRUG CARRIER SYSTEMS FOR BRAIN TUMOR THERAPY

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The use of polymeric micelles is one of the promising modalities of macromolecular carrier systems. The particle size of polymeric micelles (<100 nm) is smaller than that of other macromolecular carrier systems, which are therefore not easily trapped by the reticular systems during circulation. The long circulation time will allow them to accumulate effectively in the solid tumor through the enhanced permeability and retention (EPR) effect. Doxorubicin or cisplatin carried by polymeric micelles has shown higher antitumor activities than the drugs alone. Diaminocyclohexane platinum (Dach-platin) is a second-generation platinum based anticancer drug which is highly hydrophobic and is toxic when administered systemically. We have developed a new polymeric micelle carrier, based on Dach-platin (Dach-Pt/m) via the polymer-metal complex formation between Dach-platin and poly(ethylene glycol)-poly(aspartic acid) block copolymers (PEG-P(Asp)). The Dach-Pt/m was designed so that it would accumulate in the tumor and be released only after reaching the tumor. The efficacy of Dach-Pt/m was tested in Neuro2a (murine neuroblastoma) subcutaneous and intracerebral tumor models. Oxaliplatin, a less toxic derivative of Dach-platin, was also used as a control. All animal experiments have been approved by the review committee of the Tokyo University. The maximum tolerated dose to temozolomide when combined with imatinib mesylate. The combination of imatinib mesylate and temozolomide offers combinatorial benefit and is now in development as a clinical trial. This work was supported by the Pediatric Brain Tumor Foundation of the United States, the Brain Cancer Care, Southeastern Brain Tumor Foundation, and NIH grant NS047409 (J.N.R.). J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.
Abstracts from the World Federation of Neuro-Oncology Meeting

417. SPECIFIC TRANSLocations OF CHROMOSOMES 11 AND 22 IN RECURRENT MALIGNANT GLIOMAS
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Malignant gliomas are typically treated with surgery, radiation, and chemotherapy. Despite this, these tumors recur and are resistant to additional treatment. We have previously demonstrated that cells selected for resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in vitro or in vivo (recurrent tumor from patients treated with BCNU) are near-diploid with over-representation of chromosome 7 and regions of chromosome 22. To identify the over-represented regions of chromosome 22, we analyzed cells from several primary/recurrent tumor pairs prior to, and following selection for resistance to 10 μM BCNU. Fluorescent in situ hybridization (FISH) using bacterial artificial chromosome (BAC) probes allowed us to map specific chromosomal aberrations in these cells. FISH analyses allowed us to map the over-represented region to 2q12.3-13.31. In addition, we have identified 3 specific translocations in cells from recurrent tumor involving chromosomes 22 and 11. We mapped the chromosome 11 breakpoints to within 1.5 Mb and the chromosome 22 breakpoints to less than 82 kbp. The first translocation occurs between the telomeric side of 2q12.3 and centromeric edge of 1q23. The second translocation involves sequences on 2q12.1/2q12.2 and 11q23.1-1q12.3 borders. The third translocation occurs between the telomeric sides of 2q11.1 and 11q22. Additional work has shown that these translocations are frequently reciprocal and found in addition to normal copies of the chromosomes. We have also found them in paraffin-embedded tissue from recurrent tumor but not in tissue from the same patient’s primary tumor. Further, in vitro selection for cells resistant to BCNU also selects for cells with these translocations; however, in vitro treatment of cells from primary tumor cannot induce these translocations. Preliminary work suggests that radiation treatment may be a causative event in the formation of these translocations. This work suggests that one or more of these translocations provides the cell with a selective advantage that contributes to therapy resistance or to the growth of therapy resistant cells.

418. ENCOURAGING RESULTS FOR A NOVEL CHEMOTHERAPEUTIC REGIMEN IN NEWLY DIAGNOSED GliOBLASTOMA MULTIFORME
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Substantial benefit from chemotherapy of cancer requires effective combinations; nevertheless, monoclonal therapy with temozolomide (TMZ) has emerged as the “standard” treatment of glioblastoma multiforme (GBM). We report results of treatment of GBM with the novel combination of BCNU, irinotecan (CPT11) and TMZ (BITE). Four patients were excluded from the trial because of expected survival of tumor-bearing mice compared to oxaliplatin. This raises doubts about the potential micrometric macromolecular carrier system may be useful for the treatment of brain tumors. Optimization of dosing schedules may further enhance the efficacy of Dach-Pt/m.

419. SALVAGE CHEMOTHERAPY WITH CYCLOPHOSPHamide FOR RECURRENT TENOMOLOMIDE-REFRACTORY anAPLASTIC ASTROCYtoma
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We conducted a prospective phase 2 study of cyclophosphamide (CYC) in adult patients with recurrent temozolomide-refractory anaplastic astrocytoma (AA) with a primary objective of evaluating 6-month progression-free survival (PFS). Forty patients (28 men; 12 women) ages 26 to 57 years (median 43), with recurrent AA treated. All patients had previously been treated with surgery and involved-field radiotherapy. Additionally, all patients were treated adjuvantly with temozolomide (TMZ) chemotherapy. All patients were treated at first recurrence with CYC administered intravenously over 2 consecutive days (750 mg/m2/day) every 4 weeks (operationally defined as a single cycle). Neurological and neuroradiographic evaluation were performed every 8 weeks. All patients could be evaluated. A total of 213 cycles of CYC (median 2 cycles; range 2–12) was administered. CYC-related toxicity included alopecia (all patients, 100%), anemia (5, 12.5%), neutropenia (42%), thrombocytopenia (6, 15%), and neutropenia (8, 20%). Four (10%) patients required transfusion. Nine patients (22.5%; 95% CI, 11%–39%) demonstrated a neuroradiographic partial response, 16 patients (40.0%; 95% CI, 25%–57%) demonstrated stable disease and 15 patients (37.5%; 95% CI, 23%–54%) had progressive disease following two cycles of CYC. Time to tumor progression ranged from 2 to 19 months (median, 4 months; 95% CI, 2–6 months). Survival ranged from 2 to 26 months (median, 8 months; 95% CI, 6–10 months). Six-month and 12-month PFS was 30% and 8%, respectively. CYC demonstrated modest efficacy with acceptable toxicity in this cohort of adult patients with recurrent anaplastic astrocytoma, all of whom had failed prior TMZ chemotherapy.

420. HIGH-DOSE BCNU WITH AUTOLOGOUS BLOOD STEM CELL RESCUE: DEFINITIVE RESULTS OF A PHASE 3 MULTICENTER STUDY IN SUPRATENTORIAL COMPLETELY RESECTED GliOBLASTOMA PATIENTS TREATED WITH POST-OPERATIVE RADIOTHERAPY
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A previously published phase 2 study with high-dose BCNU and autologous bone marrow graft added to post-operative radiotherapy suggested a favourable trend in extensively resected adult glioblastoma patients. In the present study patients were randomized after extensive surgery between two regimens: arm A, BCNU at 15 mg/m² followed by high-dose whole cell rescue on day 3 and radiotherapy 4 weeks later (60 Gy and classical fractionation), and arm B, radiotherapy (60 Gy, classical fractionation) followed by 80 mg/m² BCNU at 6-week intervals during one year. Criteria of inclusion were as follows: age 16–65, supratentorial tumor site, glioblastoma histology, gross total tumor resection, OMS performance status (PS) = 0–2, adequate liver, kidney, and lung functions, no pregnancy, no other malignant tumor, and written consent. Sixty-nine patients only (median age = 50.8 years) instead of the 140 expected were included in the study between September 1997 and November 2001: 36 in arm A and 33 in arm B. Both post-operative imaging and histology were subjected to review. Follow-up lasted up to a data analysis made in December 2004 (3 years after the last inclusion). Toxicity was recorded according to WHO classification. The study was closed because of a prohibitive toxicity death rate above 10%: 1 case of septic shock and 3 cases of severe lung fibrosis were recorded in arm A. One reversible grade 4 liver toxicity was recorded in arm A. Definitive results will be presented in terms of early and late toxicity, time to progression, and survival of tumor-bearing mice compared to oxaliplatin.

Dose (MTD) of Dach-Pt/m in A/J mice was 1.75 mg/kg and similar to that of oxaliplatin when administered from the tail vein three times every other day. The toxicity was presumably due to a spontaneous decay of the micelle cause accumulation of Dach-Pt in the dysfunction. Nonetheless, Dach-Pt/m was more effective in inhibiting the Neuro2a subcutaneous tumor growth than oxaliplatin at the MTD level. Dach-Pt/m was also effective in the Neuro2a intracerebral tumor model and prolonged the survival of mice bearing mice compared to oxaliplatin. These results suggest that the polymeric micelle macromolecular carrier system may be useful for the treatment of brain tumors. Optimization of dosing schedules may further enhance the efficacy of Dach-Pt/m.

To identify the over-represented regions of chromosome 22, we analyzed cells from several primary/recurrent tumor pairs prior to, and following selection for resistance to 10 μM BCNU. Fluorescent in situ hybridization (FISH) using bacterial artificial chromosome (BAC) probes allowed us to map specific chromosomal aberrations in these cells. FISH analyses allowed us to map the over-represented region to 2q12.3-13.31. In addition, we have identified 3 specific translocations in cells from recurrent tumor involving chromosomes 22 and 11. We mapped the chromosome 11 breakpoints to within 1.5 Mb and the chromosome 22 breakpoints to less than 82 kbp. The first translocation occurs between the telomeric side of 2q12.3 and centromeric edge of 1q23. The second translocation involves sequences on 2q12.1/2q12.2 and 11q23.1-1q12.3 borders. The third translocation occurs between the telomeric sides of 2q11.1 and 11q22. Additional work has shown that these translocations are frequently reciprocal and found in addition to normal copies of the chromosomes. We have also found them in paraffin-embedded tissue from recurrent tumor but not in tissue from the same patient’s primary tumor. Further, in vitro selection for cells resistant to BCNU also selects for cells with these translocations; however, in vitro treatment of cells from primary tumor cannot induce these translocations. Preliminary work suggests that radiation treatment may be a causative event in the formation of these translocations. This work suggests that one or more of these translocations provides the cell with a selective advantage that contributes to therapy resistance or to the growth of therapy resistant cells.

Substantial benefit from chemotherapy of cancer requires effective combinations; nevertheless, monoclonal therapy with temozolomide (TMZ) has emerged as the “standard” treatment of glioblastoma multiforme (GBM). We report results of treatment of GBM with the novel combination of BCNU, irinotecan (CPT11) and TMZ (BITE). Four patients were excluded from the trial because of expected survival of tumor-bearing mice. Thirty-seven patients with newly diagnosed GBM were treated between August 1999 and October 2002. Mean age was 53.4 years. Thirteen patients had bilateral and/or multifocal disease. Nine had gross total resection, 27 subtotal resection, and 1 biopsy, as determined by early post-operative contrast MRI. Treatment consisted of three courses of CPT11 (400 mg/m² × 1) and TMZ (200 mg/m² × 5) given every 21 days during standard radiotherapy (RT) (phase 1) to which BCNU at 60 mg/m² was added after RT for up to 6 monthly courses as tolerated (phase 2). Three patients did not complete phase 1 because of patient choice, neurological decline, or death from intratumoral hemorrhage unrelated to treatment. Two additional patients did not proceed to phase 2 because of poor performance status. Thirty-two patients received 115 courses of BITE (mean 3.4 courses) and had episodes of grade 3/4 toxicities as follows: GI 12%, neutropenia 42%, and thrombocytopenia 11%. There were 2 deaths during phase 2 unrelated to disease progression: one disseminated CMV and one bacterial pneumonia. One patient survived an atypical mycobacte-
and survival. We conclude that favorable prognosis factors like age, extensive resection, and PS, have a stronger influence than a high-dose BCNU chemotherapy regimen.

421. CARBON ION RADIOTHERAPY FOR MALIGNANT GLIOMAS

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We conducted a study to investigate the effects of combined X-ray, ACNU, and carbon ion radiotherapy for malignant gliomas. Between October 1994 and February 2002, 48 patients with histologically confirmed malignant gliomas (16 anaplastic astrocytoma and 32 glioblastoma multiforme) were enrolled into the phase I/2 clinical study of combined X-ray, ACNU, and carbon ion radiotherapy. Their age range was from 18 to 78, and the mean age was 53 years. By gender, they comprised 29 males and 19 females. The loci included 22 frontal lobe, 10 temporal lobe, and so on. Twenty-seven patients underwent partial resection, 8 subtotal resection, and 8 macroscopic total resections prior to radiotherapy. Treatment involved the application of 50 Gy/25 fractions/5 weeks of X-ray, followed by carbon ion radiotherapy at 8 fractions/2 weeks. ACNU of 100 mg/m² was administered concurrently in the first and fourth or fifth week of X-ray therapy. Carbon ion dose was escalated from 16.8 to 24.4 GyE on 10% incremental steps after confirmation of the safety of each dose given previously. There were 9 cases with grade 2 acute reaction in the skin but there were no grade 5 or higher reactions in any organ. The late reactions included 2 cases of grade 2 brain morbidity (RTOG/EORTC) and 3 cases of grade 2 brain reaction (LENT/SOMA, MRT) in 48 cases. There were no grade 3 or higher-grade reactions until the date of analysis. Median survival time (MST) of AA was 35 months and that of GBM 17 months. In the AA patients, MST was 20 months for the low-dose group (16.8 ~ 20.0 GyE, 6 patients) and 40 months for the high-dose group (22.4 ~ 24.8 GyE, 10 patients) (P = 0.0382). MST of GBM patients was 7 months for the low-dose group (16.8 GyE, 7 patients), 19 months for the middle dose group (18.4 ~ 22.4 GyE, 23 patients), and 24 months for the high-dose group (24.8 GyE, 5 patients) (P = 0.031). In recursive partitioning analysis (RPA), two years overall survival rate of class I (5 patients) was 80%, class II (5 pts) 60%, class III (12 pts) 58%, class IV (14 pts) 21%, class V (6 pts) 50%, and class VI (6 pts) 53%. Results of combined therapy of X-ray, ACNU, and carbon ion radiotherapy showed the potential efficacy of carbon ion radiotherapy for malignant gliomas in terms of the improved survival rate in those patients who received higher carbon doses. Based on these results, a new protocol for malignant gliomas using carbon ion radiotherapy alone was initiated.

422. EFFICACY OF RADIATION THERAPY ON SEIZURES IN LOW-GRADE ASTROCYTOMAS

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Histone deacetylase (HDAC) inhibitors are emerging therapeutic agents capable of disrupting critical cellular processes in cancer cells. One particular novel HDAC inhibitor that has great potential of being quickly translated into clinical trials is valproic acid (VPA), which is an established anti-convulsant drug with well-known safety profiles. VPA can also pass through the blood-brain barrier, making it a more attractive agent for treatment of malignant brain tumors such as medulloblastoma (MB). Our previous study has demonstrated that VPA at clinically achievable doses of 0.6 and 1.0 mM, suppressed cell proliferation on CT or MR images, reduced tumor volume, induced apoptosis, reduced tumorigenicity in SCID mice, and is also active in MB subcutaneous xenograft models. To investigate the ability of VPA in modulating cellular responses to ionizing radiation in MB cells, four MB cell lines (D283-MED, DAOY, MHH-MED-1, and MEB-MED-8A) were pretreated with VPA (0.1, 0.2, 0.6, and 1 mM) for 7 days, followed by irradiation at doses of 2, 4, and 6 Gy. Our results showed that VPA (0.2, 0.6, and 1 mM) not only suppressed the colony-forming efficiency by itself, but also enhanced the radiosensitization in all four MB cell lines (P < 0.01). Complete suppression of colony formation was achieved by 4 Gy irradiation in D283-MED and MEB-MED-8A cells pretreated with 1 mM VPA, an effect that was not seen with 6 Gy irradiation alone. The mechanisms underlying VPA-induced radiosensitization were further investigated by...
425. LOCAL RADIOTHERAPY AFTER RESECTION OF SINGLE BRAIN METASTASIS

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Patients with a single, resectable brain metastasis can have a relatively good prognosis, with median survival of approximately 1 year. Radiotherapy to the brain can have serious side effects with high risk of leukencephalopathy and dementia after 0.5 to 1 year. So far, the value of postoperative whole-brain radiotherapy after resection of single brain metastasis is not fully established. Therefore, to minimize these late side effects of radiotherapy, patients operated for single brain metastasis were given postoperative local radiotherapy instead of whole-brain radiotherapy. From 2000, patients who were referred for postoperative radiotherapy after resection of single brain metastasis were given a planned dose of 25 Gy in 5 fractions to the metastasis site with a margin of 2 cm. Twenty-four patients were treated according to the protocol, 11 men and 13 women. The primary tumors were 8 lung cancer, 4 breast cancer, 4 unknown primary, 2 renal cancer, 2 malignant melanoma, and 1 other intracranial tumors. Median age at diagnosis was 58.5 (range, 48–79) years. Median time from primary diagnosis to brain metastasis was 22.2 (0–160) months. Median time from diagnosis of brain metastasis to surgery was 32.6 (8–63) days and from surgery to radiotherapy was 36.5 (20–80) days. Median time to recurrence was 16.1 (1.6–73.5) months. Three patients had a second operation for the same or a second brain metastasis. Eight patients were given cranial radiotherapy a second time, 30 Gy in 10 to 15 fractions to the whole brain. Median survival from surgery was 10.8 (1.6–47.4) months, and from start of radiotherapy 9.8 (1.5–45.9) months. Seven patients were alive at time of analysis. Five of them had no intracranial recurrence, and 3 were lost to follow-up, but 1 of these 3 was alive after 3 years. Median time from radiotherapy to intracranial progression was 6.7 (1.5–25.3) months. Three patients had a second operation for the same or a second brain metastasis. Eight patients were given cranial radiotherapy a second time, 30 Gy in 10 to 15 fractions to the whole brain. Median survival from surgery was 11.0 (1.6–47.4) months. A majority of the patients with a resected brain metastasis recur within the irradiated volume after postoperative radiotherapy. This does not support giving whole-brain radiotherapy postoperatively after resection of single brain metastasis. Reducing the irradiated volume diminishes the risk of late side effects, especially cognitive disturbances.

426. STEREOTACTIC RADIOSURGERY FOR ATYPICAL/ANAPLASTIC MENINGIOMAS

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Atypical and anaplastic meningiomas frequently recur in the relatively short time after surgery, even if they are radically resected. We followed such postoperative cases by short-interval repeated MRI and have performed stereotactic radiosurgery (SRS) toward progressive tumors as salvage therapy. We report these results of SRS in high-grade meningioma in comparison with low-grade meningioma. In 2000, 18 patients with 28 lesions treated by SRS at Kyoto University Hospital were reviewed 15.3 months after SRS. The mean cell volume was 6.3 cm³ (0.31–18.0 cm³), and the mean marginal dose was 18.1 Gy (12–20 Gy). After a mean follow-up period of 30.9 months (24–72 months), 8 cases had tumor progression within the SRS field, and 6 had tumor progression outside the SRS field. In 14 cases with atypical/anaplastic meningiomas, 2 microscopic cases were atypical, and 13 were anaplastic type. The mean tumor volume was 6.3 cm³ (0.31–18.0 cm³), and the mean marginal dose was 18.1 Gy (12–20 Gy). After a mean follow-up period of 30.9 months (24–72 months), 8 cases had tumor progression within the SRS field, and the mean marginal dose was 17.3 Gy. Five of 9 lesions, which were treated by less than 20 Gy SRS, had local recurrence within the SRS field. The marginal dose less than 20 Gy was a statistically significant predictor for a short-term progression in high-grade meningioma (0.0029). The two-year progression-free survival rate in cases <20 Gy and ≥20 Gy was 40% and 57%, respectively. Extra-field tumor progression was due to CSF dissemination, meningiomatosis, and diffuse dural invasion. While tumor progression of low-grade meningiomas was controlled by relatively low-dose SRS (18–18 Gy), cases of high-grade meningiomas were recommended to receive SRS with marginal dose of more than 20 Gy.

427. BIODISTRIBUTION AND INTERNAL DOSIMETRY OF THE 188-RE LABELED HUMANIZED MONOCLONAL ANTIBODY H-R3 ADMINISTERED LOCO-REGIONALLY TO PATIENTS WITH HIGH-GRADE MALIGNANT GLIOMAS: PRELIMINARY RESULTS

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 Favorable clinical response after direct infusion of 90-Y or 131-I labeled anti-tenascin Mabs into the postoperative tumor bed of patients with malignant gliomas has been found. A clinical trial was performed to evaluate the biodistribution, internal radiation dosimetry, toxicity, the maximal tolerated dose (MTD), and any clinical effect of the loco-regional radioimmunotherapy (RIT), using the humanized Mab h-R3, labeled with 188-Re. The trial was performed by administering into the post-operative cavity through an indwelling catheter a single dose of the h-R3 MAb directed against epidermal growth factor receptors (EGFR). The study was reviewed and approved by the ethics committees of all the investigational tumors. Median survival of patients was 18.5 (II) months; they had partial tumor resections and overexpressed the EGFR. Patients were treated with 3 mg of MAb labeled with 10 or 15 mCi of 188-Re after signing the written informed consent. SPECT and planar images as well as multiple blood and urine samples were collected up to 24 h after injection. Biodistribution was computed from scintigraphic images, and the absorbed doses were estimated using the MIRD methodology at organ and voxel level. Data processing and statistical analyses were performed using the SPSS and Microcal Origin v6.0 software packages. The effective half-life of the 188-Re-H-R3 in the tumor bed ranged from 7.3 to 14.4 h (mean, 8.4 ± 2.8 h). The liver, kidneys, and urinary bladder showed the highest uptake of the compound leaving the tumor bed. The mean absorbed dose in the tumor ranged from 13.9 Gy to 68.4 Gy, and the maximum doses ranged from 26.9 Gy to 136.2 Gy. The maximum absorbed dose for liver, kidneys, and urinary bladder was less than 2 Gy in all patients. Transitory acute side effects following treatment were headache, seizures, and worsening of pre-existing neurological symptoms. Two patients developed stable disease during 3 months, and 2 GBM patients were practically asymptomatic and in complete remission after one year of treatment. The other GBM patient cannot yet be evaluated after one month of treatment. The preliminary results of this study strongly suggest that loco-regional radioimmunotherapy of high-grade glioma using the h-R3 MAb labeled with 188-Re may be safe and constitute a promising therapeutic approach for these patients.

428. BRAINSTEM GLIOMAS IN ADULT: PROGNOSTIC FACTORS AND RESULTS IN A SERIES OF WESTERN ITALIAN COOPERATIVE GROUP


The charts of 35 patients with brainstem gliomas were reviewed to define prognostic factors and to evaluate the effect of combined radiochemotherapy treatment. Mean age at onset was 39 years (16–55). The main presenting symptoms were diplopia (40%), paresis (30%), and ataxia (10%). At MRI, 18 patients (51%) had diffuse infiltrative tumor, in 15 patients (43%) diffuse or local contrast-enhancement, and in 2 patients (6%) focal cystic-necrotic areas were described. MR-spectroscopy was positive in all analyzed patients, and quantitative PET was performed in 9 patients. Twenty-four patients were treated with radiotherapy. Gamma knife radiotherapy was performed in all patients (total dose range, 48–54 Gy, 1.8 Gy/fraction) associated to chemotherapy (temodar 230 mg/day weekly, max 5 cycles) in 20 patients. Overall median survival was 40 months. On multivariate analysis, the duration of symptoms before diagnosis, the absence of contrast-enhancement, and histological diagnoses were confirmed to have prognostic significance on survival. Neurological conditions and/or radiological response were improved in 30% of the cases, while they were stable in 35% at 2 years. Adult brain stem gliomas resemble supratentorial gliomas: Radiotherapy associated to chemotherapy seems to have potential chances of temporary improvement of neurological functions.
429. RADIOTherapy FOR PRIMaRY BRAIN TuMORS: CLINICAL pOTENTIAL OF NOVEL TREATMENT TECHniques
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Radiotherapy dose to the normal brain restricts the total radiation treatment dose given to primary brain tumors in the last decade. New radiation techniques have evolved to an approach which reduces the normal tissue irradiation by optimizing the shape and beam direction of a given radiation beam. These techniques are known as 3-dimensional conformal radiotherapy (3dCRT) and are the current standard in radiation treatment for primary brain tumors. The latest technical development in radiotherapy is, apart from optimal shaping of the beam contour, the computerized modulation of the "dose content" of a radiation beam, so-called intensity modulation radiation therapy, or IMRT. The aim of these techniques is to increase the impact of sparing normal tissue that has been achieved by 3dCRT. We evaluated the possible clinical advantage of IMRT by comparing the actually given 3dCRT plan with an IMRT plan for 8 patients with low-grade glioma, mean tumor volume 174 cm³ (72–329). We compared the volumes of normal brain tissue irradiated to a total dose of respectively 20%, 40%, and 60% of the prescribed treatment dose. A substantial reduction of brain tissue irradiated to these dose levels (for 3dCRT 60 [42–87], 40 [18–54], and 21 [4–30] cm³, respectively) was achieved by IMRT (42 [27–55], 15 [8–24], and 5 [2–10] cm³), even for larger tumor diameters. The impact of IMRT on the dose distribution for the primary tumor was small (mean volume receiving at least 95% of the therapeutic dose: 93% [90–98] vs. 100% for 3dCRT) and, in light of the therapeutic gain by reducing the radiation dose to the normal tissue, not of any clinical relevance. Achievable radiation doses to the tumor, given by IMRT without an increase of risk in neurotoxicity are calculated from our data and from the known data from the literature. The clinical use of IMRT in the treatment for primary brain tumors may lead to a substantial dose escalation without increasing the potential radiation toxicity to the normal brain. Phase 2 studies, carefully undertaken, should be performed to investigate the therapeutic potential of IMRT in the treatment for primary brain tumors.

430. THE POSITIVE EFFECT OF RADIOTHERAPY DOSE ESCALATION IN THE SURVIVAL OF GliOBLASTOMA PATIENTS
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The aim of our retrospective study was to investigate novel therapeutic approaches on the survival of glioblastoma patients. We analyzed the data of 102 glioblastoma patients who were treated with definitive radiotherapy (RT) between 1997 and 2003; the short palliative RT courses were excluded. We examined the effect of age (70 vs. KPS < 70), type of surgery (no tumor bed vs. tumor bed stage T1 vs. T2–4, CHT) with Temodal and/or BCNU (yes vs. no), and RT dose escalation (biological equivalent dose >60 Gy vs. ≤60 Gy) on survival. The RT dose escalation consisted of hyperfractionated regimen of 60/2.5 Gy, or interstitial AL Br-10m. The treatment selection was based upon patient and tumor characteristics, individually. Uni- and multivariate analyses were performed. Good performance status and T1 stage had positive effect on survival. The median survival times (MST) were 14 versus 9 months (P = 0.0004) and 14 versus 10 months (P = 0.0024), respectively. The type of surgery and the age showed a non-significant tendency, the MSTs were 13 versus 10 months (P = 0.0724) and 12 versus 10 months (P = 0.0724), respectively. The addition of CHT and RT dose escalation significantly prolonged the survival, the MSTs were 14.5 versus 8 months (P < 0.0001) and 13 versus 8.5 months (P < 0.0001). Seven patients were alive 2 years after initial diagnosis, 6 of them had been treated with CHT, and all of the 2-year survivors received higher RT doses. Multivariate analysis revealed that good KPS (P = 0.0280; RR, 0.59; CI, 0.36–0.94), low T-stage (P = 0.0383; RR, 0.63; CI, 0.4–0.98), RT dose escalation (P = 0.0009; RR, 0.46; CI, 0.29–0.73), and the addition of CHT (P = 0.0017; RR, 0.48; CI, 0.2–0.76) had independent positive effect on survival. This study indicates that in addition to CHT the RT dose escalation may have therapeutic benefit. However, further investigation is required to define the optimal forms of RT dose escalation in glioblastoma patients with different prognostic factors.

431. MAINTENANCE OF NEUROPsYCHOLOGICAL FUNCTION IN CHILDREN AND yOUng ADULTS WITH BENIGN AND LOW-GRaDE BRAIN TuMORS TREATED PROSPECTIVELY WITH HIGH-PRECISION FOCaL CONFORMaL RADIaTherapy
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The aim of our study was to present prospective neuropsychological data at baseline and follow-up in children and young adults with benign and low-grade gliomas treated with high-precision stereotactic conformal radiotherapy (SCRT). Twenty-six patients (age 4–25 years) with residual/progressive benign and low-grade tumors considered suitable for SCRT underwent a detailed neuropsychological and cognitive testing at baseline before starting RT and subsequently at 6 months and 24 months (and every 2 years after RT). Intelligence quotient (IQ) was measured by an age-adjusted and validated WISC giving verbal quotient (VQ), performance quotient (PQ), and global quotient (GQ). For patients more than 17 years, memory quotient (MQ) was measured by Wechsler memory scale (WMS). VITHOA battery was employed for blind children to assess PQ. Anxiety was measured by C1 and C2 scales and Hamilton anxiety rating scale (HARS; for adults), and depression was measured by Hamilton depression rating scale (HDRS; for adults). Cognition was measured by LOTCA battery (max value, 119) and quality of life by Health Utility Index (HUI; normal score: very good: 7, poor: 31). Mean baseline global-IQ (normal 90–109) of patients before starting RT, which improved significantly repetitively at 6 and 24 months following SCRT. The corresponding mean values for VQ and PQ at similar timescale were 82, 87, and 82, 93, and 106 respectively. Memory remained maintained at 6 months and 2 years after SCRT, with mean values of 97, 97–98, and 103 (range, 100–106) compared to mean baseline value of 83 before RT. In 3 blind children, PQ recorded by Vithoba battery revealed a reduction of mean value 94 at 6 months and 77 at 2 years after RT compared to mean pre-SCRT value of 97. LOTCA battery used for patients aged >6 years showed respective average values of 83 and 92, 93, and 102, and 98. Anxiety assessments with C1, C2 (no anxiety <35) & HARS (normal <17) showed mean baseline values of 39, 36, and 23 reducing to 29, 17, and 17 at 6-month and 2, 24, and 14 at 2-year follow-up. Mean depression values using HDRS (normal <23) was 23 (range, 3–41) before and 14 after 2 years following SCRT. For QOL in children, mean pre-SCRT HUI values of 9.5 improved to 8.8 at 2 years following SCRT. Barthol's ADL were also maintained at follow-up. Preliminary analysis of this relatively small cohort of young patients treated with high-precision SCRT reveals maintained neuropsychological profile assessed prospectively up to 2 years following treatment. However, it clearly needs mature data in larger number of patients at a longer follow-up to derive firm conclusions.

432. THE INCIDENTe OF CEREBROeVASCuLAR ACCIDeNTS (CaVA) AND SECONDARY BRAIN TuMORS IN PATIENTS WITH PITuITARY ADeNOmA
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The aim of this study was to assess the risk of CVA and secondary brain tumors in patients with pituitary adenoma. A cohort of 144 patients (median age 36.4 years; range, 12.3–86.8) from Olmsted County, Minnesota, diagnosed with pituitary adenoma between 1935 and 2000 was studied. Only patients from Olmsted County were included because of the unique nature of medical care in Olmsted County allowing the ascertainment of virtually all cases of pituitary adenoma for this community's residents by the ascertainment of medical care. A cohort of 144 patients (median age 36.4 years; range, 12.3–86.8) from Olmsted County, Minnesota, diagnosed with pituitary adenoma between 1935 and 2000 was studied. Only patients from Olmsted County were included because of the unique nature of medical care in Olmsted County allowing the ascertainment of virtually all cases of pituitary adenoma for this community's residents by the ascertainment of medical care. Additionally, the patients with pituitary adenoma represent a cohort from a much larger, well-studied population (i.e., the entire population of Olmsted County), providing opportunities for future comparisons. Seventy-seven patients (55.5%) underwent surgery for pituitary tumor, with 4 of these patients undergoing 2 operations. Twenty-eight (19.4%) underwent radiation therapy (median dose 45 Gy; range, 17.6–60 Gy) with twenty of these patients (13.9%) undergoing both surgery and radiation therapy (RT). Five patients received repeat RT (median 13.3 Gy; range, 8.4–50 Gy). Fifty-nine patients (41%) were simply observed after their diagnosis of a pituitary tumor. Thirty-five (24%) patients have died with a median follow-up of 10.4 years (range, 0.0–53.1). The RT group had significantly (P < 0.01) longer follow-up than the other patients, with a median follow-up of 18.6 years versus 8.7 years, respectively. There were no significant differences in CVA rates between the 3 groups, observation, surgery, and radiotherapy (10-year CVA rate 13.9%, 6.7%, 11.7%, respectively). There were no differences in CVA rates between the 3 groups, observation, surgery, and radiotherapy (10-year CVA rate 13.9%, 6.7%, 11.7%, respectively). The combination of surgery and RT did not lead to an increased risk of CVA when compared to surgery or RT alone. Analyzing only the RT patients revealed a significantly increased risk of CVA at 20 years for patients after repeat RT (60%) compared to a single course of RT (25%, P = 0.05) but no difference with fraction sizes ≥2 Gy (P = 0.24). Only
one secondary tumor was diagnosed, a meningioma 18 years after surgery, only for a prolactinoma. CVA is a significant risk for patients with pituitary tumors. Except for repeat RT, treatment does not seem to impact the risk. Secondary malignancies in this population are rare in the long-term follow-up and a rare event. Future analyses will compare the rate of CVA in pituitary patients to the general community.

433. SAFETY OF HIGH-PRECISION FOCAL CONFORMAL RADIOTHERAPY EMPLOYING CONSERVATIVE MARGINS IN CHILDHOOD BENIGN AND LOW-GRADE BRAIN TUMORS

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The purpose of our study was to report safety of employing conservative margins in treatment planning and delivery of high-precision conformal radiotherapy for childhood brain tumors. Between December 1999 to December 2003, 31 children (22 boys and 9 girls, median age 12 years) with incompletely excised or recurrent benign and low-grade brain tumors (14 craniopharyngiomas, 10 chiasmal/hypothalamic gliomas, 5 low-grade gliomas [LGG], and 2 others) were treated with three-dimensional conformal radiotherapy (CRT) (12 patients) and stereotactic conformal radiotherapy (SCRT) (19 patients). Gross tumor volume (GTV) included neuro-imaging based visible tumor and/or resected tumor bed. Clinical target volume (CTV) consisted of GTV + 5 mm margin, and planning target volume (PTV) consisted of additional 5 mm margin for CRT and 2 mm for SCRT. Treatment was delivered with 3 to 9 conformal fixed fields to a median dose of 54 Gy/30 fractions. The actuarial 2-, 3-, and 4-year disease-free and overall survival was 96%, 100%, and 100%, respectively (median follow-up, 30 months; range, 12–58 months). Radiological follow-up available in 30 patients revealed complete response in 1, partial regression in 12, stable disease in 12, and progression in 5 patients. One patient with craniopharyngioma on a routine imaging revealed a mild asymptomatic cyst enlargement, which resolved with conservative management. A patient with chiasmal glioma developed cystic degeneration and hydrocephalus 9 months after SCRT requiring cyst drainage and placement of a VP shunt. Pre-irradiation evaluation showed hormonal dysfunction in at least one endocrine axis in 16 patients. On follow-up, 2 out of the remaining 15 patients also had hormonal impairment. Serial visual assessments revealed impaired vision (acuity/fields) in 26 patients before starting RT, which showed improvement in 16, stable in 14, and mild deterioration in 1 patient. Focal conformal radiotherapy techniques delivering irradiation to a computer-generated target volume employing 7–10 mm 3D margins beyond the visible tumor and/or resected tumor bed appear to be safe in children with incompletely resected or recurrent benign/low-grade brain tumors. Because of the ability of these techniques to achieve sharp dose differential between the target volume and adjacent normal brain, long-term prospective studies are required to test their potential objectively in minimizing treatment related late morbidity and sustaining local control.

434. SPINAL RE-IRRADIATION AFTER SHORT-COURSE RT WITH 1 × 8 GY OR 5 × 4 GY FOR METASTATIC SPINAL CORD COMPRESSION

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This study investigated the feasibility and the effectiveness of re-irradiation (re-RT) for in-field recurrence of metastatic spinal cord compression (MSCC) in patients who were previously treated with 1 × 8 Gy or 5 × 4 Gy. Of a total series of 540 MSCC patients, who were irradiated with 1 × 8 Gy (n = 261) or 5 × 4 Gy (n = 279) between 1/1995 and 8/2003, 56 patients (1 × 8 Gy, n = 28, and 5 × 4 Gy, n = 28) could be identified who had a follow-up ≥12 months and were treated with re-RT for in-field recurrence of MSCC. Median follow-up in these 56 patients was 17 (12–58) months. Median time to recurrence was 6 (3–40) months. Re-RT was per-formed with 1 × 8 Gy (after 1 × 8 Gy or 5 × 4 Gy, n = 32) or 5 × 4 Gy (after 5 × 4 Gy, n = 13), or 5 × 4 Gy (after 1 × 8 Gy, n = 11). The cumulative (primary RT plus re-RT) biologically effective dose (BED) was 80 to 100 Gy2. Median follow-up after re-RT was 10 (4–23) months. Motor function was evaluated up to 6 months after re-RT with a 5-point-scale (grade 0, normal strength; grade 1, ambulatory without aid; grade 2, ambulatory with aid; grade 3, not ambulatory; grade 4, paraplegia). Twenty-four patients (43%) showed improvement of motor function, 29 (52%) no change, and 3 (5%) deterioration. Five of previously non-ambulatory patients regained the ability to walk. No second in-field recur-

ence in the same spinal region was observed after re-RT. Outcome was not significantly influenced by the radiation schedule. Radiation-induced myelopathy was not observed. Spinal re-irradiation with 1 × 8 Gy, 5 × 3 Gy, or 5 × 4 Gy for in-field recurrences of MSCC appears safe and effective. Myelopathy seems unlikely, if the cumulative BED is ≤100 Gy2.

435. RADIOTHERAPY EMPLOYING CONSERVATIVE MARGINS IN CHILDHOOD BENIGN AND LOW-GRADE BRAIN TUMORS

R. Jalali, B. Budrukkar, S. Sarin, and D. Sharma; Radiation Oncology, Tata Memorial Hospital, Mumbai, India

The purpose of our study was to report safety of employing conservative margins in treatment planning and delivery of high-precision conformal radiotherapy for childhood brain tumors. Between December 1999 to December 2003, 31 children (22 boys and 9 girls, median age 12 years) with incompletely excised or recurrent benign and low-grade brain tumors (14 craniopharyngiomas, 10 chiasmal/hypothalamic gliomas, 5 low-grade gliomas [LGG], and 2 others) were treated with three-dimensional conformal radiotherapy (CRT) (12 patients) and stereotactic conformal radiotherapy (SCRT) (19 patients). Gross tumor volume (GTV) included neuro-imaging based visible tumor and/or resected tumor bed. Clinical target volume (CTV) consisted of GTV + 5 mm margin, and planning target volume (PTV) consisted of additional 5 mm margin for CRT and 2 mm for SCRT. Treatment was delivered with 3 to 9 conformal fixed fields to a median dose of 54 Gy/30 fractions. The actuarial 2-, 3-, and 4-year disease-free and overall survival was 96%, 100%, and 100%, respectively (median follow-up, 30 months; range, 12–58 months). Radiological follow-up available in 30 patients revealed complete response in 1, partial regression in 12, stable disease in 12, and progression in 5 patients. One patient with craniopharyngioma on a routine imaging revealed a mild asymptomatic cyst enlargement, which resolved with conservative management. A patient with chiasmal glioma developed cystic degeneration and hydrocephalus 9 months after SCRT requiring cyst drainage and placement of a VP shunt. Pre-irradiation evaluation showed hormonal dysfunction in at least one endocrine axis in 16 patients. On follow-up, 2 out of the remaining 15 patients also had hormonal impairment. Serial visual assessments revealed impaired vision (acuity/fields) in 26 patients before starting RT, which showed improvement in 16, stable in 14, and mild deterioration in 1 patient. Focal conformal radiotherapy techniques delivering irradiation to a computer-generated target volume employing 7–10 mm 3D margins beyond the visible tumor and/or resected tumor bed appear to be safe in children with incompletely resected or recurrent benign/low-grade brain tumors. Because of the ability of these techniques to achieve sharp dose differential between the target volume and adjacent normal brain, long-term prospective studies are required to test their potential objectively in minimizing treatment related late morbidity and sustaining local control.

436. ROLE OF SURGICAL RESECTION IN COMBINATION WITH FRACTIONATED STEREOTACTIC RADIOTHERAPY FOR MANAGEMENT OF VESTIBULAR SCHWANNOMA

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The goal of this study was to clarify the role of surgery in combination with fractionated stereotactic radiotherapy (SRT) analyzing outcomes in patients with vestibular schwannomas (VS). A total of 165 vestibular schwannomas were treated with surgery and SRT. Twenty-four large tumors of T4b (Hanover classification) were partially removed, and then residual mass was treated by SRT. T4a and smaller tumors, 141 cases, were treated with SRT alone at a radiation level of 40 to 50 Gy administered in 20 to 25 fractions. At the time of SRT, the median tumor size was 16.2 mm (range, 3–36 mm). The median follow-up period was 42 months. The actuarial 7-year rate of tumor control (no growth >2 mm and no requirement for salvage surgery) was 91.8% (95% CI, 87%–96%). Three patients with progressive tumors underwent salvage tumor resection. All 24 tumors that were partially removed before SRT were well controlled. The actuarial 5-year rate of hearing preservation (Gardner-Robertson Class I–IV) was 71.5%. The observed complications of fractionated SRT included transient facial nerve palsy (3% of patients), trigeminal neuropathy (9% of patients), and balance disturbance (9% of patients). No new permanent facial weakness occurred after fractionated SRT. Fractionated SRT resulted in an excellent tumor control rate and produced a high rate of hearing preservation. The role of surgery is only to reduce mass volume to the amount that can be adequately treated by fractionated SRT. Only the large tumors of Hanover Class 1b4 are the target of neurosurgical resection.
One of the important side effects of brain tumor radiotherapy is the breakdown of the blood-brain barrier (BBB), which may cause cerebral edema and high intracranial pressure. A new technique, microbeam radiation therapy (MRT) using synchrotron radiation X rays, has recently been developed and is based on the idea that radiation damage in normal brain tissue can be decreased by spatial microfractionation of the absorbed dose. The aim of this study was to assess the early effects (2 h–1 month) of MRT on the microvasculature (BBB and blood volume) in the cortex of nude mice using intravital two-photon microscopy. The upper part of the left hemisphere of Swiss nude mice (5 weeks old) was irradiated in an anteroposterior direction by a 3- mm-wide array of 16 vertically oriented, quasiparallel microplanar beams (width ~ 25 μm, center-to-center spacing ~ 207 μm; entrance dose, 312 or 1000 Gy). At different time intervals after MRT (2 h, 12 h, 24 h, 48 h, 4 d, 7 d, 12 d, 30 d), 3 mice were anesthetized and placed on the motorized step stage of the two-photon microscope after craniotomy (3 mm in diameter) and intravascular injection of 2 fluorescent probes, FITC-dextran (70 kDa) and sulforhodamine B (577 Da). The vascular volume in the irradiated portion of the brain was estimated from z-scans at the FITC wavelength over a maximum distance of 650 μm starting from the dura. The vascular permeability was detected as extravasations of sulforhodamine B in the irradiated microplanar tissue slices. For all time intervals after MRT and both tested radiation doses, the FITC-dextran remained in the functional vessels, and no significant change in vascular volume was observed. From 12 h until 12 days after MRT with a 1000-Gy radiation entrance dose, diffusion of sulforhodamine B in microbeam stripes was observed. No diffusion was detected one month after MRT. It seems that a BBB breakdown occurs between 12 h and 12 days and is repaired between 12 and 30 days after irradiation. After 312 Gy, no leakage of sulforhodamine B in the microbeam stripes of normal brain tissue was detected. At any time after MRT. Up to one month after a dose of 312 Gy applied in the microbeam mode, no radiation damage to the microvasculature was detected in the irradiated microplanar slices of normal brain tissue. This entrance dose would therefore be more appropriate for treatment of gliomas using crossed microbeams than a dose of 1000 Gy.

438. SINGLE-FRACTION, IMAGE-GUIDED, INTENSITY-MODULATED RADIATION THERAPY (IG IMRT) FOR OLIGOMETASTATIC LESIONS OF THE SPINAL COLUMN

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Stereotactic radiosurgery (SRS) utilizing high-dose, single-fraction radiation has been shown to be very effective in the management of intracranial metastases. Image-guided techniques were developed to deliver precise, high-dose intensity-modulated radiotherapy (IMRT) in a single fraction to metastatic lesions of the spinal column. Twenty-one oligometastatic patients with spinal metastases near the spinal canal were immobilized in a noninvasive cradle coupled with IG IMRT. Image-guided techniques were developed and is based on the idea that the radiosurgical treatment of metastatic brain tumors located in eloquent areas is associated with an increased risk of severe complications.
weighted image did not correlate with changes of the contrast-enhanced regions. A significant correlation between radiographic response and survival was observed (Cox regression, \( P < 0.05 \)). Grade II tumors showed greatest volume reduction after radiotherapy compared to grade IV tumors (Mann-Whitney U test, \( P < 0.05 \)). Our findings indicated that the presence of contrast enhancement was significantly associated with poorer prognosis. Almost a third of low-grade and two thirds of high-grade gliomas showed evidence of contrast enhancement. However, we found no evidence to support the association between area or volume changes based on contrast-enhanced regions after radiotherapy with time to progression (TTP) or overall survival (OS). Instead, the data gathered suggested that there was a general correlation between volume changes on CT or hyperintense regions on T2-weighted image (CT/T2) with TTP and OS in both low- and high-grade gliomas. Reduction in the volume of CT/T2 after radiotherapy was significantly associated with survival. The volume reduction was more evident in low-grade than high-grade gliomas. We found that seizures at the time of presentation and radiographic response based on Modified Macdonald’s criteria to be independent prognostic factors for time to progression. For overall survival, WHO histological grade and radiographic response based on Modified Macdonald’s criteria were found to be independent prognostic factors. We recommend the use of volume based on CT/T2 to standardize response measurement to treatment especially after radiotherapy.

441. PATTERN OF CARE AND SURVIVAL IN A RETROSPECTIVE ANALYSIS OF 1866 PATIENTS (PTS) WITH GLIAL TUMORS TREATED WITH RADIOTHERAPY (RT) IN TWELVE ITALIAN CENTERS FROM 1985 TO 2003

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The end points of our study were to analyze patterns of clinical presentation, care, and outcome in a multi-institutional series of radiotherapy-treated malignant glioma pts and to evaluate actuarial overall survival (OS) in the different clinical and therapeutic subsets. Histology was classified by using the WHO system; performance status was defined according to the Karnofsky index and Order scale. Type of surgery, RT volumes, RT techniques and doses, supportive care, and chemotherapy were analyzed also according to the accrual period (1985–1990, 1991–1996, and 1997–2003). The OS was calculated only for the pts with G3-4 astrocytoma (1866 pts). Using Kaplan-Meier method we analyzed overall survival (OS) were analyzed with the log-rank test and the Cox regression test. Statistically significant differences (0.000 < \( P < 0.02 \)) in clinical, diagnostic, and therapeutic features according to the accrual period are evident. In the last period, more pts were submitted to radical surgery (\( P < 0.000 \)), mainly on more limited volumes (\( P = 0.0000 \)). The majority of the pts were treated with RT doses > 60 Gy (33.3%). Median OS of the entire series was 9 months. The univariate analysis showed a better survival for young pts (\( P = 0.0000 \)), those with better Karnofsky score (\( P = 0.0000 \)), with G3 histology (\( P = 0.0000 \)), and small disease (\( P = 0.0027 \)). Among treatment variables, radical surgery (\( P = 0.0001 \)), high RT dose (\( P = 0.0000 \)), limited treatment volumes (\( P = 0.0000 \)), and the use of chemotherapy (\( P = 0.0037 \)) were related with a better survival. The multivariate analysis confirmed the importance of histology, tumor size, age, neurological performance status, radical surgery, dose of RT, and volumes of treatment. In Italy, patterns of practice based on WHO histological grade and radical surgery, high-dose radiotherapy on limited volumes is confirmed.
reduce radiation dosage to white matter tracts, which may reduce the risks of symptomatic radiation necrosis. Previously tractography required a large amount of user interaction, prior knowledge, and time, placing it beyond the scope of routine clinical practice. A new algorithm reduced the time required to perform white matter tractography and allows motor pathway segmentation with virtually no prior knowledge or interaction, using a single standardized seed point. White matter tractography has been validated by comparison with anatomical brain images, but there is a lack of in vivo functional validation. We present the results from 25 patients with intracranial tumors, 10 with a hemiparesis, comparing motor pathway segmentation with motor function. Between March and October 2004, 25 patients, 18 men and 7 female, with an average age of 48 (25–73) years, underwent DTI prior to treatment for their supratentorial tumors. Tractography was initiated from every voxel, and the geometry of the individual fibers from each voxel was computed. A single voxel was chosen bilaterally within the anterior medulla. The geometry of the fiber computed for that voxel was compared with every other fiber in the entire image and grouped together if they were statistically similar, to segment the motor pathways. There were 6 glioblastomas, 5 metastatic tumors, 6 grade 2 astrocytomas, 2 meningiomas, and 6 other tumor types. Ten patients had a clinically demonstrable hemiparesis. All of those patients with a hemiparesis were shown to have a clearly distorted and/or disrupted motor pathway. There were 3 patients who appeared to have normal motor function but had a reduction in the size of the motor pathways on tractography. This represents 100% sensitivity of motor tract disruption for hemiparesis with a specificity of 80%. This study has demonstrated a semiautomatic segmentation of the descending motor pathways with minimal user interaction or prior anatomical knowledge, bringing the technique closer to being practical within a clinical setting. We have shown that motor pathway disruption is highly sensitive to hemiparesis, validating motor pathway segmentation by tractography and clearly demonstrating its potential for radiotherapy treatment planning.

446. IS THERE AN ASSOCIATION BETWEEN LOSS OF HETEROZYGOITY (LOH) ON CHROMOSOMES 1p/19q AND IMAGING FEATURES OF LOW-GRADE OLIGODENDROGLIOMAS (LGO)?
S. Lieberman,1 I. Lavon,2 J. Gomori,1 and OF HETEROZYGOSITY (LOH) ON CHROMOSOMES

447. PERFUSION-WEIGHTED IMAGING IN THE ASSESSMENT OF BRAIN GLIOMAS
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Because brain gliomas are histologically heterogeneous neoplasms, biopsy on targeting must be based on neuroimaging findings and on the location of malignant areas on imaging. Considering the reported differences in relative cerebral blood volume (rCBV) among tumor grades, we evaluated whether preoperative perfusion-weighted imaging (PWI) could improve the detection of areas better suited for radiosurgery. We studied a consecutive group of patients with newly diagnosed brain gliomas. All patients underwent preoperative MR imaging and PWI. Surgery consisted of stereotactic biopsy in 29 cases and open craniotomy in 24 cases. Patients were followed up to 5 years after the diagnosis to evaluate their final survival. Our experience, PDWI added to standard MR imaging improved the choice of targets for biopsy and the diagnostic accuracy, as confirmed by the clinical follow-up. Despite a less precise demarcation of the tumor borders, it allowed a better localization of its most malignant parts. Among the PWI parameters, we found that only maximal rCBV ratios were clinically useful. PWI was a valuable implement in the imaging assessment of brain gliomas, discriminating high-grade from low-grade tumors. PWI correlated significantly with the tumor grade and the final outcome (P < 0.01).

448. CORRELATION OF HYPOXIC CELL FRACTION WITH GLUCOSE METABOLIC RATE AND MARKERS OF ANGIOGENESIS AND PROLIFERATION IN GLIOMAS
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In vitro studies suggest 18F-fluorodeoxyglucose (FDG) uptake in tumor cells may reflect both aerobic and anaerobic glycolysis. 18F-Fluoromisonidazole (FMISO) has been shown to selectively identify hypoxic but viable tissue, which may contribute to chemoresistance in gliomas. The aim of this study was to map and correlate tumor hypoxia with glucose metabolic rate, angiogenesis, and proliferation in patients with low- and high-grade gliomas. Seventeen patients with newly diagnosed primary brain tumors were studied prospectively with 18F-FMISO and 18F-FDG positron emission tomography (PET), and MP-RAGE MRI, prior to surgery. All FMISO and FDG PET images were co-registered with the MRI for stereotactic comparison of relative uptake. Biopsy samples at surgery were histologically examined for expression of GLUT1, Ki67, HIF1a, VEGF, VEGFR1, and microvessel density (MVD). In the 17 patients studied there were 7 grade IV, 3 grade III, 4 grade II gliomas, and 2 metastatic adenocarcinoma. 18F-FMISO uptake was found to correlate with tumor grade and was often highly suggestive in the tumor. In high-grade gliomas 18F-FMISO uptake overlapped regions of maximal 18F-FDG uptake. Low-grade gliomas uniformly did not take up 18F-FMISO and were negative on 18F-FDG PET. GLUT1 expression correlated with 18F-FDG uptake. Both 18F-FMISO and 18F-FDG uptake were associated with a significant increase in Ki67 and VEGFR1 markers in tumor tissue (18F-FMISO, P < 0.0001 and < 0.0014; and 18F-FDG, P < 0.0001 and < 0.0012, respectively). There was a trend for increased HIF1a expression with high 18F-FMISO uptake. Expression of HIF1a was associated with increased Ki67, VEGF, and VEGFR1 and reduced MVD (P < 0.05). 18F-FMISO uptake correlates with glioma tumor grade and 18F-FDG uptake. Cellular markers of hypoxia, proliferation, and angiogenesis are strongly associated with hypoxic cell fraction and glucose metabolic rate in gliomas. Further studies are planned to define the biology of hypoxia in high-grade gliomas and the predictive ability of 18F-FMISO scans in glioma patients during therapy.

449. DIFFUSION-WEIGHTED MRI IN THE EARLY DIAGNOSIS OF MALIGNANT GLIOMA
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The diagnosis of glioblastoma multiforme is based upon imaging findings and histopathological features that are usually readily identified in incidence and 7 female with an average age of 52. A small subset of patients comes to medical attention before the masses show rim enhancement and central necrosis. As in those cases the tumors are typically located in eloquent areas of the brain, tissue diagnosis is obtained through stereotactic biopsy and not uncommonly is unsuccessful or does not allow for accurate grading. We
conducted this study in order to identify imaging characteristics of early stages of malignant gliomas. This is a retrospective analysis of patients with newly diagnosed malignant glioma seen on the Neuro-Oncology Service at the National Cancer Institute between 2002 and 2004. Patients with a clinical radiographic presentation (large rim-enhancing masses on T1-weighted MR images after administration of gadolinium) were excluded. Medical records and magnetic resonance imaging studies of patients were reviewed. Eight patients meeting the above inclusion criteria were identified. Diffusion-weighted imaging (DWI) showed areas of increased signal intensity in all cases. Low signal on apparent diffusion coefficient maps (ADC) indicative of restriction of proton diffusion in corresponding areas was present in six patients, whereas in the other two, ADC maps were not obtained. In five patients, patchy or small nodular enhancing lesions without central necrosis were identified. Biopsy was performed in six patients at the time of the above-described imaging findings. Diagnosis of a malignant glioma could only be established prior to further tumor growth in two cases. Glioblastoma can be a challenging diagnosis in a small subset of patients. Those may particularly benefit from early diagnosis and initiation of treatment, as tumors are frequently located in a functionally important area in which even minimal progression can give rise to substantial disability. Information obtained through DWI and ADC maps should be incorporated in the clinical decision-making process. For patients with masses displaying restricted proton diffusion indicating high cellularity, even in the absence of contrast enhancement, a biopsy should not be delayed.

450. PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA – VOLUMETRIC ANALYSIS OF RESPONSE TO CHEMOTHERAPY: IS THERE PREDICTIVE VALUE TO A “RESPONSE NOMOGRAM”? M.M. Mrugala, S. Schreiber, C.B. Knobbe, A. Muzikansky, G.J. Harris, J. Rabnow, F.H. Hochberg, Stephen E. and Catherine Pappas Center for Neuro-Oncology, Radiology, and Biostatistics Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

Primary central nervous system lymphoma (PCNSL) is a non-Hodgkin’s B-cell lymphoma. Over 52% of patients achieve a complete response (CR) following receipt of parental high-dose methotrexate (HD-MTX). We prospectively collected MRT (16/m2) every 14 days until a complete response (CR) was achieved or disease progression is observed. Of importance to us is the separation of early responder patients from those who will not achieve CR with this therapy. We have been unable to use as predictors of CR the following variables: age, performance status at diagnosis, or MTX pharmacokinetics. The development of a “response nomogram” would provide early identification of patients likely to achieve a CR and to separate these individuals from those with either progressive disease (PD) or partial response (PR) for whom phase 1/2 trials would most appropriate. We performed a retrospective analysis of 20 patients whose PCNSL completely responded to MTX in the absence of XRT or other chemotherapeutic agents. Ten male and 10 female immunocompetent patients with median age at diagnosis of 64.5 years were included. All patients received MTX therapy, the protocol of diminishing steroid dosing, for measurable disease (MRI-T1 gadolinium-enhancing masses). Contrast-enhanced MRI scans were performed prior to treatment and thereafter every 2 cycles. Tumor volumes (T1 gadolinium-enhancing, contrast-enhanced T1 and T2 images and FLAIR images) were calculated by using Vision 2 software and a semi-automated edge detection system. The median baseline, pretreatment, T1 post-gadolinium-enhancing tumor volume was 7.28 cm3, and median baseline FLAIR volume was 64.49 cm3. At CR the enhancing tumor volume was 0 and the median FLAIR volume was 29.52 cm3. Twenty percent of patients achieved CR after 4 cycles, a further 15% after 3 cycles, and an additional 13% after 5 cycles. Ten percent achieved CR following 6, 7, or 8 cycles. The remaining 5% of completely responsive patients required 9 to 12 cycles to achieve remission. The median time to achieve 50%, 75%, and full reduction of the enhancing tumor volume from the start of therapy was 1 cycle (14.3 days), 2 cycles (28 days), and 3.5 cycles (76 days), respectively. We present (A) the relationship of baseline tumor volume to therapeutic response, (B) the relationship between response criteria based on T1-gadolinium and FLAIR volumes, and (C) correlates of MRI volumetric response including initial tumor volume, number of lesions, and patient age. We conclude that construction of a nomogram, based on volumetric analysis, can permit (A) rapid identification of non-responsive patients who will be eligible for early-phase drug trials and (B) identification of populations of MTX-responsive and non-responsive patients as the basis for pharmacogenomic and chromosomal studies of drug response correlates.

451. SYSTEMATIC REVIEW OF THE DIAGNOSTIC ACCURACY OF THALLIUM-201 SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY IN THE DETECTION OF RECURRENT GLIOMA M. Vou, B. Tony, O. Hoekstra, T. Postma, J. Heimans, and L. Hoof; Neurology, Nuclear Medicine, and Clinical Epidemiology and Biostatistics, VU Medical Centre, Amsterdam, The Netherlands

The aim of this study was to determine the diagnostic accuracy of thallium-201 single-photon emission computed tomography (201TI SPECT) in the detection of tumor recurrence in patients with previous radiotherapy for supratentorial glioma. The databases of Embase and Embase were searched for relevant studies. Two reviewers independently selected and extracted data on study characteristics, quality, and accuracy of studies. Studies were included if they comprised at least six eligible patients, who underwent 201TI SPECT (index test) and in whom (histo)pathological confirmation (reference test) of the suspected brain lesion was obtained. Because of the methodological and statistical heterogeneity of the included studies, a quantitative meta-analysis was not performed. Instead, for every individual study, the sensitivity, specificity, and diagnostic odds ratio (DOR) of 201TI SPECT was calculated. Eight studies met the inclusion criteria. Only one study was considered of high methodological quality. Methodological limitations referred most notably to blinding and patient selection. The DOR was greater than 1 in all included studies, with a broad range (2.1–350.9) and relatively wide 95% confidence intervals. The sensitivity of 201TI SPECT ranged from 0.43 to 1.00, and the specificity from 0.25 to 1.00. We conclude that 201TI SPECT is a valuable modality in the detection of tumor recurrence in patients treated with radiotherapy for supratentorial glioma. However, the evidence is not very robust because of the low quality and high heterogeneity of the studies included. Future studies are warranted to further explore the diagnostic potential of 201TI SPECT, and to determine optimum thresholds for the detection of glioma recurrence.

452. TAURINE DETECTED BY 1H MAGNETIC RESONANCE SPECTROSCOPY IS INDICATIVE OF SUPRATENTORIAL PRIMITIVE NEUROECTODERMAL TUMORS (PNETS) H.J.G. Krouwer,1 R.W. Proost,2 W.M. Mueller,3 G.P. Sisson,3 C.J. Schultz,3 and K.-C. Ho3; Departments of 1Neurology, 2Neurosurgery, 3Radiology (Neurooncology), and 4Biostatistics (Neurooncology), Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Proton (1H) magnetic resonance spectroscopy (MRS) is an noninvasive imaging technique to characterize intracranial lesions. With this modality, medulloblastomas have recently been found to demonstrate elevated taurine levels. Taurine is a non-proteinogenic amino acid present in high concentrations in fetal neural tissues and decreasing thereafter. We extend this observation to include other primitive neuroectodermal tumors (PNETs) with 2 cases of disseminated pineoblastomas. Spectra were acquired on a 0.5 T clinical system with a quadrature transmit/receive head coil using an elliptical excitation chemical shift imaging (CSI) sequence with TE = 46 ms and TR = 1000 ms. Taurine resonances were detected at 3.63 ppm. This resonance becomes more detectable at 0.5T because of the collapse of its complicated resonance in a manner similar to what has been shown for glutamate (Proost et al., Magn. Reson. Med. 37, 615, 1997). Two male patients, 32 and 47 years of age, presented with pineal region masses, both with extensive leptomeningeal dissemination (4th ventricle and anterior spinal cord at C3-C4; auditory internal canals bilaterally, cerebellar folia, along cervical and thoracic spinal cord and conus medullaris, as well as all nerve roots) as assessed by conventional imaging studies. In both, beta-HCG levels in CSF were slightly elevated, and AFP levels in CSF were normal. MRS studies demonstrated, in addition to the resonances assigned to myo-inositol, choline, creatine, N-acetylaspartate, glutamine/glutamate, and lipid/lactate, a resonance at 3.36 ppm, compatible with taurine. Histo-pathological specimens obtained from a drop metastasis at T10-T11, and from the 4th ventricular mass, respectively, showed pineoblastoma. These findings support the hypothesis that detection of taurine by 1H-MRS in an intracranial mass, supra- or infratentorial, may indicate a PNET histology. Whether taurine as detected with the 0.5 T system is indeed specific for PNETs requires further study.
453. COMPARISON OF GENOTYPE, HISTOPATHOLOGICAL GROWTH PATTERNS, AND THE MR IMAGING CHARACTERISTICS OF OLIGODENDROGLIAL NEOPLASMS

The goal of this study was to investigate the relationship between genotype and histopathological growth patterns of oligodendroglial neoplasms. The study was performed on 45 patients diagnosed with CT-guided serial stereotactic biopsy. The position of calcification and the growth pattern (solid, mixed, infiltrative) were recorded in relation to the biopsy tract, enabling comparison with the histopathology of each case. The study aimed to determine if there is a correlation between genotype and the development of radiological findings.

454. IN VIVO MAGNETIC RESONANCE IMAGING OF β-GALACTOSIDASE IN A RAT C6 GLIOMA MODEL

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This study evaluated the potential of a novel MRI probe which allows for in vivo measurement of reporter gene expression. The probe was developed for the measurement of the change in blood-tumor permeability, via the determination of the uptake of radiolabeled fluorodeoxyglucose (FDG-PET) in a patient affected by GBM who had had previous surgery and chemotherapy. The technique was useful for patients for whom MR is not suitable.

456. THE ROLE OF 111 INDIUM-OCTREOTIDE BRAIN SCINTIGRAPHY IN THE DIAGNOSIS OF MENINGIOMAS FROM OTHER CRANIAL BASED DURAL LESIONS

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Meningiomas are common extra-axial brain tumors with somatostatin receptors that bind octreotide. We report the use of 111 indium-octreotide brain scintigraphy (Oct) in the non-invasive differentiation of meningiomas from other cranial-based pathology. A retrospective analysis of our experience with Oct in the non-invasive differentiation of meningiomas was performed. A neuroradiologist, blinded to the clinical data, used a standardized grading scheme to define the uptake of octreotide and the pattern of enhancement performed at 6 and 24 h post-administration. Correlation of [111]fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), MRI scans, and octreotide uptake was performed. The cohort consisted of 49 patients (34 female; 15 males) with a mean age 62.4 years and a median follow-up of 24 months. Management consisted of biopsy (n = 3), resection (n = 10), observation (n = 15), and radiation therapy (radiosurgery n = 21; external beam n = 3). Oct was correlated with FDG-PET brain studies (n = 37), histology (n = 13), and angiography (n = 1). The sensitivity, specificity, accuracy, and positive predictor value for Oct were 100%, 60%, 85%, and 80%, respectively. The test successfully differentiated meningiomas from a dural-based venous anomaly except for 2 false patients (metastasis; chronic inflammation). The addition of PET did not improve the specificity of Oct. The assessment of the clinical role of Oct is important in the management of Oxs and MR images. MRI vs. PET (P = 0.0011), MRI vs. PET (P = 0.0218), and Octrode vs. PET (P = 0.0071) revealed a significant relationship. Octrode scintigraphy together with FDG-PET scanning increases the diagnostic specificity of conventional neuroimaging when differentiating meningioma from other dural-based pathology.
457. SENESCEENCE, AUTOPHAGY, MORPHOLOGICAL PLASTICITY, AND SURVIVAL OF GLIOMA CELLS EXPOSED TO IONIZING RADIATION

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Resistance to ionizing radiation (IR) and apoptosis is one of the key factors contributing to the poor prognosis of patients with glioblastoma. However, the mechanism underlying this resistance remains poorly understood. Two approaches were used in this study to better identify relevant events involved in radioresistance of glioma cells. First, glioma cells with different p53 status (U87 wt, T98 mut, LN-Z308-null) were distributed into 100-mm plates, in triplicate. Cells were treated with single doses of IR (6 Gy), administered five consecutive times every six days. For each time, samples were separated for protein extraction and further maintenance in culture for immunocytochemistry. A second approach was aimed to select highly radioresistant cells. Thus, cells were treated with high-dose radiation (60 Gy), administered twice within a period of 6 days. Cells were analyzed for morphological changes by optical and electronic microscopy; proliferation rate and viability were assessed by MTS assay and trypan blue; IR resistance was monitored by clonogenic assay; changes in cell cycle were determined by flow cytometry, and alterations in protein profile followed by Western blot and immunocytochemistry. Antibodies to both p53 and p53-induced proteins, as well as to differentiation markers and radiation-resistant-related proteins, were used. U87 glioma cells (wt p53) were more radioresistant than T98G (mut p53). Exposure to consecutive doses of 6 Gy IR decreased proliferation and increased apoptosis to the unirradiated control, yet proliferation and apoptosis and cells accumulating in G1 of the cell cycle followed a dose-dependent manner. However, with higher doses, surviving populations were able to resume their normal rate of proliferation comparable to cells that did not receive IR. Resistant U87 cells exposed to high-dose IR exhibited a striking change in morphology characterized by increase in size (10-15-fold larger than untreated controls), increase in cytoplasm vacuolization that preceded the formation of neurite-like projections and aneuploidy. These cells entered a senescence state and survived for at least one year with reduced proliferation. At the molecular level, reduced expression of p53 and p21(waf) protein, decreased expression of the undifferentiated marker NEST, reduced levels of Rb protein but increased levels of survivin. These cells also showed aberrant expression of the neuronal marker beta III tubulin in addition to the astrocytic marker GFAP. In contrast, T98G cells showed no alteration in the levels of these proteins but did not survive as long as U87.

In conclusion, our results suggest that radiation resistance may be in part associated with the plasticity of some glioma cells to undergo senescence and terminal differentiation followed autophagy. Furthermore, the p53 and Rb pathway with participation of DNA repair and anti-apoptotic proteins such as survivin are important players in radioresistance.

458. BIOLOGICAL EFFECTS OF PROTON BEAM IRRADIATION ON GLIOMA CELL LINES IN VITRO

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Gliomas are considered to be one of the most radioresistant and least curable tumors, and although many patients show an initial response to radiotherapy, the majority recur within or adjacent to the target volume. Proton therapy used in the treatment of some gliomas enables higher doses of conformal irradiation to be given, while sparing critical normal tissue. Various molecular genetic characteristics were used to investigate the in vitro responses to proton beam irradiation, suggesting that depending on their genetic subtype, gliomas may display differential intrinsic radiosensitivity. Through expression profiling we aim to identify factors that contribute to radiation resistance.

459. GROWTH DELAY OF HUMAN GLOILOBLASTOMA MULTIFORME SPHEROIDS AFTER THERMAL NEUTRON IRRADIATION WITH GADOLINIUM (GDNCT) OR BORON (BNCT)

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The survival of patients with GBM continues to be dismal despite various modalities of treatment. Neutron capture therapy (NCT) is an experimental treatment modality: Following thermal neutron irradiation of nuclear elements with a high propensity to capture neutrons, such as boron-10 (10B) or gadolinium-157 (157Gd), a high dose of secondary radiation is released according to the nuclear reaction: 10B(n, α)7Li and 157Gd(n, α)122Gd. Certain compounds, such as boron phenylalanine fructose (BPA-F) and Gd-DTPA, can accumulate locally in a brain tumor. Neutron irradiation of a patient receiving boron (BNCT) or gadolinium (GdNCT) may reach a tumoricidal local irradiation. In this study we investigated if the theoretical effect population of BNCT and GdNCT works in the in vitro GBM spheroid model. Multicellular spheroids of the human GBM cell line Gli-6 were cultivated up to a diameter of about 300 microns. Clinically achievable concentrations of gadolinium-DTPA (Magnevist; 2.5 mM) and BPA-F (30 ppm) were used as neutron capture agents. The spheroids were irradiated with thermal neutrons in a dose of 0.3 Gy and 0.6 Gy at the Low Flux Reactor (NRG, Petten) or with cesium-137 X rays (photons) up to 8 Gy (AMC, Amsterdam). The size of the spheroids was measured for some weeks. Irradiation of spheroids with X-ray-photons shows a dose-dependent growth delay; Gd-DTPA or BPA-F did not alter the tumor response to X rays. Irradiation of spheroids with thermal neutrons gives a dose-dependent growth delay. The radiosensitization (RE) of thermal neutrons is about 5- to 10-fold that of X-ray photons; combination with Gd-DTPA or BPA-F caused a significant prolonged growth delay. The prolongation is somewhat more pronounced in Gd-DTPA treated spheroids, both after 0.3 Gy neutron irradiation, and more pronounced after 0.6 Gy neutron irradiation. Both Gd and B, at a clinically feasible concentration, produce a strong growth delay of GBM spheroids after neutron irradiation. This radiosensitization by Gd and B was, as expected, not observed after spheroids were irradiated with photon irradiation. The NCT-effect of Gd cannot be explained by the release of secondary Auger electrons (as these do not penetrate more than 1 or 2 cell layers), and should be attributed to the secondary release of longer range photons and nuclear conversion electrons.

460. ABSOCOPAL EFFECT ON SUBCUTANEOUSLY IMPLANTED N29 RAT GLIOMA TUMORS, FROM CONTRALATERAL TREATMENTS WITH PULSED ELECTRIC FIELDS, RADIATION THERAPY, AND IMMUNIZATION WITH SYNGENEIC INTERFERON-GAMMA-SECRETING TUMOR CELLS

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Effects of radiation therapy on cancer tumors outside the radiation field are a phenomenon known as the abscopal effect. The aim of the present study is to study the effect of immunization with syngeneic interferon-gamma (INFγ)-secreting cells on abscopal regression of contralateral, subcutaneously implanted rat glioma N29 tumors. The N2-25 model was established in Balb/c mice using the subcutaneous implantation of glioma cells. Animals were arranged into one group of controls and 3 groups of various treatments, receiving 10 Gy irradiation. In contrast, U373, also with mutant p53, showed an increased expression of p53 and p21cip/waf protein, decreased expression of the undifferentiated marker NEST, reduced levels of Rb protein but increased levels of survivin. These cells plating in monolayers at 5 × 10⁶ cells per 35-mm dish were positioned in the modulated Bragg peak and obtained 0, 2, 4, 6, 8, or 10 Gy proton irradiation (60 MeV) at a dose rate of 36 Gy/min. Following irradiation, Annexin-V staining was added to the media to assess apoptosis, and time-delay confocal microscopy was performed over 65 h with an image recorded every 15 min. Flow cytometric analysis was performed 65 h post-irradiation. Monolayer cultures were maintained post-irradiation to determine if populations surviving sequential fractions of proton irradiation could be established. T98G with mutant p53 was the most resistant cell line, with little change in proliferation and apoptosis and cells accumulating in G0 of the cell cycle following 10 Gy irradiation. In contrast, U373 showed complete inhibition of proliferation, and cells accumulated in G2/M after 65 h. U-87 MG with wild-type p53 showed a similar cell cycle distribution and rate of apoptosis to the unirradiated control, yet proliferation was inhibited. U373 showed a 3-fold increase in apoptosis at the level of apoptosis, with a 9-fold increase in Annexin-V staining at 65 h post-irradiation, and surviving cells accumulated in G2 of the cell cycle. All cell lines produced populations that survived a single fraction of 10 Gy protons. Surviving populations were established following 2 × 10⁶ for U-87MG and 3 × 10⁵ for T98G. Expression profiling using oligonucleotide microarrays to investigate differential gene expression in these cells lines in response to irradiation is currently being undertaken. The four cell lines with their different molecular genetic profiles showed very different responses to proton beam irradiation, suggesting that depending on their genetic subtype, gliomas may display differential intrinsic radiosensitivity. Through expression profiling we aim to identify factors that contribute to radiation resistance. 
461. CORRELATIVE MR IMAGING WITH GBM CANCER GENOMICS
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Glioblastoma multiforme (GBM) is characterized by genetic instability and an extremely variable appearance on magnetic resonance imaging (MRI). We sought to determine whether characteristic GBM imaging defining features seen on MRI could capture significant alterations in intrinsic gene expression signatures identified by gene expression microarrays. Four imaging parameters were defined a priori to reflect fundamental GBM-defining MR imaging characteristics (degree of contrast enhancement, necrosis, T2 heterogeneity, mass effect). MR images (T1, T2, contrast enhanced) of 20 GBMs in 20 patients were evaluated and scored across these 4 parameters by 2 radiologists in consensus and without knowledge of the matching GBM genomic information. Unsupervised and supervised analyses were performed for correlation of the imaging parameters against the corresponding matched GBM microarray data (each microarray containing 23,000 clones) to identify relationships between imaging traits and tumor gene expression. Four of four imaging traits demonstrated statistically significant correlations with large-scale alterations in gene expression: degree of contrast enhancement (P < 0.01), necrosis (P < 0.01), T2 heterogeneity (P < 0.001), and mass effect (P < 0.005). Further, there was significant enrichment of genes identified by 3/4 imaging traits in several fundamental GBM gene expression signatures: Degree of contrast enhancement (P < 0.01), necrosis (P < 0.01), T2 heterogeneity (P < 0.001), and mass effect (P < 0.005). The coordinated imaging and gene expression analyses validate this novel method for linking clinical imaging traits to fundamental GBM genomic information.

462. IMAGING PRIMARY BRAIN TUMORS WITH [18F]-3-FLUORO-AMINOCYCLOBUTANE CARBOXYLIC ACID (FACBC) PET: A PILOT STUDY
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In the management of primary brain tumors, new or expanding lesions observed on MRI may represent recurrent tumor, treatment effect, or necrosis. [18F]-methyl-methionine ([18F]-MET) PET imaging has several advantages over [18F]-fluorodeoxyglucose PET, but is contoured by the presence of radiolabeled metabolites. Logistically, [18F]-MET is more challenging because of a shorter half-life (20 min vs. 110 min for [18F]). [18F]-MET requires on-site production and is less widely available. For these reasons and substantial cost savings, 3-fluoro-aminocyclobutane carboxylic acid ([18F]-FACBC) PET studies may be superior to both [18F]-fluorodeoxyglucose and [18F]-MET PET. We report interim results of a pilot study evaluating dosimetry and imaging characteristics of [18F]-FACBC in comparison to [18F]-MET PET. Patients with previously treated primary brain tumors and a suspicion for recurrent or progressive disease were included. All patients had [18F]-FACBC, [18F]-MET PET, and tumor. Results of the MIB-1 labeling index of 1 to 3.5 months. Neuro-Oncology

463. IMAGING CORRELATES OF MOLECULAR SIGNATURES IN OLIGOASTROCYTOMAS
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In order to improve therapeutic management of oligoastrocytomas (OA) we investigated the relationship between magnetic resonance imaging (MRI) features on the pre-operative scan, the histopathological diagnosis, and the genetic signature of the tumors. Sixty-six pre-operative tumors were analyzed by histopathology as grade II (n = 31) or grade III OA (anaplastic OA, AnOA; n = 35). Loss of heterozygosity (LOH) with different microsatellite markers was studied on chromosomes 1p, 1q, 9p, and 9q. On each MRI examination the following parameters were analyzed: location of the tumor, sharpness of tumor border on T1-weighted images, homogeneity of the tumor signal on T1 and T2 weighted images, mass effect and presence of contrast enhancement. In a series of 62 cases we observed that 1p and/or 19q LOH correlates with homogeneous T1 and T2 appearance (P < 0.008) and with decreased frequency of temporal location (P = 0.002). Indistinct border, lack of T1 and T2 homogeneity, and presence of enhancement were significantly associated with LOH on 10q (P < 0.001). These findings indicate that molecular alterations associated with cancer may confer physical or biochemical characteristics to the tumor that can be imaged. In oligoastrocytomas, LOH on 10q, a marker of astrocytoma progression, correlates well with MRI findings of high-grade gliomas (indistinct border, T1 and T2 lack of homogeneity, and presence of enhancement). Genotypic assessment, in addition to MRI and histological evaluation, may improve the management of patients with oligoastrocytomas.

464. IMAGING TUMOR PROLIFERATION: VALIDATION OF 3'-DEOXY-3'-[F-18]FLUOROTHYMIDINE (FLT) POSITRON EMISSION TOMOGRAPHY IN CEREBRAL GLIOMAS
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Uncontrolled cellular proliferation is one of the cardinal features of malignant tumors. 3'-Deoxy-3'-[F-18]fluorothymidine (FLT) is a compound that is taken up into dividing cells and phosphorylated by thymidine kinase 1 (TK1), which leads to intracellular trapping. FLT activity is therefore a measure of cellular TK activity and hence cellular proliferation. Its location within the body can also be imaged by using positron emission tomography (PET). Twelve patients with cerebral gliomas (mean age, 49.6; 4 WHO grade IV, 4 WHO grade III, and 4 WHO grade II tumors) were studied pre-operatively; 200 MBq of FLT was given intravenously and a 3D dynamic PET study performed. Venous blood sampling was performed throughout the study. Maps of FLT uptake were generated and correlated to an MR study used for biopsy planning. All patients underwent an image-guided biopsy, with biopsies taken at intervals along the biopsy tract. Biopsies were paraffin embedded and sections used for regular histopathological analysis and immunohistochemistry using the MIB-1 antibody as a marker for cellular proliferation. The coordinates for all biopsy sites were determined, and uptake values for FLT were determined. There was little uptake of FLT in either the normal brain or the low-grade gliomas, except in one case where the tumor uptake was 12% without the expected morphologic transformation. In 3 of the WHO grade III tumors the MRI showed little enhancement, but there was local increase in FLT in these cases. In all grade IV tumors there was marked uptake of FLT. The maximum FLT uptake from all biopsy regions correlated with the maximum MIB-1 labeling index.
(Pearson’s correlation coefficient $\tau = 0.69; P = 0.03$). By using a threshold of $1.5 \times 10^5 \text{ml}_{\text{lesion}} \text{mm}^{-3} \text{min}^{-1}$ for the individual biopsy sites, FLT could detect tumor with a sensitivity of 92% and a specificity of 64%; it was marginally more sensitive but less specific than either $T_1$- or contrast-enhanced $T_2$-weighted MRI. FLT is a good imaging marker of tumor proliferation. It may be especially useful in monitoring response to therapy and guiding image-guided biopsies in minimally enhancing tumors.

465. ADVANCES IN MANAGEMENT OF MALIGNANT MENINGITIS
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Neoplastic meningitis (NM) is a common problem in neuro-oncology, occurring in approximately 5% of all patients with cancer, and is the third most common site of central nervous system (CNS) metastases. NM is a disease affecting the entire neuraxis, and therefore clinical manifestations are pleomorphic, affecting the spine, cranial nerves, and cerebral hemispheres. Because of craniospinal disease involvement, staging and treatment needs encompass all cerebrospinal fluid (CSF) compartments. Treatment of NM utilizes involved-field radiotherapy of bulky or symptomatic disease sites and intra-CSF drug therapy. The inclusion of concomitant systemic therapy may benefit patients with NM and may obviate the need for intra-CSF chemotherapy. At present, intra-CSF drug therapy is confined to three chemotherapeutic agents (i.e., methotrexate, cytosine arabinoside, and thio-TEPA) administered by a variety of schedules either by intralumbar or intraventricular drug delivery. Although treatment of NM is palliative, with an expected median patient survival of 2 to 6 months, it often affords stabilization and protection from further neurologic deterioration in patients with NM.

466. ADVANCES IN MANAGEMENT OF BRAIN METASTASIS
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The management of patients with brain metastases has improved over time, because of advances in technology and a better knowledge of prognostic factors, leading to a more accurate patient selection. In the diagnostic setting, MRI is the gold standard, and MRI diffusion/perfusion and MRS in certain circumstances improve the diagnostic accuracy. A tissue diagnosis is needed in patients with unknown primary tumor or with absent/controlled systemic disease when a long interval has elapsed since the initial cancer diagnosis or with active but treatable systemic disease when the radiologic appearance is atypical. Whole-body F18 PET is a sensitive tool to detect pulmonary foci as probable primary tumors in patients with biopsy-proven brain metastasis and a negative cancer history. Neurocognitive tests are a relatively sensitive measure of brain functioning, being important in predicting survival and monitoring treatment effects and tumor progression.

The combination of surgical resection and WBRT is superior to WBRT alone for the treatment of single brain metastasis in patients with limited or absent systemic disease and good performance status. Radiosurgery yields results that are comparable to those reported after surgery, provided that the lesion’s diameter does not exceed 3 to 3.5 cm. Radiosurgery combined with WBRT (“radio-surgical boost”) is superior to WBRT alone in single but not in multiple brain metastases. Hypofractionated stereotactic radiotherapy can be an alternative to radiosurgery. Still controversial is the need for WBRT after surgery or radiosurgery: Local control on MRI is significantly better after the combined approach, but overall survival does not improve. Phase 3 studies (RTOG, EORTC, etc.) are ongoing, trying to identify which subgroup of patients are candidates for WBRT after local treatments. A new form of brachytherapy (Glasiat Radiotherapy System) is now being investigated in patients who have undergone surgical resection. Novel radiation sensitizers (Motexafin gadolinium, RSR 13) have some activity in conjunction with WBRT. The response rate of brain metastases to chemotherapy is similar to that of the primary tumor and extracranial metastases. Promising results have been recently reported with temozolomide in melanoma, capetcitabine in breast cancer, and gefitinib (ZD 1839), an EGFR tyrosine kinase inhibitor, in NSCLC. Chemotherapy may represent the initial treatment (with or without subsequent WBRT) in patients with multiple brain metastases from NSCLC and breast cancer who are asymptomatic, whereas WBRT remains the treatment of choice in symptomatic patients. Cognitive deficits in long-surviving patients after WBRT are a real concern: Acetylcholinesterase inhibitors (donepezil, memantine) could be of some efficacy in improving cognitive functioning.

469. TEMOZOLOMIDE AS PRIMARY CHEMOTHERAPY FOR LOW-GRADE OLIGODENDROGLIAL TUMORS (LGOT): PREDICTIVE IMPACT OF CHROMOSOME 1P LOSS ON RADIOGRAPHIC RESPONSE
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Temozolomide (TMZ) has recently been shown to be active as initial treatment for low-grade oligodendroglial tumors (LGOT). Preliminary results suggested that chromosome 1p loss may predict the response to TMZ (Hoang-Xuan, K. J. Clin. Oncol. 22, 3133, 2004). The objectives of our study were to confirm in a larger series and with a longer follow-up (1) the efficacy of TMZ on LGOT and (2) the predictive value of 1p loss on the radiographic response. Patients suffering from WHO grade II oligodendroglioma and mixed glioma, with progressive disease on MRI, were eligible for the study. TMZ was delivered at the starting dose of 200 mg/m²/day for 5 days, repeated monthly. Response was evaluated by MRI (T2–FLAIR weighted sequences). Deletion on 1p was searched by the LOH technique. From 1999 to 2004, 99 consecutive patients were included in the study (median age, 44 years; median Karnofsky score, 90). Median follow-up was 22 months (6–54), and the median number of TMZ cycles was 18 (6–26). The response rates were as follows: partial response (PR), 25%; minor response (MR), 14%; stable disease (SD), 52%; and progressive disease (PD), 9%, respectively. Grade 3–4 toxicity occurred in 6% of patients. The median PFS was 31 months (95% CI, 19–∞). Blood and tumor DNA pairs from 50 patients were available for LOH analysis. LOH 1p was present in 22 tumors (corresponding to 15 PR + MR, 7 SD, 0 PD) and absent in 28 tumors (corresponding to 4 PR + MR, 20 SD, 4 PD) ($P = 0.0001$). We confirm in a larger series that TMZ provides a substantial rate of objective response in LGOT and that LOH 1p is closely correlated with radiographic response.
470. PHASE 2 MULTICENTER STUDY OF DOSE-INTENSE TEMOZOLOMIDE IN PATIENTS WITH NEWLY DIAGNOSED PURE AND MIXED ANAPLASTIC OLIGODENDROGILIA (AO/MAO) WITH NO PRIOR CHEMOTHERAPY OR RADIATION THERAPY. All pathology had central review of the molecular genetics of the tumor. Due to their chemo responsiveness, this trial has used a dose-intense regimen of temozolomide and reserved RT for patients with disease progression. Our objectives were to determine the progression-free survival, response rate, and quality of life (QOL) in patients with newly diagnosed AO/MAO treated with temozolomide every other week and to determine outcomes according to tumor cytogenetic status. Eligible patients had newly diagnosed AO/MAO with no prior chemotherapy or radiation therapy. All pathology had central review and tumor assay for 1p deletion using FISH (fluorescent in situ hybridization). Treatment was stratified by 1p status. Temozolomide was given 150 mg/m², days 1–7 and 15–26, every 28 days. Therapy was given for up to 8 cycles in the absence of progressive disease. Responses and QOL (FACT-BR and EORTC brain module) were evaluated every 8 weeks. To date, 41 patients have been enrolled from 7 centers. Four patients are too early to evaluate. Median age is 42 (range, 20–83); Karnofsky PS, 100 in 10 pts. In 1 pt, 3 and 7/100 of 1p loss at MAO, and 1p deletion in 1 pt (15/21 with AO and 6/13 with MAO). Patients have received 0 to 8 cycles (median 8) of temozolomide (21 patients have completed all 8 cycles, 7 withdrew consent for toxicity or patient choice prior to completion, 3 withdrew for progressive disease prior to completion, 2 continue with stable disease in cycle 6, 1 withdrew prior to receiving treatment). Only 1 patient has required dose reduction for toxicity (20% reduction for thrombocytopenia). Nineteen patients remain free from progression with a median progression-free survival of 10 months (range, 2–25). The overall survival is 14 months (5–34). Of the 32 patients with response data available, 2 (AO with 1p loss) (6%) achieved complete remission. Patients with 1p loss had a median PFS of 8 months compared with 6 for 1p gain or 1p neutral. Toxicities were limited to myelosuppression and gastrointestinal symptoms. Activity is seen in TMZ refractory GBM, and the reasons may include suboptimal TMZ schedule, alternative mechanisms of resistance, and AGT mutations.

471. TEMOZOLOMIDE (TMZ) WITH O6-BENZYL GUANINE (BG) FOR TMZ-RESISTANT MALIGNANT GLIOMA: A PHASE 2 TRIAL A. Desjardins, J. Quinn, D. Reardon, J. Rich, S. Sathornsumetee, J. Vredenburgh, A. Walker, S. Tourt-Uhlig, D. Bigner, and H. Friedman; Duke University Medical Center, Durham, North Carolina, USA

TMZ is a methylating agent with confirmed activity in recurrent and newly diagnosed malignant glioma. One of the major mechanisms of resistance to TMZ is determined by the DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT), which removes the methylation damage from O6 position of guanine. BG inhibits AGT by acting as an AGT substrate. We performed a phase 2 study of TMZ combined with BG in patients with TMZ-resistant malignant glioma. Eligibility criteria were as follows: histologically proven glioblastoma multiforme, because single drugs have not been sufficiently effective. Baseline characteristics for the 21 patients enrolled on this study were as follows: median age 52 years (19–77), 15 males and 6 females, Karnofsky PS 100 in 10 patients, 35 patients had a new diagnosis, 16 of 17 patients had progression in < 3 months, and 2 patients had no prior therapy. TMZ was administered at 150 to 200 mg/m² days 1–5 and lonafarnib at 150 mg BID on days 8–28 of a 28-day cycle. Response was evaluated every 2 cycles. Standard response criteria established by MacDonald were used. The 6-month PFS rate was 13%, median PFS was 16 weeks, and overall survival from study entry was 38 weeks. Treatment was well tolerated. One patient had grade 3 granulocytopenia, thrombocytopenia, and anemia, one patient had grade 4 granulocytopenia, and one patient had grade 4 thrombocytopenia. One patient developed grade 3 diarrhea. The combination of TMZ with lonafarnib using a conventional TMZ dosing schedule was well tolerated, but these data do not support improved efficacy over TMZ alone. Recent reports suggest that the “dose-densitv" of TMZ can be safely increased, and this may enhance activity. Additionally, modifying the dose and schedule of the lonafarnib may improve efficacy when combined with the dose-intensified TMZ regimen. A phase 1 study is under way to optimize the TMZ-lonafarnib combination.

472. A PHASE 2 STUDY OF TEMOZOLOMIDE (TMZ) AND THE FARNESYLTRANSFERASE INHIBITOR (FTI) LONAFARBIN (SARAZARTM, SCH66336) IN RECURRENT GLIOBLASTOMA M. Gilbert,1 K. Hess,1 V. Liu,2 M. Groves,1 V. Puduvalli,1 V. Levin,2 C. Conrad,1 H. Colman,1 S. Hsu,2 M.M. Sugrue,2 and W.K.A. Yung;1 Neuro-oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; 2Schering-Plough Research Institute, Kenilworth, New Jersey, USA

Farnesylation is an essential step in the post-translational modification of several proteins that play a role in cell proliferation and growth, including Ras, Rho, centromere binding proteins (CENP-E/CENP-F), lamin B, and protein tyrosine phosphatase (PTP). Inhibition of farnesylation may inhibit tumor cell proliferation. Preliminary testing demonstrates antiproliferative effects of FTIs in a variety of tumor cell lines and xenograft models. Additional laboratory data from animal models demonstrated enhanced efficacy of combining lonafarnib with TMZ compared with TMZ alone. Our objective was to determine the efficacy as measured by response rate and 6-month progression-free survival rate of the combination of TMZ with lonafarnib. Eligibility criteria were as follows: patient choice prior to completion, 5 withdrew for progressive disease prior to completion, 2 continue with stable disease in cycle 6, 1 withdrew prior to receiving treatment). Only 1 patient has required dose reduction for toxicity (20% reduction for thrombocytopenia). Nineteen patients remain free from progression with a median progression-free survival of 10 months (range, 2–25). The overall survival is 14 months (5–34). Of the 32 patients with response data available, 2 (AO with 1p loss) (6%) achieved complete remission. Patients with 1p loss had a median PFS of 8 months compared with 6 for 1p gain or 1p neutral. Toxicities were limited to myelosuppression and gastrointestinal symptoms. Activity is seen in TMZ refractory GBM, and the reasons may include suboptimal TMZ schedule, alternative mechanisms of resistance, and AGT mutations.
$m^2$), carboplatin ($110$ mg/m$^2$), vincristine ($0.6$ mg/m$^2$) and interferon-beta (300 million/body) were administered in day 1, concomitant with fractionated radiotherapy ($63$ Gy total dose; $1.8$ Gy x 5 days for 7 cycles) and vincristine ($0.6$ mg/m$^2$) and interferon-beta (300 million/body) in day 7 and 15, interferon-beta (300 million/body) three times for a week during the radiation course. Two months after the radiation, ACNU ($60$ mg/m$^2$), carboplatin ($110$ mg/m$^2$), vincristine ($0.6$ mg/m$^2$) and interferon-beta (300 million/body) were administered in day 1 and vincristine ($0.6$ mg/m$^2$) and interferon-beta (300 million/body) in day 7 and 15, every 58 days. The primary end points were safety and tolerability, and the secondary end points were time to progression and overall survival. Nonhematologic toxicities were rare and mild to moderate in severity. During the radiation treatment phase, grade 3 toxicities in neurocytopenia, or thrombocytopenia, or both were observed in $23\%$ of patients. Grade 4 hematological toxicities were not observed. During adjuvant maintenance chemotherapy after radiation, $7\%$ of all cycles were associated with grade 3 toxicities in neurocytopenia or thrombocytopenia. None of the cycles were with grade 4 toxicities. Time to progression (TTP) was $11$ months, and median survival was $16$ months. VAC-feron-R is safe and well tolerated. This regimen of concomitant chemo-radiation therapy followed by adjuvant maintenance chemotherapy may prolong the survival of patients with GBM. Further investigation is warranted.