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The introduction of gadolinium-enhanced MRI into medical and surgical practice is among the most important of all advances in the care of patients with metastatic tumors of the nervous system. This presentation considers technical aspects of differential diagnosis, staging, surgical planning, and determination of response to therapy. Neuroimaging enables the refinement of the differential diagnosis of a newly detected mass lesion in a patient without known cancer. Many nonneoplastic processes can mimic metastatic deposits in the brain, including enhancing arterial infarcts, venous infarcts, hematomas, abscesses, and demyelinating lesions. Recent MRI data have shown that while metastases are multifocal in two-thirds of patients, malignant gliomas are multifocal in a much higher fraction (30%) of patients than previously realized, and this factor should be taken into account in differentiating metastasis from primary tumors. T2*-weighted GRE imaging is useful in suggesting metastatic melanoma as the source of metastasis of undetermined primary. The combination of brain MRI and chest CT are sufficient to enable biopsy site selection in almost all patients with a newly detected mass lesion, providing a strategy to optimize initial histopathologic diagnosis of patients whose cancer presents as a brain mass. Tumor staging is best performed with gadolinium-enhanced MRI and in melanoma patients should include T2*-weighted images. Neurosurgical and radiosurgical planning relies heavily on anatomical data from high-resolution 3-D MRI but increasingly on functional MRI for motor and language mapping and diffusion tensor imaging for analysis of white matter tracts. A recently described imaging feature of great significance is the high frequency of peritumoral infarcts following resection of brain tumors. These infarcts can explain some postoperative deficits and often show focal enhancement on follow-up scans, with the latter feature producing the potential for inappropriate diagnosis of progressive tumor. The evaluation of response and progression of brain metastasis during and following therapy is evolving toward the more common use of computer-assisted volumetrics, although the RECIST and two orthogonal diameter methods are still in widespread use.

Brain metastases represent one of the most frequent and serious clinical conditions in the management of malignant diseases. They occur in up to 40% of cancer patients with increasing frequency. The most common primary diseases are lung and breast cancer, followed by malignant melanoma and cancer of unknown primary. The dismal outcome mandates further research.

In multiple brain metastases, radiation therapy of whole brain is the treatment of choice. Control of symptoms can be expected in approximately 80% of cases. The prognosis for these patients is poor, with a median survival time of one to two months with corticosteroids only, which can be improved by two to four months with whole brain irradiation. In solitary brain metastases, local tumor control (achieved either by radiosurgery or neurosurgical resection) can improve survival from approximately six months to 10–12 months. Radiosurgery offers the advantage of treating patients with surgically inaccessible metastases. According to the RTOG 9503 trial, radiosurgery is additionally superior to whole brain irradiation alone in terms of providing a stable or improved Karnofsky performance status. The addition of whole brain irradiation to local treatments, however, is controversial. It might prolong intracranial disease-free survival, including prevention of neurological symptoms caused by intracranial recurrent disease, whereas the impact on overall survival is unclear. The positive effect of whole brain irradiation after local treatment for solitary brain metastases is of particular importance in cases without extra CNS tumor activity. This question has currently addressed in the ongoing EORTC 22982–26001 trial, in which the role of additional whole brain irradiation is investigated in a randomized setting and tested against local treatments only (surgery or radiosurgery) in patients with controlled extra CNS disease. The impact of whole brain irradiation as supplementary treatment in patients with extra CNS tumor activity is difficult to assess with respect to varying clinical conditions and often concomitant systemic treatments.

Prognostic factors that have an impact on the selection of treatment modality are a high performance status, solitary brain metastases, absence of systemic tumor activity, controlled primary tumor, and a younger age. The now widely accepted RPA classification enables a clinically relevant categorization and is a useful tool in deciding on the selection of treatments (whole brain irradiation, surgery, neurosurgery). The standard fractionation and total dose for whole brain irradiation is 30 Gy in 10 fractions. The decision on fractionation schedule has to consider the prognostic factors with shorter overall treatment times including higher fractionated doses in the unfavorable prognostic group and lower fractionated doses in the favorable prognostic group.

The addition of systemic treatments to whole brain irradiation in terms of radiosensitizers becomes an open question. EGFR-associated thymidine kinase inhibitors might enhance the antitumor activity of radiation therapy and warrants further research. Experimental radiosensitizing agents like motexafin gadolinium and elaproxil might positively influence quality of life or achieve even a possible survival advantage. Prophylactic cranial irradiation provides a significant survival advantage and prolongs progression-free survival in small cell lung cancer. In non-small-cell lung cancer a corresponding beneficial effect might be present.

The long-term neurotoxicity of whole brain irradiation depends on the radiotherapeutic treatment schedule, with a lesser risk for neurocognitive dysfunction in reduced fractionated doses.

The impact of medical treatments on the outcome of brain metastases is limited so far. New treatment options include new drugs, alone or in combination with radiotherapy, new methods of drug delivery, and new approaches to treat or avoid late neurotoxicity from WBRT. Among new drugs, temozolomide alone is of limited efficacy, but dose-dense schedules in combination with other cytotoxic or cytostatic drugs (capetitabine in breast metastases, thalidomide in melanoma metastases, pegylated doxorubicin, vinorelbine) are ongoing. Experimental data suggest a potential synergism between temozolomide and compounds with radiosensitizing properties (motexafin gadolinium) can modulate resistance mechanisms (PARP/PARG inhibitors). The correlation between TMZ activity and level of MGMT in the different primary tumors and brain metastases is being investigated. The role of targeted therapies (in particular EGFR and VEGFR inhibitors, GW-572016 and CI-1033) is emerging, but translational approaches are needed. Local treatments to circumvent the blood-brain barrier are now available: apart from intra-arterial chemotherapy, direct drug delivery with drug-impregnated polymer wafers (Gliadel, etc.) and convection-enhanced delivery appear promising in small subsets of patients. Treatment of cognitive dysfunctions after WBRT with neuroactive compounds (donepezil, memantine) is being investigated both in Europe and the United States. Last, new radiation techniques (the Gliaste technique, hippocampus avoidance by intensity-modulated radiotherapy) could better spare those brain areas that are “critical” for cognitive dysfunctions.

K3. NEW TREATMENT OPTIONS FOR BRAIN METASTASES
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The role of targeted therapies (in particular EGFR and VEGFR inhibitors, GW-572016 and CI-1033) is emerging, but translational approaches are needed. Local treatments to circumvent the blood-brain barrier are now available: apart from intra-arterial chemotherapy, direct drug delivery with drug-impregnated polymer wafers (Gliadel, etc.) and convection-enhanced delivery appear promising in small subsets of patients. Treatment of cognitive dysfunctions after WBRT with neuroactive compounds (donepezil, memantine) is being investigated both in Europe and the United States. Last, new radiation techniques (the Gliaste technique, hippocampus avoidance by intensity-modulated radiotherapy) could better spare those brain areas that are “critical” for cognitive dysfunctions.

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K4. TRANSLATING RESULTS FROM PHASE II TO PHASE III IN GLIOMA: PITFALLS AND LESSONS FROM THE RECENT EORTC/NCIC TRIAL EXPERIENCE

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Chemotherapy has been extensively investigated for the treatment of malignant glioma, and most agents have not demonstrated measurable antitumor activity. Nevertheless, a meta-analysis on over 2000 patients indicated a significant benefit with the administration of chemotherapy. Recently, our randomized phase III EORTC/NCIC trial demonstrated improved survival with the administration of temozolomide and radiotherapy (TMZ/RT) in newly diagnosed glioblastoma. To some, these results were unexpected, because TMZ shares similar mechanisms of action with nitrosoureas and other alkylating agents. The objective response rate of TMZ in recurrent disease was less than 10%.

Many factors beyond the choice of the treatment agent contribute to the outcome of patients with malignant glioma. Pretreatment prognostic factors and patient selection may have a much greater impact on outcome than does the treatment investigated. The time of the intervention may also play an important role. If chemotherapy is administered too late in the disease course, treatment may be too brief to induce a measurable antitumor effect. Similarly, associated toxicity may preclude sufficient exposure to the therapy.

In 1993 (validated in 1998), the RTOG presented a model of recursive partitioning analysis (RPA) based on over 1500 patients enrolled in clinical trials that characterized six prognostic classes of malignant glioma: classes III to VI being applicable to glioblastoma. Main criteria in this model are histological grade, performance status and neurological function, age, surgical debulking, and administration of radiotherapy. This classification provided a valuable tool for analyzing and comparing results from a number of phase II studies. For many “promising” studies, analysis and comparison according to the RTOG RPA classification suggested no benefit from the new treatment and further investigation was not recommended. In our phase II pilot study demonstrating the safety and feasibility of concomitant TMZ/RT, overall survival for each RPA class was also analyzed. For classes III and IV, a clear survival advantage both at the median and at two years was demonstrated, which was the impetus of conducting a large and definitive randomized trial. A detailed analysis of prognostic factors and an updated RPA classification for glioblastoma patients treated with TMZ/RT within the EORTC/NCIC trial was recently presented (Mizrami et al., J. Clin. Oncol., 2006). The probability of survival at two years increased to 43%, 28%, and 17% for classes III, IV, and V, respectively, compared to the RTOG database with 35%, 15%, and 6%. Often only a subgroup of patients with malignant glioma respond to a specific chemotherapy agent. Thus, verifying the presence of the treatment target or possible resistance mechanisms may enable the selection of patients based on their individual molecular characteristics. A retrospective analysis of the recently completed randomized EORTC/NCIC trial demonstrated that, almost exclusively, patients with a methylated (silenced) MGMT tumor gene promoter benefited from the addition of TMZ chemotherapy. The MGMT gene codes for the DNA repair enzyme methylguanine-methyltransferase (MGMT). MGMT expression will revert the cytotoxic methylation at the O6 position of guanine, thus conferring a mechanism of resistance to TMZ. The absence of MGMT methylation is the strongest predictor of response to TMZ therapy and increased probability of survival at two years. In addition to RPA classes, future trials will need to stratify for MGMT promoter methylation when evaluating new agents in conjunction with standard TMZ/RT.

K5. DEVELOPMENT OF MOLECULAR TARGETED THERAPIES IN MALIGNANT GLIOMAS: LESSONS LEARNED FROM OTHER TUMOR TYPES IN MEDICAL ONCOLOGY

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Recent advances have been made in medical oncology using molecular targeted therapies that block cell signaling by disrupting ligand-receptor interactions of multiple receptor tyrosine kinases, such as EGFR, HER2, VEGF/VEGFR, PDGFR, KIT, and FLT3, among many others. Most recent advances have aimed to interact with downstream signaling proteins that transmit the signal through three main signaling pathways: RAS/MEK/ERK, PI3K/Akt/mTOR, and PKC. Among a number of molecular targeted agents, human monoclonal antibodies blocking EGFR, HER2, and VEGF were shown to improve survival when combined with chemotherapy or radiotherapy in patients with colon, breast, and head-and-neck carcinomas. In addition, small molecule blocking of the inner ATP-binding domain of EGFR/VEGFR/PDGFR/KIT tyrosine kinases was also shown to be of benefit, either alone or in combination with chemotherapy, in the treatment with lung, pancreas, renal cell, and colorectal tumors. A lesson learned from the development of those agents is that, in clinical trials, highly selective or specific blocking of only one of the kinases involved in these signaling pathways has been associated with limited or sporadic responses. Improved understanding of the complexity of signal transduction processes and their roles in cancer has suggested that simultaneous inhibition of several key kinases at the level of receptors and/or downstream serine/threonine kinases may help to optimize the overall therapeutic benefit associated with molecularly targeted anticancer agents. Using targeted agents to inhibit multiple signaling pathways has emerged as a new paradigm for anticancer treatment based on preclinical and clinical data showing potent antitumor activity of single drugs inhibiting multiple molecules. Using targeted agents in combination with multiple drugs with selective or narrow target specificity. Preclinical and clinical studies point to molecules on vascular endothelial cells and pericytes as being important targets for anticancer therapies, as well as molecules on or within tumor cells themselves. This suggests that optimal therapeutic approaches to cancer may involve targeting multiple molecules found both in the tumor and supportive tissues. We use the most recent preclinical and clinical data to describe this emerging paradigm for anticancer therapy that involves targeting multiple signaling pathways with tyrosine or serine/threonine kinase inhibitors. New parameters used for investigating the antitumor effects, surrogate endpoints of activity, and usefulness of target expression in selecting patients for individualized therapeutics are discussed in the context of their potential application in the treatment of malignant gliomas.

K6. NEXT STEPS IN GlioBLASTOMA AND OTHER GILOMAS

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In recent years, important data from biology, imaging, and clinical trials have profoundly changed and significantly improved our knowledge and practice in the treatment of gliomas, but also raised numerous unanswered questions and opened new directions for clinical research.

Improvement in diagnosis and prognosis is still a valuable goal in the treatment of gliomas—even in glioblastomas in which age and Karnofsky performance score remain the mainstays of therapeutic decision making. While no clear factor can be used to predict extreme early or long-term survival in gliomas (WHO grade II and III), the profound unreliability of grading has led to the definition of new types of prognosis by including clinical, radiological, and molecular parameters such as 1p/19q deletions. However, aggressive grade II gliomas share with 1p-intact grade III gliomas a similar prognosis, while indolent grade II gliomas exhibit an expected survival close to that of 1p-deleted grade III tumors. Access to and progress in metabolic imaging of grade II and III tumors led to its increased use, but their additive benefit has yet to be validated.

Recently, on the impact of MGMT status on the benefit of combining chemotherapy and radiotherapy has also offered the field of the use of predictive factors in therapeutic decision making. Other candidates have also been proposed, but, for all these markers, the technical and methodological process of validation needs to be performed before they can be used in routine practice. As in other fields of oncology, translational research will become a critical issue in future trials. Improvement in the treatment of gliomas is the ultimate goal of research. Results observed with the use of radiotherapy combined with temozolomide administration is the strongest predictor of response to TMZ therapy and increased probability of survival at two years. In addition to RPA classes, future trials will need to stratify for MGMT promoter methylation when evaluating new agents in conjunction with standard TMZ/RT.

K7. ONGOING GLIOMA STUDIES

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At present, many of the clinical trials on high-grade glioma are either fine-tuning concurrent chemoradiation with temozolomide or are trying to improve on this regimen by adding novel targeted compounds that inhibit proteins or receptors that are presumed to play a pivotal role in glioma tumorigenesis. In the latter studies, knowledge obtained in laboratories or in preclinical studies on overexpressed or downregulated proteins is taken to the clinical arena. Targeted agents are added to the standard treatment in either single-arm or randomized phase II trials to determine whether the outcome is improved as compared to historical controls or a preset surrogate end point.
point. A multitude of trials are ongoing, even with agents that failed to produce significant clinical activity when given in recurrent disease.

One of the important questions that arises is the clinical decision about which regimen is considered interesting enough to warrant further clinical trials. In recurrent GBM, a multitude of trials with targeted agents and antiangiogenic agents are ongoing. These trials are indicated to develop new treatment strategies for patients failing combined chemoradiation therapy and to identify new active agents. In the coming months, results are expected of many CED trials with targeted agents. The results will show whether this approach holds promise. Trials on anaplastic glioma are now being scheduled depending on their 1p/19q status and aim at establishing whether combined chemoradiation is also the treatment of choice for these tumors. Trials on low-grade glioma mainly focus on the role of chemotherapy in this disease, either given in lieu of radiotherapy or with radiotherapy. Since these trials will take considerable time to complete, intergroup cooperation is being developed.

ORAL PRESENTATIONS

01. HYPOXIA INDUCES MET EXPRESSION AND ENHANCES SF/HGF-INDUCED MIGRATION OF GLIOMA CELLS
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Scatter factor/hepatocyte growth factor (SF/HGF) is a pleiotropic growth factor with motogenic, mitogenic, and angiogenic properties. Both SF/HGF and its receptor, the proto-oncogene–encoded tyrosine kinase receptor MET, are strongly overexpressed in human malignant gliomas. In carcinoma and sarcoma cell lines, MET was shown to be inducible by hypoxia. The MET promoter contains several binding sites for the hypoxia-inducible factor 1 alpha (HIF-1α). Since hypoxia is an important driving force in glioma angiogenesis and invasion, we investigated whether hypoxia upregulates MET expression in glioma cells and whether it affects SF/HGF-induced cell migration. We found that MET protein levels were increased after 48 h of hypoxia in 9 of 18 (50%) glioblastoma cell lines and in five of seven (71%) glioblastoma primary cultures. Upregulation of MET was also detected at the transcriptional level by using quantitative PCR; typically, two expression peaks were detected at 4 and 16 h. Cell lines with comparatively high levels of MET already under normoxic conditions tended not to show further upregulation under hypoxia. In 16 of 18 (89%) cell lines, HIF-1α was induced by hypoxia. All cell lines in which MET was inducible by hypoxia also showed upregulation of HIF-1α, so that MET induction did not occur without HIF-1α induction. Transfection of siRNA against HIF-1α abrogated the hypoxic induction of HIF-1α as well as of MET, suggesting that MET expression is upregulated by a HIF-1α–dependent mechanism. In a spheroid migration assay, hypoxia sensitized glioma cells in three of three glioblastoma cell lines for the stimulating effects of SF/HGF. To conclude, MET expression is inducible by hypoxia in many but not all glioblastoma cell lines, and even more so in primary cultures. Hypoxia may be a key factor in sensitizing glioblastoma cells for the motogenic effects of SF/HGF.

02. TARGETING RAC1 GUANINE NUCLEOTIDE EXCHANGE FACTORS BLOCKS THE MIGRATION AND INVASION OF GLIOMAS
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The invasion of tumor cells into regions of normal brain is arguably one of the main reasons why patients with malignant astrocytomas frequently fail therapy. We have recently shown that Rac1 is a key mediator of glioma cell migration and invasion. Accordingly, microarray analysis that we have now performed on 111 human malignant astrocytoma specimens show that significant alterations are noted in transcripts of upstream activating proteins of Rac1 known as the Rac guanine nucleotide exchange factors (Rac GEFs), while Rac1 mRNA levels are similar across the panel examined. By principle component analysis, increased expression of Rac GEFs is associated with poor survival and advanced astrocytoma grade. Of 26 possible Rac GEFs examined, three were identified that displayed consistent association with poor outcome: Ect2, Trio, and Vav1. We have performed quantitative PCR to validate the expression of these genes in clinical specimens and established astrocytoma cell lines. Interestingly, decreasing the expression of these Rac GEFs by small interference RNA inhibits the migratory and invasive properties of glioma cell lines in 2-D migration and brain–slice assays. These data suggest that targeting Rac GEFs may be a novel strategy by which astrocytoma migration and invasion can be inhibited.

03. THE HUMAN MAJOR VAULT PROTEIN (MVP) CONTRIBUTES TO THE MALIGNANT GROWTH OF HUMAN GLOBLASTOMA MULTIFORME
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Vaults are ubiquitously expressed and highly abundant ribonucleoprotein multimeric complexes consisting of three proteins (MVP, vPARP, and TEP1) and a small untranslated RNA (vRNA). The major vault protein (MVP) and/or vaults have been implicated in the regulation of multiple cellular processes, including transport mechanisms, chemoresistance, and several signaling cascades, including the MAPK pathway, src-derived signals, and the tumor-suppressor phosphatase PTEN. While in normal brain the expression of MVP, representing more than 70% of the vault particle mass, is very low, we have previously shown that MVP expression is strongly and consistently upregulated in all forms of astrocytomas, including glioblastoma multiforme. To investigate the significance of MVP activation in primary brain tumors, we have analyzed the consequences of (1) ectopic MVP overexpression (stable clones and adeno viral constructs) in MVP-negative H7 cell lines established from a highly dedifferentiated human glioma and (2) inhibition of MVP expression by adenosine shRNA constructs in several glioblastoma cell lines. While ectopic MVP in H7 cells only marginally enhanced drug resistance to diverse chemotherapeutics, it led to a massively increased proliferative (growth curves) and migratory (scratch assays and Boyden chambers) potential in vitro. Cytoskeletal plasticity and actin filament rearrangement in response to phorbol ester was increased in MVP-overexpressing subclones (immunofluorescence). Moreover, MVP-transgenic cells were significantly resistant to cell death induced by serum starvation. In vivo, subcutaneous tumor growth in SCID mice was significantly enhanced in all MVP-overexpressing H7 subclones as compared to vector controls. Specific inhibition of MVP expression by shRNA induced cell cycle delay and/or programmed cell death in 9T8G and U373 glioblastoma cells. In summary, our data suggest a significant contribution of vaults like MVP to the malignant phenotype of human glioblastoma cells and implicate suppression of MVP as a new therapeutic strategy against glioblastoma.

04. THE ANALYSIS OF EFPR INH-B LIGAND TYROSINE KINASES IN GLIOMA INVASION
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The erythropoietin-producing hepatocellular (Eph) receptors and their ligands, ephrins, represent the largest known family of tyrosine kinase receptors in the human genome, with 16 Ephs and nine ephrins. The signaling via Eph/ephrin is involved in various aspects of the nervous system’s development, including axon guidance regulation, cell migration, morphogenesis, and vascular formation. Both Ephs and ephrins are transmembrane proteins and transduce signals bidirectionally when Ephs bind with ephrins. The mutual activation of Eph/ephrin causes permissive effects upon cell–cell contact. Previously, we have shown that overexpression of EphB2/ephrin B3 in glioma cells increases cell invasion. We further investigated the clinical relevance of ephrin B by analysis of ephrin-B mRNA expression in 24 normal and 111 brain tumor specimens annotated with clinical outcome. The levels of ephrin B1 and B2 mRNA were significantly higher in glioblastoma samples (n = 82) compared to normal brain specimens (P < 0.01). Kaplan-Meier analysis demonstrated that ephrin-B2, but not ephrin-B1, expression level is a powerful predictor of a short survival in malignant astrocytomas (n = 97, P = 0.016). Highly invasive glioma cell line U87 expressed a high level of ephrin B2 compared with relatively low invasive glioma cell lines (U251, T99G, and SF767). The invasion of U87 was accelerated by the addition of EphB2/Fc chimera, which activates ephrin B. U87 cells transfected with ephrin-B2 siRNA decreased invasion in vitro, while ephrin-B1 siRNA did not affect the invasion activity. Depletion of endogenous ephrin B2 expression abrogated the increase of invasion by EphB2/Fc stimuli, indicating increased invasion is dependent on ephrin-B2 activation. Concomitant with this data, Akt was phosphorylated by EphB2/Fc, and the phosphorylation of Akt by addition of EphB2/Fc, suggesting that ephrin B2 increases invasion via...
O5. A GENETIC VARIANT OF METHIONINE METABOLISM (MTR c.2756A>G) IS ASSOCIATED WITH GliOblastOma INCIDENCE

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Objective: Glioblastoma multiforme (GBM) is the most common primary brain tumor in humans, accounting for up to 15% of all intracranial neoplasms. Polymorphisms of methionine metabolism have been associated with the incidence of various cancers and toxic side effects of chemotherapy. In the present study, the authors investigated whether a germ-line variant of the MTR gene (methionine synthase, MTR c.2756A>G) may influence the risk of developing GBM.

Methods: The MTR c.2756A>G polymorphism was genotyped in 213 GBM patients and in 400 healthy population controls without a history of cancer. Differences between genotype frequencies were tested for statistical significance using the chi-squared test (Pearson’s P). Significance was set at P < 0.05.

Results: A highly significant association between the MTR c.2756A>G polymorphism and GBM incidence was observed (x² = 13.86; P < 0.001).

Conclusion: This is the first report linking a genetic variant of methionine metabolism to the risk of developing a GBM. The MTR c.2756A>G polymorphism has been shown to influence DNA methylation and may play a role in chromosomal stability. One is tempted to speculate that (e.g., dietetic) interventions aimed at modifying MTR activity may help to reduce GBM incidence.

O6. GENETIC AND EPIDEMGENIC MARKERS IN RECURRENT OLIGODENDROGLIAL TUMORS (OT): WHAT DOES IT TELL US?

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Introduction: Allelic losses on chromosomes 1p/19q are well recognized in OT while 10q loss is frequent in high-gradeastrocytomas. MGMT promoter methylation (PM) is associated with an improved survival in GBM treated with alkylating agents. In OT, the status of MGMT PM and its association with other genetic alterations is not well characterized, and even less is known about temporal evolution of such changes detected at tumor progression (TP).

Objective: To evaluate the status of chromosomes 1p/1q/10q and MGMT PM in the early phase of OT and again at TP.

Methods: A total of 46 tumors of 23 patients were obtained at an early phase of the disease and again at TP. Initial diagnosis included 17 OT and six oligoastrocytomas. 1p/19q13 and 10q22–26 status were evaluated from paired tumor-blood DNA samples by PCR-based microsatellite analysis. MGMT promoter status was determined by methylation-specific PCR analysis.

Results: At initial evaluation, 61% tumors were low grade (WHO II), compared to only 17% grade II tumors at TP (P < 0.001). OT tumors that initially were described as OT, 13 (76.5%) remained in this registry, regardless of their grade, while 23.5% changed to primarily astrocytic tumors. Of the six mixed tumors, four (67%) transformed to astrocytic tumors (P = 0.02). The cell type of all 10 tumors initially characterized as OT remained unchanged if they contained a 1p/19q deletion, while only one of six mixed tumors with a 1p/19q deletion remained phenotypically unchanged (P = 0.008). Of the 15 tumors with early 1p loss, 80% remained OT at TP as compared with the eight tumors without 1p loss, 75% of which changed to astrocytic phenotype (P = 0.01). Loss of 10q was uncommon in both phases. The proportion of MGMT PM increased from 19% in the early phase to 71% at TP (P = 0.0000). MGMT was uniformly unmethylated in early tumors with an intact 1p, whereas 31% of tumors with a 1p deletion contained MGMT PM (P = 0.04). The proportional gain in MGMT PM at TP was limited to 31% for tumors with a 1p deletion, unlike tumors with an intact 1p, which had an 87.5% gain of MGMT PM at TP (P = 0.006).

Conclusions: Our findings indicate that OT with a 1p/19q deletion tend to retain their cell phenotype and genetic profile at TP, unlike tumors with no deletions. MGMT PM is more pronounced at TP particularly in tumors with an intact 1p. It is suggested that the chemosensitivity of OT is not related to MGMT PM, and there should be other contributing factors, yet to be discovered.

O7. MULTIPOTENTIAL NEURAL PROGENITORS THAT MIGRATE EXTENSIVELY IN THE ADULT BRAIN CAN BE DERIVED FROM HUMAN WHO IV ASTROCYTOMAS

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To explore the multipotentiality and migratory potential of neurosphere-forming cancer, stemlike cells isolated from human glioblastoma fresh surgical specimens from six patients aged 55 to 65 were used. After enzymatic digestion, the tissue was finely minced and dissociated and then filtered through a 40-μm filter. The tissue was resuspended in serum-free medium with EFG and FGF at a density of 100 cells/μm. The cells were fed every seven days. Spheroid bodies were formed over a period of five to six weeks and were dissociated and reseded (passaged) at 100 cells/ml.

After one to three passages (6 to 18 weeks) in culture, the spherical bodies were plated onto coverslips coated in laminin. Mitogens were removed from the culture medium, and 1% fetal calf serum was added. The spherical bodies were exposed to this differentiation medium for up to five days. Immunohistochemical studies identified cells with a neuronal (TuJ1), astroglial (GFAP+), and oligodendrocyte (O4+) phenotype. Confocal microscopy of these spheroids demonstrated that the neural lineages were homogeneously distributed within the spheres which had little or no internal necrosis on TUNEL and H&E staining. Whole genome microarray analysis of the neurospheres confirmed it was derived from human glioblastoma.

Oligo- and astrocytosis-forming stemlike cells from two patients were each implanted into the forebrain of three CB17/Cr-Pkradexed/Crl (SCID) mice at a density of 5 × 10⁶ cells/m. The cells were traced immunohistochemically by using an antibody against human nuclear antigen. After six weeks of survival, these human derived stemlike cells did not form large tumors. They were identified within the corpus callosum extending into the contralateral hemisphere, in the septum, rostral migratory stream, and olfactory bulb.

These data suggest that human glioblastomas contain a subpopulation of cells that exhibit certain characteristics of neural progenitors and are capable of widespread migration in vivo. Grant support: MRC U.K. grants 60773 (C.W.) and 67437 (C.W./J.F.).

O8. LOSS OF 1P/19Q IN INFILTRATING GLIOMAS: PCR MICROSATellite AMPLIFICATION AND FISH DETERMINATION

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Background: Patients with anaplastic oligodendroglioma harboring 1p or 1p/19q losses live significantly longer and their tumors are more sensitive to therapeutic agents than are neoplasms without these alterations. Determination of 1p/19q status may also help to subclassify oligoastrocytoma (some tumors are probably oligodendroglioma in nature and behavior, whereas others are similar to astrocytomas) and to distinguish glioblastoma from anaplastic oligodendroglioma. Fluorescence in situ hybridization (FISH) and microsatellite amplification by PCR are the two main techniques used for 1p/19q loss determination.

Objective: To compare the validity of FISH and PCR in 1p/19q loss determination, we have contrasted the results obtained with both techniques in a series of 48 glioma samples obtained between 1992 and 2003. These included nine grade II oligodendroglialomas (OGs), eight grade III oligodendroglialomas (OADs), two grade II astrocytomas (GAs), three grade III oligoastrocytomas (OAAs), four grade III astrocytomas (AAs), and 22 glioblastomas (GBs). In every case, 1p and 19q losses were independently investigated with both methods and the findings were not compared until the end of the study. In regard to FISH, for each chromosome we used a probe specific for the potentially deleted arm and a probe for the opposite arm as control. A total of 300 cell nuclei were evaluated per slide. As for PCR, four microsatellites were amplified for each chromosome: D1S199, D1S124, D1S508, and D1S234 for 1p; and D19S112, D19S219, D19S412, and D19S536 for 19q. LOH was analyzed with GeneScan. Peripheral blood or normal tissue from the same patient was used as control.

Results: In 41 of the 48 cases, FISH and PCR results were completely coincident. In four cases (three ODs and one OAD), tissue was insufficient for FISH evaluation. In one case (AA), PCR showed a 1p deletion detected by FISH, and in two cases, PCR suggested 1p and 19q losses not shown by PCR. Combined 1p/19q losses were present in four ODs, three OADs, one OA, and two GBs, by either FISH or PCR, or by both. Isolated 1p loss was seen in one AA and four GBs, and isolated 19q loss in one OAD and five GBs.

Conclusions: Our findings indicate that FISH and PCR provide virtually coincident results in regard to determination of 1p/19q loss. It thus seems that the experience and facilities available should be the main considerations to be taken into account by each institution when deciding which method to implement for the study of 1p/19q status.
O9. ESTABLISHMENT AND CHARACTERIZATION OF HUMAN GliOBLASTOMA STEM CELL LINES

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Glioblastomas contain a small subpopulation of cells that display a stem cell–like phenotype. Serum-free culture conditions with appropriate growth factors can support the survival of neural stem cells and were recently shown to also support the growth of glioblastoma stem cells. We used established protocols to generate permanent glioblastoma stem cell lines from tumors of eight different glioblastoma patients. All eight cell lines have now been grown for at least 12 passages, and for up to 25 passages (over 1.5 years in culture), and are stably expandable. Most cell lines grow entirely as neurospheres, but three cultures grow partly adherent. In all cultures, expression of the neural stem cell markers nestin, CD133, musashi-1, SOX2, and Bmi-1 was detected by immunocytochemistry and/or RT-PCR, and most cells coexpressed GFAP. Under differentiating conditions, expression of the neuronal and oligodendroglial markers MAP-2 and GalC, respectively, was inducible, whereas nestin expression was lost by most cells. More than 50% of the cells were clonogenic and formed new secondary spheres in limiting-dilution assays. Upon intracerebral injection into nude mice, the cells formed diffusely invasive tumors. Exposure to standard chemotherapeutics in an in vitro survival assay revealed the relative chemoresistance of glioblastoma stem cells in comparison to tumor cells that do not display stem cell–like properties. To conclude, long-term propagation of glioblastoma stem cells as permanent cell lines is feasible. These cells can be used to develop specifically tailored cyrotactic therapies and to test anti-invasive strategies in a highly invasive murine model.

O10. PRESERVATION OF CANCER GENOMIC PROFILE IN ORGANOTYPIC GLIOMA SPHEROIDS, BUT NOT IN PRIMARY CELL CULTURE

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The prognosis for patients with glioblastoma multiforme (GBM) remains poor despite surgical resection, radiotherapy, and temozolomide chemotherapy. As such, there is a continuous need for valid experimental models to screen novel therapeutics efficiently. For this purpose, established cell lines and primary cell (PC) cultures are customarily used because of their ease of culture and quantification of response. However, the correlation between in vitro and in vivo responsiveness is poor. The organotypic spheroid model provides a more complex biological system that maintains the cell-cell interactions, extracellular matrix, and cellular heterogeneity. Copy-number abnormalities represent an important aspect of tumor biology because they are correlated with prognosis and therapeutic response in patients. The present study compared the GBM genomic profiles in both OS and PC cultures from the same tumor material.

For this purpose, surgical material was collected from five GBM patients. Both OS and PC culture were grown using standard procedures. After two weeks, OS and PC were harvested (passage 2). DNA was extracted from the original tumor, OS, and PC cultures.

Copy-number abnormalities were determined throughout the genome by array comparative genomic hybridization at high resolution (500-kib) and completely sequenced. The DNA microarray. The log2 ratios of Cy3/Cy5 signals were median normalized and ordered by chromosomal position. Gains and losses of genomic regions were determined with the clac algorithm. The cancer genomic profiles were compared with unsupervised cluster analysis.

The genomic profiles of the original tumor, OS, and PC cultures clustered together for all five patients. In five of five, the OS genomic profiles clustered close to the tumor profile. In only three of five, the PC culture’s genomic profiles clustered close to the tumor profile, whereas, in two, the profile changed dramatically, showing loss of relevant gains and restoration of relevant losses of chromosomal regions compared to the original tumor.

In conclusion, the GBM genomic profile is preserved in the OS model but not in short-term PC culture, providing a more representative model of the tumor biology. The altered genomic profiles in two of five short-term PC cultures is in support of clonal selection, possibly explaining the discrepancy between response to treatment in vitro and in vivo.

O11. A RECOMBINANT VESICULAR STOMATITIS VIRUS (VSVδM51) PROLONGS SURVIVAL AND TARGETS MULTIFOCAL GLIOMAS AND INVASIVE CELLS WHEN ADMINISTERED INTRATRAINOSLY

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An ideal oncolytic virus to treat gliomas would replicate preferentially in glioma cells, target multifocal gliomas and invasive glioma cells, and be delivered systemically. While wild-type VSV has demonstrated some antitumor potential in animal models of systemic cancer and gliomas, it has had limited efficacy or unacceptable toxicities. To enhance VSV’s selectivity for tumor cells, we constructed a mutant with a deletion in its M protein we call VSVδM51. We then evaluated VSVδM51 in various experimental models of human MGs. We found that all 14 glioma cell lines tested were susceptible to VSVδM51 infection, including two that were resistant to reovirus type 3. Normal human cells (HS68 and NIH3T3) were resistant to both VSVδM51 and reovirus type 3. VSVδM51 also killed glioma cells very rapidly and at low multiplicities of infection, resulting in marked gene expression, which could be used in test anti-invasive strategies in a highly invasive murine model.
distribution of NSCs in intracranial glioma. Our study serves as a model for future studies that seek (1) to gain more insight into the biochemical factors involved in the NSC-tumor tropic phenomenon, and (2) to better define the optimal conditions for NSC delivery of specific therapeutic genes for cancer treatment.

013. NEUROTROPHINS AND NEUROTROPHIN RECEPTORS EXPRESSION IN BRAIN METASTASIS FROM DIFFERENT HUMAN MALIGNANCIES

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A major cause of death in cancer patients is brain metastasis, which occur in 20% to 40% of cases. Brain invasion by tumor cells depends on diffusion through the blood barrier and on response to growth factors. Neurotrophins (NTs) are growth factors that play an important role in invasion, proliferation, and apoptosis. NTs may take part in tumorigenesis in nonneural tissues, and their expression and role have been investigated in several nonneuronal carcinomas as markers in the clinic. NTs are a family of secreted proteins consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5). These molecules bind two types of cell surface receptors: the Trk receptors and the common neurotrophin receptor p75. The Trk family of receptors has tyrosine kinase activity and is made up of three members, each with different specificities: Trk-A binds NT-3, NGF, Trk-B binds BDNF and NT-4/5, and Trk-C binds NT-3. NTs promote invasion of responsive tumor cells through the blood-brain barrier by enhancing the production of basement-membrane-degradative enzymes. Our study investigated the expression and distribution of some NTs and their receptors in brain metastasis from different human malignancies by immunohistochemistry, and evaluated a possible clinical role. The specificity of anti-NT and anti–NT receptor antibodies was determined by Western blot analysis. We used antibodies directed against NGF, NT-3, Trk-A, and Trk-C in 63 surgically resected brain metastasis from different types of malignant tumor, with the following origin: lung (20), breast (20), kidney (10), colon (5), ovary (3), prostate (2), and thyroid (1). Immunoreactivity for NTs and NT receptor was detected with different expression patterns in vessel walls and sometimes within neoplastic cells. Lung, breast, and thyroid carcinomas displayed high levels of Trk-A and Trk-C in 40% to 60% of the cases. A moderate expression of NGF and NT-3 was observed in about 30% of the cases, mainly in lung carcinomas. A partial correlation was found between the immunohistochemical expression and the pattern of nervous tissue infiltration. The overall survival of the patients was evaluated, and the correspondence with the NT and NT receptor expression did not reach statistical significance. Our results suggest that NTs and NT receptors may be involved in growth and invasion of several brain metastasis.

014. CLINICAL ANALYSIS OF PATIENTS WITH BRAIN METASTASES WITH PREVIOUSLY UNDIAGNOSED PRIMARY MALIGNANCY

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Background: Brain metastases are common as the first symptom in systemic cancers. We intended to determine the clinical and histopathological aspects, frequency of brain metastases with unknown origin, and related survival.

Methods: A total of 105 consecutive patients (mean age, 52.6 years) with brain metastasis admitted to four neurosurgical centers of Tehran University of Medical Sciences were studied. Age, gender, site of the metastasis, neurological manifestations, histological pattern, origin, survival, and treatment were investigated. The previously undiagnosed primary tumors were included (n = 53). Survival was determined by Kaplan-Meier method.

Results: Of the 53 cases (50.5% of all; male–female ratio of 2:1), 47 (88.7%) had solitary lesions. The most frequent symptom was local pare-sis. The primary origins were diagnosed in 36 cases, respectively, as lung (30.2% adenocarcinoma and squamous carcinoma), kidney, and colon. In 17 (32.1%), the primary origin remained unknown, of which 7 were adenocarcinoma, 5 squamous carcinoma, and 5 undifferentiated metastatic cells. The mean survival was 13 months with radiotherapy after resection. The mean survival was 11 months in all 105 cases compared to 13 months in 53 cases with unknown primary origin, and the difference was not statistically significant. We have also compared the cases based on the treatments, survival, and primary tumor.

Conclusion: Despite the high frequency of cases with previously undiagnosed origin, the rate of finally unknowns is 16.2% of all 105 cases. This shows the short metastasis interval of tumors such as those of the lung, with some symptoms probably inadvertently ignored by patients and physicians.

015. THE INTERDISCIPLINARY CONCEPT AND OUTCOME IN TREATING PATIENTS WITH MALIGNANT ANTERIOR SKULL BASE TUMORS

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Objectives: Malignant tumors of the anterior skull base are complex lesions that require an understanding of the tumor pathology, principles of resection, and nonsurgical therapeutic modalities. To justify a complex interdisciplinary surgical approach, the indications and contraindications for resection should be influenced by prognostic factors and anticipated outcome.

Methods: A 10-year retrospective analysis was performed on a homogeneous group of 40 patients with malignant anterior skull base tumors metastases, intrasellar metastases, ethmoidal adenocarcinomas, sarcomas, chordomas, and malignant meningiomas). Recursive partitioning analysis (RPA) criteria for metastases used for class assignment were Karnovsky performance status (KPS), primary tumor status (PD), presence of extracranial system metastases (SD), and age.

Results: Median follow-up was 41 months (range, 3–124). Median age at diagnosis was 49.6 years (range, 3–91). Median KPS was 70 (range, 60–90). The median survival of patients with anterior skull base metastases was 16 months (range, 3–48), contrary to that for patients with intrasellar metastases, who had a median survival of 14 months (range, 10–19). Median survival of patients with ethmoidal adenocarcinomas was 10 months (range, 8–12); sarcomas, 10.5 years and 5.3 years (still under observation); malignant meningiomas, 19 months (range, 12–25); and chordomas, 23 months (range, 8–51).

Conclusions: Carefully selected patients will benefit from surgical treatment. The outcome and survival time is comparable to those for patients with brain metastases; in our analysis, even better. RPA classes are valid for malignant anterior skull base tumors and may also serve as a basis for historical comparisons.

016. TEMOZOLOMIDE IN ADVANCED MALIGNANT MELANOMA WITH SMALL BRAIN METASTASES: CAN WE CANCEL CRANIAL IRRADIATION?

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Purpose: To evaluate the efficacy of treatment with temozolomide in patients with metastatic melanoma, including brain metastases, who do not require immediate cranial irradiation, and to evaluate the feasibility of postponing or canceling cranial irradiation.

Patients and methods: Patients with brain metastasis were identified from prospective studies of temozolomide (with or without immunotherapy) for metastatic melanoma. Brain metastasis larger than 2 cm, extensive edema, and localization in the brain stem were excluded. Previous stereotactic RT, whole brain RT within 6 weeks prior to start of temozolomide, and leptomeningeal metastasis were excluded. In patients with systemic response or stabilization to temozolomide, the response of brain metastases and necessity of palliative cranial RT were evaluated.

Results: From 179 patients treated for advanced melanoma, 52 patients with brain metastasis (29 without immunotherapy and 23 with immunotherapy) were evaluable. Stabilization of systemic metastasis was noted in 7 (13%) of the 52 patients and a response in 6 patients (5 PR and 1 CR) (11%). In these 13 patients, stabilization of brain metastasis was observed in 6 patients (11%) and a response in 5 patients (2 PR and 3 CR) (9%). Immunotherapy did not affect response. Median time to neurological progression was 7 months (range, 2–15 months). Cranial RT for cerebral relapse was required in 2 patients. Median overall survival of patients with brain metastases was 5.6 months (95% CI, 4.4–6.8). In the 11 patients with neurological response or stabilization, median survival was 9.3 months (95% CI, 9.2–9.5), compared to 4.7 months (95% CI, 4.0–5.4) in the 41 nonresponders. Hematologic toxicity included grade 3–4 leukopenia (6%) and grade 3–4 thrombocytopenia (6%). Intracranial hemorrhagic complications were not observed.

Conclusions: It is feasible to treat patients with advanced melanoma and small brain metastasis with temozolomide as single treatment. Patients with systemic response usually show a durable stabilization or response of the small brain metastases. With this approach, neurological disease can be controlled and cranial RT canceled in most of these patients.
A07. CORRECT CEREBRAL NEURONS: TARGETS FOR CYTOTOXIC T CELLS IN ANTI-YO—ASSOCIATED PARANEOPLASTIC SYNDROME (PNS)

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A 66-year-old man was admitted to our department because of progressive gait ataxia, acute vertigo, and incipient slurred speech and language disturbance. Three weeks earlier, the patient, who had smoked heavily for 40 years, underwent biopsy of enlarged bronchial lymph nodes. Invasion of non-small-cell lung cancer cells was found. The results of physical and neurological examination were normal except for motor tetraparesis, gait ataxia, and dysthria and mild aphasia. Computed tomography and MRI investigation of the brain, both performed with contrast media, showed normal results.

While gait ataxia declined progressively, dysthria and aphasia were fluctuating, thus seeming to change “from day to day.” The results of radiographic follow-up investigations were repeatedly negative. Pneumonitis was suspected and then confirmed by antineuronal antibodies—anti-Yo—in the patient’s serum. Although chemotherapy with cisplatin/vinorelbine was started immediately, the patient’s general condition and neurological symptoms deteriorated rapidly, and he died of septic complications 5 months after onset of neurological symptoms.

Autopsy revealed an adenocarcinoma of the lung with infiltration of mediastinal lymph nodes only. Further detailed examination confirmed paraneoplastic cerebellar degeneration with complete loss of Purkinje cells, but also loss of cerebral cortical neurons. These changes were accompanied by infiltration of cytotoxic T lymphocytes and glia.

In conclusion, we provide further evidence for the occurrence of anti-Yo PNS in male and in nongynecological tumors. In addition, besides already well-described cerebral cortical degeneration, we found cortical cerebellar degeneration. Our data suggest that highly specialized neurons other than Purkinje cells may serve as targets for direct cytotoxic T-cell attack.

A08. PARANEOPLASTIC NEUROLOGICAL SYNDROMES FROM EURONEURONETWORK DATABASE: A DATABASE

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Objective: This study is focused on characterization of the clinical profile of paraneoplastic neurological syndromes (PNS) retrieved from the PNS Euroneuronetwork Database.

Background: PNS are rare diseases, and thus multicentric studies are the only way to collect an adequate number of patients to conduct rigorous studies. In 2002, a network was developed (with a grant awarded by the European Commission) involving 20 leading European researchers on these diseases.

Design: A database has been produced in which new cases are inserted by all the centers, following a standardized method, and new diagnostic criteria have been defined (Graus et al., JNPN, 2004). This represents an operating instrument for conducting new studies on these neurological diseases.

The database now contains new cases of PNS from 2000 onward. Most cases of sensory neuropathy (125 patients), followed by paraneoplastic cerebellar degeneration (123), limbic encephalitis (57), paraneoplastic encephalomyelitis (42), and dysautonoma (42). The clinical profile has been characterized for each syndrome by analyzing the principal symptoms and immunological and tumor-related data. Interestingly, a large number of cases with sensory-motor neuropathy (47) and chorea (9) have also been collected. Antibodies against onconeural antigens have been found in 523 cases (86%); anti-Hu was the most frequent (41.4%), followed by anti-Yo (11.7%). In all lung cancer was the malignancy most associated with PNS, while 81 patients were affected by tumors more rarely associated with PNS.

Conclusions: The clinical characteristics of the individual syndromes are discussed in relation to the data in the literature. However, according to the data included in the database, the total spectrum of PNS is higher than previously thought.

A09. CSF PROTEIN PROFILING: A POTENTIAL NEW DIAGNOSTIC AND PROGNOSTIC TOOL FOR PATIENTS WITH LEPTOMENINGEALE METASTASES

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Background: Leptomeningeal metastases (LM) occur in 0.8% to 8% of cancer patients and are associated with a poor prognosis. The diagnosis of LM is based on clinical symptoms, MRI of brain and spine, and cytological analysis of CSF. The clinical picture of LM is highly variable, and both cytological CSF analysis and contrast-enhanced MRI are limited in sensitivity. More sensitive tools are welcomed to diagnose LM. Furthermore, to make a better estimation of the survival of LM patients so as to decide on treatment, additional prognostic indicators are needed.

Methods: Using multiplex immunosassay, we measured a profile of nine proteins involved in adhesion and inflammation in the CSF of LM (n = 57) and in control patients (systemic malignancy [n = 20], asymptomatic patients [n = 11], and other neurological diseases [n = 19]) and determined their potential diagnostic and prognostic value.

Results: We found high CSF levels of soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1), interleukin 8 (IL-8), pulmonary and activation regulated chemokine (PARC), interleukin 18 (IL-18), and interleukin γ-inducible protein (IP-10) in patients with LM. The CSF protein profile in patients with LM differed significantly from the profile found in control patients. Multivariate logistic regression and ROC analysis showed that the MIA-measured CSF protein profile has an additive discriminating value for LM above standard CSF parameters. A combination of total protein, glucose, IL-8, PARC, and IP-10 CSF levels proved to be most discriminative between LM and non-LM patients. Multivariate Cox proportional hazard regression analysis further demonstrated that high IL-8 CSF levels in patients with LM predicted short-term survival.

Conclusions: Our data indicate a potential diagnostic and prognostic value of CSF protein profiling for patients with LM. The results need to be confirmed in a prospective setup before they can have clinical implications.
Although flow cytometry is generally more sensitive than cytromophogy, a number of patients have negative flow-cytometric results in the presence of cytologically proven leptomeningeal involvement. Similarly, although the probability of CSF localization is increased in the cases of CSF lymphocytic or elevated protein, normal cell counts and protein concentrations do not rule out the possibility of a CSF localization. Thus, CSF cytromophogy and evaluation of cell counts and protein concentration remain valuable adjuncts to flow cytometry.

O21. POLYMORPHISMS OF METHIONINE METABOLISM AND THE CLINICAL COURSE OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Methotrexate (MTX) is the most efficient cytostatic drug in primary central nervous system lymphoma (PCNSL). It directly interferes with folate and methionine metabolism. We analyzed the relevance of nine polymorphisms with influence on folate and methionine metabolism for the clinical course of PCNSL patients treated with a MTX-based polychemotherapy (MTX, cytarabine, and prednisolone).

Higher age (multiple nominal logistic regression, P = 0.090 for trend), male gender (P = 0.008), and the genotypes of DHFR c.594+59del19bp (P = 0.016), MTTHFR c.1298A>C (P = 0.004), and Tc2 c.776C>G (Kaplan-Meier, P = 0.036 for the CG genotype vs. CC/CG).

We conclude that polymorphisms of methionine metabolism modify both the vulnerability of white matter and the clinical outcome in PCNSL patients treated with high-dose MTX-based polychemotherapy. A detailed analysis of the impact of individual conditions of methionine metabolism for effects and side effects of MTX may help to improve MTX-based therapy regimens.

O22. ISOLATED CENTRAL NERVOUS SYSTEM POSTTRANSPLANT LYMPHOProliferATIVE DISORDER (CNS-PTLD): THE INTERNATIONAL PRIMARY CNS LYMPHOMA (IPCL) COLLABORATIVE GROUP EXPERIENCE

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We reviewed records of 24 patients diagnosed with PTLD restricted to the CNS following solid organ transplantation to characterize clinical features and response to therapy of this rare disorder.

A total of 24 patients (18 male) had a median age of 40 (range, 5–74). Transplanted organs included 2 hearts, 2 lungs, 1 liver, 3 pancreas, and 2 kidneys. Median time from transplant was 32 months; 6 patients developed CNS-PTLD within 1 year after transplantation, and 1 patient was treated with rituximab before transplantation and presented with SD. The genotypes of the IL-28B rs12979860 were available in 12 patients, and all but one were C/C (rs12979860).

Conclusion: Long-term survival is attainable in CNS-PTLD and is much more likely to be seen in younger patients. Several treatment strategies demonstrated some efficacy; in some cases, the combination of rituximab and decreased immunosuppression was sufficient to control CNS-PTLD. Further studies will focus on histopathology and microarray analyses.

O23. TEMOZOLOMIDE AS SALVAGE TREATMENT IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS

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The empirical combination of high-dose methotrexate (HD-MTX) with other drugs has not produced any survival advantage over single-agent treatment against primary central nervous system (PCNSL) lymphomas. Prospective information on activity of antineoplastic agents is valuable because it may help to develop more effective up-front therapeutic combinations. We report the final results of the first phase II trial assessing activity of a single agent, temozolomide, against PCNSL. Entry criteria included failure after HD-MTX, including treatment and/or radiotherapy, histological or cytological diagnosis of NHL, presence of at least one biddimensionally measurable target lesion, negative HIV serology, and ECOG performance status (PS) of <4. Temozolomide was administered at 150 mg/m2 day for 5 days every 4 weeks until progressive disease (PD), unacceptable toxicity, or patient’s refusal. In case of disease stabilization (SD), the patient was treated for a maximum of six cycles. In patients with objective response, at least two cycles of temozolomide were administered after maximum response. Thirty-six patients (24 men; median age, 59 years; age range, 34–81) were registered between January 2000 and June 2005. PS was >2 in 26 patients; 31 patients had received previous MTX-containing chemotherapy, 6 patients had received more than one line of chemotherapy, and 31 patients had received radiotherapy (all but one in association with chemotherapy). After enrollment of 36 patients, nine complete responses (25%) and two partial responses (6%) were observed, and the study was accordingly closed as the target of 10 patients with objective response had been reached. Median complete response duration was not reached at 7 months (range, 2–70). Five patients had SD, 14 had PD, and 6 did not have response assessment due to neurological deterioration attributed to PD. Twenty-nine patients died, five were alive and free of disease at 4 to 72 months after initial failure (median, 18), and two with PD were lost at follow-up at 22 and 25 months. Actuarial 1-year overall survival was 31%. Altogether, 126 cycles (median, 2; range, 1–12) of temozolomide were delivered. Toxicity was mild, with 17 patients with grade 1 toxicity and 15 with grade 2 toxicity. Two patients had grade 4 neutropenia, and one patient had grade 3 vomiting in a single cycle. Temozolomide is an active agent against PCNSL and may be proposed, apart for salvage treatment, for testing as part of induction, consolidation, maintenance, or radioimmunotarget.
Purpose: To evaluate whether intraventricular treatment is dispensable, we omitted chemotherapy via an Ommaya reservoir in a phase II trial otherwise maintaining the systemic treatment.

Methods: Fifty patients with histologically confirmed PCNSL were enrolled onto a phase II study evaluating chemotherapy without radiotherapy. A high-dose methotrexate (MTX) (cycles 1, 2, 4, and 5) and cytarabine (ara-C) (cycles 3 and 6)–based systemic therapy (including dexamethasone, vincas alkaloids, ifosfamide, and cyclophosphamide) was administered.

Results: Thirty-five of 50 patients (18 patients less than 60 years of age, and 17 patients 60 years of age or older) were assessable for response after a median follow-up of nine months (range, 1–26 months). Of these, 66% achieved tumor remission (CR in 46% and PR in 20%). Median time to treatment failure (TTF) was eight months, both in patients over 59 years and under 60 years of age. Median overall survival is not reached for either age group. Median progression-free survival (PFS) in younger patients was only seven months according to frequent early relapses.

Conclusions: Due to an extremely short median PFS in patients under age 60 years (7 months) compared to the results of the original pilot/phase II study (PFS not reached after a median follow-up of 26 months), the current study was terminated. These preliminary results support the assumption that intraventricular treatment is essential to achieve sustained remissions after successful treatment of PCNSL.

O25. COMPUTERIZED ASSESSMENT OF QUALITY OF LIFE IN PATIENTS WITH BRAIN TUMORS

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Aims: Assessment of quality of life (QoL) in patients with brain tumors is an increasingly important issue to optimize treatment. The aim of this project was to implement a software program for computerized collection, processing, and presentation of longitudinal QoL data in patients with brain tumors.

Methods: Since July 2005, a total of 50 brain tumor patients (main diagnoses: glioblastoma, meningioma; 42.6% female; age, 47.6 ± 14.0 years) treated at the neuro-oncological outpatient unit of Innsbruck Medical University were consecutively included in the project. Inclusion criteria were a diagnosis of primary brain tumor, age between 18 and 80 years, German speaking, expected survival time of more than six months, and informed consent. QoL (EORTC QLQ-C30/BN20 brain tumor module) was computer-assisted assessed using a specially designed software tool called CHES (Computer-based Health Evaluation System). QoL was assessed 3.4 times on average per patient and 170 times in total. Age and sex–matched group of healthy subjects served as controls. With the aim of CHES, the EORTC subscales can be automatically calculated and presented as a graphic bar chart. The longitudinal graphic presentation enables physicians to detect QoL deficits (e.g., emotional problems and specific symptoms) at a single glance.

Results: The software was well accepted by both the patients and the physicians. Only two patients (4%) were not able to complete the computer-based questionnaires because of hemiplegia in one patient and ambiopha in the other. The software-generated graphic QoL profiles were found to be an important tool for screening patients for clinically relevant problems. The QoL of the neuro-oncological patients was significantly reduced compared to an age and sex–matched group of healthy controls. The highest correlation (Pearson’s r) with global QoL could be found for the EORTC subscale “Future uncertainty” (0.52, P < 0.001). Other symptoms that are moderately correlated with overall QoL are fatigue (0.41, P < 0.001), motor dysfunction (0.40, P < 0.001), leg weakness (0.35, P < 0.001), and financial impact (0.39, P < 0.001).

Conclusion: For QoL assessment, user-friendly software (CHES) was successfully implemented and tested in the clinical setting of a neuro-oncological outpatient unit. Computer-assisted QoL data can serve two purposes: to optimize treatment in individual patients and to better address scientific QoL issues systematically.

O26. IMPACT OF KARNOFSKY PERFORMANCE STATUS (KPS) ON OUTCOME OF ELDERLY PATIENTS WITH GLOBLASTOMA (GBM) AND ACTIVITY OF TEMOZOLOMIDE (TMZ) AS FIRST-LINE THERAPY: RETROSPECTIVE ANALYSIS OF A COHORT

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Background: Due to increasing incidence of GBM in the elderly, prognostic factors and therapeutic strategies need to be considered in this population. Recently, radiotherapy has shown survival improvement in patients with KPS of >70, while chemotherapy with TMZ can be considered as an option.

Methods: We analyzed retrospectively all patients over 70 years of age with GBM who were referred to our institution from May 1998 to October 2004; all responses to TMZ were reviewed.

Results: We identified 136 registered patients. Median age was 74 (range, 70–87), and 43% had a KPS of <70. Surgery consisted of stereotactic biopsy (SB) in 29%, partial surgery (PS) in 12%, and gross total removal (GTR) in 29% of patients. Diagnosis was strongly suggested by neuroradiology in 30% of cases. Treatment consisted of TMZ (5-day standard schedule) as first-line treatment in 89 patients (65%) (group A), radiotherapy: and/or nitrosourea-based regimens in 40 patients (30%) (group B), and best supportive care in 7 patients (5%). For the entire cohort, the median of overall survival (OS) was 7 months, strongly impacted by KPS (P < 0.001), KPS < 70 and > 70, respectively and age (6 vs. 8.2 months, P < 0.007 for age < 75 years vs. > 75). Median survival time (MST) was 6.6, 7.3, and 8.4 months in the case of SB, PS, and GTR, respectively, and was 5.2 for neuroradiologic diagnosis. In group A, median times to tumor progression (TTP) and OS were 7.3 and 14.0 months, respectively. KPS impacted TTP (2.9 vs. 5.1 months, P = 0.0002) and OS (4.9 vs. 8.7 months, P = 0.0001) for KPS < 70 and > 70, respectively. For 71 patients evaluable for response, we observed objective response (OR) in 28%, SD in 35%, and PR in 37% associated with an OS of 7.7, 11.5, and 14 months, respectively. OR was 34% for histologically proven GBM vs. 22% in cases of neuroradiologic diagnosis. In group B, TTP and OS were 4.3 and 6.7 months, respectively.

Conclusions: KPS appeared to have a major impact on outcomes in elderly patients with GBM. Future trials should take this impact into consideration. TMZ as first-line treatment appeared to be effective in elderly patients with newly diagnosed GBM. This alternative approach is currently being tested against RT alone in international trials. The impact of MGMT status in the TMZ population will be presented.

O27. NEUROCOGNITIVE FUNCTIONING IN LONG-TERM LOW-GRADE GLIOMA SURVIVORS: A SIX-YEAR FOLLOW-UP STUDY

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Purpose: Our previous findings among 195 low-grade glioma (LGG) patients regarding the effects of radiotherapy on midterm to long-term neurocognitive functioning suggest that the tumor itself has the most deleterious effect on neurocognitive functioning and that radiotherapy results in neurocognitive disability only when fraction doses exceeding 2 Gy are used. The present study aimed at determining neurocognitive functioning in very long-term LGG survivors with stable disease who had been diagnosed 13 years earlier and who had neurocognitive baseline assessment six years ago.

Patients and methods: The medical history of the 195 LGG patients was checked for stability of disease with the general practitioner. After obtaining informed consent, neurocognitive functioning was assessed. Subsequently, summary measures were calculated to detect possible deficits in neurocognitive domains of processing, psychomotor function, attentional functioning, verbal memory, working memory, and executive functioning.

Results: Since the baseline assessment, 30% of 195 patients had died, 23% had tumor dedifferentiation and/or received treatment, 8% could not be traced, 2% moved abroad, and 5% declined participation. The remaining 67 patients had a neurocognitive follow-up. Of these patients, 31% received RT and 89% of them received fraction doses of ≥2 Gy on average six years prior to baseline assessment. Repeated-measures analyses yielded interaction effects between RT use (yes vs. no) and time (baseline vs. follow-up) on tests measuring information-processing speed (P = 0.037), psychomotor function (P = 0.03), attentional functioning (P = 0.012), and working memory (P = 0.038). Post hoc analyses indicated that RT patients deteriorated over time, whereas RT+ patients had an unaffected performance or even improved in most neurocognitive domains.
Conclusions: The main results of this study are: (a) unirradiated LGG patients without tumor de-differentiation do not deteriorate in neurocognitive functioning, (b) irradiated LGG patients do show a neurocognitive decline over time, and (c) the risk of RT is not restricted to high fraction doses. We therefore conclude that the notion that only LGG patients with high fraction doses are at risk for neurocognitive deterioration might need revision.

028. THE BENEFITS AND DISADVANTAGES OF USING SELF-REPORTING MEASURES AMONG PATIENTS WITH MALIGNANT GLIOMA
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Introduction: It is widely acknowledged within the literature that more studies are needed to investigate the psychosocial issues experienced by patients who have been diagnosed as having malignant glioma (Gregor and Cull, 1996; Salander et al., 1996; Gupta and Sarin, 2002). There is some debate within the literature about what data collection techniques the health care professional should use to effectively measure the experience of a patient with a malignant glioma (Gregor and Cull, 1996; Weitzen et al., 1996). Using the findings of a study that employed both quantitative and qualitative data collection techniques to measure quality of life, anxiety and depression, and level of life disruption among patients with malignant glioma, this article discusses the advantages and disadvantages of using self-reporting measures within this group of patients.

Method: Using the HAD scale, the EORTC QLC-C30, telephone interviews, and unstructured interviews, this study investigated the anxiety and depression and quality of life of 51 patients between surgery and radiotherapy with malignant glioma.

Findings: The results obtained from the quantitative and qualitative data collection tools used within this study contradicted each other. Analysis of the HAD scores indicated that anxiety was prevalent only among 13% to 22% of patients throughout the study period, whereas content analysis of the interviews indicated 75% of patients were anxious. The HAD scores detected depression in 2% to 3% of patients. However, depressive symptoms were located in over half of the patient transcripts. The EORTC QLC-C30 indicated that few patients had quality-of-life issues, but, when asked, the majority of patients reported a disruption in family life, social life, work, and child care.

Discussion: This study indicates that using self-completion questionnaires among this group of patients is problematic. Patients who are compromised physically and/or mentally are unlikely to complete questionnaires. Additionally, self-completion questionnaires are not sensitive to the psychosocial issues experienced by patients with malignant glioma throughout their illness. As there is increasing emphasis being placed on measurement of psychosocial issues among brain tumor patients, the use of self-completion questionnaires has to be considered. The results from this study indicate that they are not always the most effective measurement.

029. THE IMPACT OF BRAIN TUMORS ON GLOBAL FUNCTIONAL NETWORKS IN THE BRAIN: A MAGNETOENCEPHALOGRAPHY STUDY
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Purpose: Patients with “local” brain lesions such as brain tumors often demonstrate nonspecific, “global” cognitive deficits, such as attention deficits, working-memory problems, reduced psychomotor speed, and executive dysfunctions. These cognitive disturbances cannot be explained by the localization of the lesion. It is hypothesized that local lesions, such as brain tumors, interfere with widespread functional networks in the brain.

In the present study, we explored the impact of focal brain lesions on the functional interactions (“functional connectivity”) between brain regions.

Patients and methods: We analyzed the synchronization likelihood (SL, a measure of generalized synchronization between MEG signals at rest) in 17 patients with a brain tumor and in 15 healthy controls. Following an approach that derives from graph theory, we also analyzed the architectural properties of the networks by computing two parameters: the cluster coefficient C and the characteristic path length L. These measures reflect the local connectedness and the global integration.
Tumor grade and tumor location are the two important factors for survival in patients with glioblastoma. The authors retrospectively analyzed patients older than 16 years of age diagnosed as having brain ependymal tumors, from 1990 to 2004; all clinical and histopathological data were centrally reviewed. Object: To identify prognostic factors and therapeutic strategies in a large series of intracranial ependymomas in an adult population. Methods: The authors retrospectively analyzed patients older than 16 years of age diagnosed as having brain ependymal tumors, from 1990 to 2004; all clinical and histopathological data were centrally reviewed.

Results: Among the 121 patients selected, 80 tumors were infratentorial (41 supratentorial (35.6% grade III) and 41 supratentorial (53.7% grade III); surgery consisted of gross-total resection (GTR) (n = 76), subtotal resection (n = 22), and partial resection or biopsy (n = 23). The median follow-up was 79 months (range, 13–168). The 5- and 10-year overall survival (OS) rates were 85% and 76%, respectively; the 5- and 10-year progression-free survival (PFS) rates were 64% and 49%, respectively. Infratentorial location and grade II were associated with better prognosis for OS and PFS; and OS was significantly higher in the case of GTR. In multivariate analysis, grade II, extent of resection, and radiotherapy were significantly predictive of improved PFS. Among patients with grade II (n = 76), 29 (38%) underwent subtotal, partial resection or biopsy. In this group, 10 received no post-surgical treatment and exhibited a 5-year PFS rate of 0%, while 19 (23%) received radiotherapy and reached 5- and 10-year PFS rates of 84% and 63%, respectively (P = 0.025). Lomustine has no influence PFS in grade III with GTR (61 vs. 33% at 5 years) (P = 0.08).

Conclusions: Tumor grade and tumor location are the two important prognostic factors with respect to patient survival and tumor recurrence. GTR should be the intent of surgery. The authors currently recommend the use of postoperative radiotherapy for grade II when GTR can’t be realized.

O32. SURVIVING GliOBLASTOMA FOR MORE THAN FIVE YEARS: THE PATIENTS’ PERSPECTIVE

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Only 2% to 5% of patients survive five years from the diagnosis of glioblastoma. In this study, we investigated the functional outcome of glioblastoma survivors from a single institution. Ten patients with a median survival of 94 months (range, 60–120 months) after the diagnosis of glioblastoma, confirmed by neuropathological reassessment, were identified. A comprehensive analysis was performed of clinical variables, imaging data, neuropsychological function, and quality of life.

At reassessment, neurological deficits were mild to moderate in most patients, but neuropsychological testing demonstrated cognitive defects in all patients. Depression and anxiety were common. Many dimensions of quality of life were affected, in particular anxiety and social functioning and work. In contrast, little reduction in mean global health status and overall quality of life was perceived. One patient is working full time, and two patients gave birth to healthy children 52 and 67 months after the diagnosis.

In conclusion, the majority of the overall few glioblastoma patients with long-term survival suffer significant impairment. Therefore, in addition to the development of more effective therapies, novel strategies aiming at the maintenance or recovery of neurological and cognitive function and the diminution of neurotoxic side effects of treatment are urgently needed.

O33. LEVETIRACETAM (LEV) USE IN GLIOBLASTOMA MULTIFORME (GBM) PATIENTS: A SINGLE INSTITUTION RETROSPECTIVE REVIEW OF 102 PATIENTS WITH SEIZURES

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Introduction: Since 1993, nine new antiepileptic drugs (AEDs) have been released for use in the United States. Many of these second-generation AEDs have limited hepatic metabolism, thereby decreasing potential drug interactions. Levetiracetam (LEV) has no protein-binding or cytochrome-P450 metabolism and is excreted in the urine. These properties make it an ideal drug to use in brain tumor patients with seizures who are often on multiple heptatically metabolized drugs, including experimental chemotherapies.

Methods: A retrospective analysis was completed using our brain tumor database that identified all GBM patients treated over the previous five years with LEV. All patients had to have had documented seizure activity. No patients were treated with prophylactic medication. Patients were evaluated for seizure type and frequency, as well as medication side effect. Seizure frequency was calculated as a percentage change from baseline averaged over three months.

Results: A total of 102 glioblastoma multiforme (GBM) patients were identified. The mean age was 57.8 years (range, 23–87) with 59 men and 43 women. LEV was used as a first-line medication in 14 patients. The most common medication conversion was phenytoin to LEV. LEV was used as an add-on medication in 22 patients, while 80 patients were maintained on LEV monotherapy. The most common seizure type was simple partial seizures (n = 76). The average initial dose of LEV was 1500 mg, and 20 patients had the dose increased. Forty-seven patients were treated on a clinical trial. Ten percent of patients reported a side effect causing a dose reduction or stoppage of medication. The average seizure frequency decreased by at least 50% in 68 patients with active seizures. Twenty-seven patients were seizure-free on their previous AED and converted to LEV secondary to side effects or entry into a clinical trial. Only one of those 27 patients experienced a worsening of seizures on LEV.

Discussion: LEV appears to be well tolerated in GBM patients with fewer than 10% of patients stopping or reducing medication. Seventy-eight percent of patients were maintained on monotherapy, and seizure control was excellent in patients with active seizures, with 68% of patients experiencing at least a 50% reduction. LEV appears to be a good AED choice in GBM patients.

O34. INCIDENCE OF EPILEPSY AS A PRESENTING COMPLAINT IN INTRACRANIAL MENINGIOMA


Objective: To evaluate the incidence of epilepsy as a presenting complaint in intracranial meningiomas.

Materials and methods: More than 1000 patients were recruited retrospectively. Patients were collected from the National Hospital for Neurology and Neurosurgery in London from 1963 to 2000 by using pathology reports and clinical notes. Patients included were those that had undergone surgery for intracranial meningiomas. We collected demographic data and information on location of meningioma, age, gender, and presenting clinical symptoms. We also evaluated the number of patients who became epileptic free after six months of the surgery. Data was analyzed using STATAview® using univariate and multivariate analyses. The study had ethics committee approval.

Results: More than 100 patients: 65.24:34.76 female–male ratio. Age ranged from 13 to 96 years. Of the meningiomas, 18.26% were located in the convexity, 18.5% in parasagittal area and 5.94% in the posterior fossa, 9.13% suprasellar, and 1.21% intraventricular. 1.98% were cavernous sinus meningiomas, 0.66% were pineal meningiomas, and 4.07% appeared in the cerebellopontine angle, 4.51% in the optic nerve and orbital region, and 11.88% in the sphenoid ridge; 14.85% were subfrontal meningiomas, 1.32% were clival meningiomas, and 2.97% were located in the petrous apex; and, finally, 5.07% were unclassified. Of the patients, 18.92% had suffered an epileptic fit as first presenting complaint. We present the incidence of epilepsy in each tumor location, as well as the different types of epilepsy described, which included tonic-clonic seizure, focal seizures, absence seizures, Jacksonian episodes, and sensorial epileptic events. Almost 100% of epileptic presentations had EEG studies registered.

Conclusion: Epilepsy is a known presenting symptom in patients who suffer from intracranial meningiomas. Our series (one of the largest meningioma studies in the literature) analyzes the incidence and multiple features of epilepsy in patients suffering from this benign pathology.

O35. A MULTICENTER STUDY OF PROGNOSIS AND TREATMENT OF ADULT BRAIN Ependymomas

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Background: Ependymomas account for 2% of all intracranial tumors in adults and generate considerable controversy with regard to their prognostic factors and therapeutic management.

Object: To identify prognostic factors and evaluate therapeutic strategies in a large series of intracranial ependymomas in an adult population.

Methods: The authors retrospectively analyzed patients older than 16 years of age diagnosed as having brain ependymal tumors, from 19 institutions, between 1990 and 2004; all clinical and histopathological data were centrally reviewed.

Results: Among the 121 patients selected, 80 tumors were infratentorial (13.8% grade III) and 41 supratentorial (53.7% grade III); surgery consisted of gross-total resection (GTR) (n = 76), subtotal resection (n = 22), and partial resection or biopsy (n = 23). The median follow-up was 70 months (range, 13–168). The 5- and 10-year overall survival (OS) rates were 85% and 76%, respectively; the 5- and 10-year progression-free survival (PFS) rates were 64% and 49%, respectively. Infratentorial location and grade II were associated with better prognosis for OS and PFS; and OS was significantly higher in the case of GTR. In multivariate analysis, grade II, extent of resection, and radiotherapy were significantly predictive of improved PFS. Among patients with grade II (n = 76), 29 (38%) underwent subtotal, partial resection or biopsy. In this group, 10 received no post-surgical treatment and exhibited a 5-year PFS rate of 0%, while 19 (23%) received radiotherapy and reached 5- and 10-year PFS rates of 86% and 63%, respectively (P = 0.012). Lomustine has no influence PFS in grade III with GTR (61 vs. 33% at 5 years) (P = 0.08).

Conclusions: Tumor grade and tumor location are the two important prognostic factors with respect to patient survival and tumor recurrence. GTR should be the intent of surgery. The authors currently recommend the use of postoperative radiotherapy for grade II when GTR can’t be realized.
O36. A CASE OF SOLITARY SCIATIC NERVE LYMPHOMA
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Lymphoma occasionally affects the peripheral nervous system, and diagnosis can be elusive since many patients present without known lymphoma. Most peripheral nerve complications are plexopathy, generalized neuropathy, or mononeuropathy.

Here we present a case of a 44-year-old woman who came to our attention in February 2005 for a six-month history of stumbling, paresthesias, and pain associated with a progressive weakness in the right lower limb. Neurological examination revealed absence of Achilles’ tendon and plantar flexor stretch reflexes on the right side, and decreased strength in all distal muscles of the right leg. A reduced soft touch and pinprick sensation was present in the thigh and leg posterior surface and in plantar and lateral foot face. Nerve conduction studies revealed absence of right tibial motor response. Right peroneal CMAP and MCV as right tibial SAP and SCV were below the lower limits of normality. EMG revealed profuse denervation in the muscles innervated by the sciatic nerve, but also in the gluteus maximus. No voluntary activity was seen. The results of lumbaroscal MRI, abdomen-pelvis TC, pelvis radiography, and CSF examination were negative, as was all laboratory workup for connective tissue, infectious, and paraneoplastic disease. The pelvis MRI showed a T2, DUAL, and STIR hyperintense signal of proximal part of right sciatic nerve that appeared edematous and showed Gd enhancement. High-dose parenteral steroid therapy was started for five days and then followed with methylprednisolone 1 g/week for the following three months with a partial resolution of pain and paresthesias and apparent strength amelioration.

Five months later, nerve conduction studies showed a worsening condition (right sural SAP and peroneal CMAP absent). Lumbosacral MRI was unchanged. In November 2005, because of progressive worsening and lack of steroid therapy response, the patient underwent a lumbar-pelvis CT and MRI that revealed a tumoral lesion with Gd enhancement extending from L4, L5, and S1 right roots to the leg along the sciatic nerve. Histologic diagnosis on a biopsy specimen of the nerve revealed a diffuse, large, B-cell lymphoma. Total body PET did not show evidence of other disease localization. Since December 2005, the patient underwent three chemotherapy cycles (CHOP schedule). A control MRI in March 2006 showed a complete disappearance of the pathological tissue. This is a case of solitary sciatic nerve lymphoma: at the moment, is it possible to exclude the beginning of neurolymphomatosis?

O37. RESULTS OF A PHASE IIIB ACTIVELY CONTROLLED CLINICAL TRIAL IN PATIENTS WITH RECURRENT MALIGNANT GLIOMA
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In three preceding phase I/II dose-escalation studies, patients with recurrent malignant glioma were treated intratumorally with the TGF-β3-specific compound AP 12009. AP 12009 proved to be well tolerated and revealed a good safety profile. Moreover, objective tumor activity including long-lasting and complete tumor remission was observed.

In the current phase IIb study G004, an international open-label, actively controlled, dose-finding trial, adult patients with histopathologically confirmed recurrent malignant glioma in 1 of 5 centers were treated to study the efficacy and safety of two doses of AP 12009 (10 and 80 μM) compared to standard chemotherapy TMZ (or PCV, if a patient had previously failed TMZ treatment). The primary end point is tumor response assessed by central MRI analysis. The secondary end point is overall survival. Survival status as well as poststudy MRI results will be continued to be collected at least twice per year.

Recruitment was completed with 145 patients being enrolled. AP 12009 was administered intratumorally by convection-enhanced delivery in patients with anaplastic astrocytoma (AA, WHO grade III) and glioblastoma multiforme (WHO grade IV) during a 7-day-on, 7-day-off treatment cycles. A total of 134 patients (35% women and 65% men; median Karnofsky performance status, 90, and range, 70–100), including 96 GBM patients (median age, 50 years; range, 20–74) and 38 AA patients (median age, 40 years; range, 22–60), were treated. A total of 89 patients received AP 12009 (10 patients, 10 μM; and 49 patients, 80 μM), and 44 patients received standard chemotherapy. Adverse events were evaluated by an independent data and safety monitoring board. Preliminary assessments show only 7 SAEs possibly related to the study drug in 89 AP 12009 patients (both AA and GBM) despite the extremely aggressive form of this neoplastic disease. Of 37 procedure-related SAEs, 89% were assessed as mild or moderate. Exact response rates will be determined after central MRI analysis is completed. Updated results from the phase IIb study in malignant glioma will be presented.

As in the previous studies, long-lasting responses were observed resulting also in an improved quality of life. Phase III clinical trials in AA and GBM patients are currently in preparation.

O38. IMPROVING LOCAL CONTROL OF MALIGNANT GLIOMAS: TARGETED RADIOPEPTIDE BRACHYTHERAPY USING DOTAGA-SUBSTANCE P
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Aim: NK-1 receptors (NK-1Rs) are consistently overexpressed in malignant gliomas. For assessment of biodistribution, toxicity, and response of DOTAGA-substance P (DOTAGA-sP), the local injections of the vector into the tumor or the resection cavity were performed. For labeling, the high-energy and low-energy beta emitters yttrium 90 and lutetium 177 and the alpha emitter bismuth 213 were used in this phase I study.

Methods: Twenty patients with glioblastoma multiforme (WHO grade IV, n = 14) and WHO grade II–III gliomas (n = 6) were enrolled. Autoradiography proved NK-1Rs to be a universal target for all gliomas. The radiopharmaceutical, exhibiting a molecular weight of 1,8 KD and physicochemical properties comparable to native substance P, was injected into an intrasellar catheter system following stereotactic insertion. Pretherapeutic assessment of dose distribution of the radiopharmaceutical was performed using 111In–DOTAGA-substance P.

Results: Of these 20 tumors, 16 were targeted using yttrium 90–labeled substance P (mean tissue range, 5 mm). In four tumors at functionally critical brain areas, the low-energy beta emitter lutetium 177 and the alpha emitter bismuth 213 (range, 80 μm) were used. Initially impaired neurological functions in 12 of 20 cases gradually improved or stabilized in 10 of 12 patients. Symptomatic transitory radiogenic edema was the major toxicity observed.

Conclusion: Targeted diffusible radiotope brachytherapy using DOTAGA-substance P has the potential to become a versatile new tool either as alpha therapy and/or beta therapy for the local control of low-grade and high-grade gliomas.

O39. HIGH-GRADE GLIOMA RADIOTHERAPY: A DOSIMETRIC COMPARATIVE ANALYSIS BETWEEN 3D CRT AND IMRT TECHNIQUES
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To evaluate the improvement of intensity-modulated radiotherapy (IMRT) versus three-dimensional conformal radiotherapy (3D CRT), 20 patients with high-grade gliomas, radically or partially resected, were selected as representative of the overall patient population in terms of tumor location, size, grade, volume, and prescription dose.

Ten patients received 3D CRT with two or three no coplanar 6-MV fields technique planned with Eclipse TPS. The second group was treated with an IMRT technique using four or more no coplanar 6-MV fields planned with BrainScan TPS.

All patients were immobilized with a thermoplastic individual face mask. To define volumes of interest, magnetic resonance images were matched with CT images by using an automated mutual information-based registration technique or pixel-level data-fusion algorithm, respectively, for BrainScan and Eclipse. CTV was defined as a 2–3 cm margin of tissue surrounding the perimeter of the CTV- and MRI-defined contrast-enhancing lesion.

PTV was determined by adding an additional 0.5 cm margin to CTV. OARs were brain stem, lens, optical nerves, eyes, and “normal brain” outside PTV. The prescription was 60 Gy in 30 fractions for both. According to RTOG protocols, for 3D CRT we
used a shrinkage field; initially the treatment volume included the contrast-enhancing zone on CT-MRI study with a 2- to 3-cm margin. Subsequently, after 46 Gy, the target volume was reduced to include the enhancing lesion only with a 1-cm margin. For IMRT plans, we used a concomitant non-integrated boost technique to give the prescribed doses. Treatments were delivered with a Linac Varian 2100 with Millennium 120MLC. With 3D CRT, any effort to decrease the treatment toxicity might result in compromised target coverage, thus increasing the risk of local recurrence. To obtain a dose homogeneity in the target, we set the opportune dose volume constraint on the plan. The IMRT treatment plans and the conventional 3D-CRT plans were compared through DVH and TCP/NTCP analysis. The IMRT approach has been demonstrated to improve tumor coverage while sparing the dose to critical structures when compared with 3D CRT, without increasing normal tissue toxicity. Therefore, the increased conformity of IMRT plans observed in this dosimetric comparison should permit escalation of tumor doses to improve local tumor control, because the predominant failure pattern in high-grade gliomas remains local.

**040. MGMT PROMOTER METHYLATION STATUS IN Glioblastomas: An implication for therapeutic decisions?**

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**Introduction:** In glioblastomas, MGMT promoter methylation has been shown to correlate with longer overall survival and significant survival benefit after treatment with temozolomide (TMZ) and radiotherapy (RT). Our study analyzed the MGMT promoter methylation status in glioblastoma primary cell cultures and its clinical relevance in the treatment of glioblastoma.

**Materials and methods:** MGMT promoter methylation was analyzed in 50 glioblastoma-derived cell cultures. DNA was modified with bisulfite, and methylation-specific PCR (MSP) was performed with primers specific for either methylated or modified unmethylated DNA. From two patients, data about postoperative course of disease were not available. Of 50 patients, 6 did not receive further therapy because of rapid worsening of performance status; 16 of 42 (38%) received RT and TMZ concomitantly (n = 16), as well as adjuvant (13 of 16); 12 of 42 patients were treated in an adjuvant setting with RT and CCNU and 13 of 42 patients received only RT, whereas in two cases it had to be aborted after 2000 cGy. One patient was treated with CCNU only. TMZ was administered at a dosage of 75 mg/m²/day during radiotherapy and/or 200 mg/m²/day over 5 days every 28 days. Treatment cycles were repeated up to six times. CCNU was applied at a dosage of 100 to 130 mg/m²/66 weeks (1–6 cycles).

**Results:** MGMT promoter methylation was observed in 32 (64%) of the 50 cell cultures analyzed, whereas 17 of 32 were exclusively methylated, and 15 of 32 displayed methylated as well as unmethylated DNA; 18 (36%) of 50 were unmethylated. The MGMT promoter was methylated in tumor cells of 81% of patients (13 of 16) who received concomitant RT-TMZ. Of 13 RT-TMZ patients, treatment is still alive, with longest post-operative survival being 33.5 months. Irrespective of treatment, Kaplan–Meier survival analysis revealed a significant difference in survival (P < 0.04) between patients with a methylated MGMT promoter and those with an unmethylated MGMT promoter.

**Summary and conclusion:** Our data demonstrate that the analysis of MGMT promoter methylation represents a tool that allows selecting a subgroup of glioblastoma patients who, to a great extent, profit from radiochemotherapy with TMZ. Furthermore, methylation of the MGMT promoter has prognostic value. Thus, the MGMT promoter methylation status should be considered in the therapeutic decision for glioblastoma patients.

**041. OPTIMAL DOSSING OF TEMOZOLOMIDE FOR EFFICACY AND TOLERABILITY IN PATIENTS WITH RECURRENT OR PROGRESSIVE Glioblastoma: A ONE-WEEK-ON/ONE-WEEK-OFF REGIMEN?**

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Clinically, sensitization to temozolomide by O6-methylguanine DNA methyltransferase (MGMT) depletion could potentially be achieved with alternating dosing schedules that deliver more prolonged exposure and higher cumulative doses than the standard five-day regimen. Recently, protracted low-dose temozolomide at 75 mg/m² was surprisingly toxic, resulting in cumulative lymphopenia and opportunistic infections. In an extension of the experience with a one-week-on/one-week-off schedule in 90 patients with primary brain tumors, we determined toxicity and efficacy of this regimen. Temozolomide was administered at 150 mg/m² on days 1 to 7 and days 15 to 21 of 28-day treatment cycles and dose adjusted according to individual myelotoxicity. In 906 treatment weeks, thrombopenia was encountered at grade 3 according to the common toxicity criteria (CTCAE) in 1.1% and at grade 4 in 0.7% of patients. Leukopenia was seen in 1.1% at grade 3 and 0.1% at grade 4. In contrast, lymphopenia was more common, with 8.5% grade 3 and 1.9% grade 4 toxicity. Looking at the individual patients’ worst toxicity, 29 patients started treatment at grade 1 or 2 and two patients at grade 3 or 4 lymphopenia. Further, 17% of all patients at least once had a grade 3 and 12% a grade 4 lymphopenia. However, there were no opportunistic infections or toxic deaths. Lymphopenia was not cumulative. There was no correlation between prior treatments and the occurrence of toxicity. Grade 3 or 4 toxicity was not predictive for progression-free survival for six months or longer. Of 64 patients treated on the progressive glioblastoma, one achieved a complete response and six a partial response. The median progression-free survival was 24 weeks. The progression-free survival at six months was 43.8%. The overall survival (OS) after the onset of temozolomide was 23% at 12 months in a cohort of patients with an OS12 from diagnosis of 77% and OS24 of 25%. These data imply that the one-week-on/one-week-off schedule is feasible and effective and clearly warrants investigation in randomized studies.

**042. A CONTROLLED, RANDOMIZED, PARALLEL GROUP, MULTICENTER STUDY OF THE EFFICACY AND SAFETY OF HERPES SIMPLEX VIRUS – THYMIDINE KINASE GENE THERAPY (CEREPRO™), WITH SUBSEQUENT GancIClovIR, FOR THE TREATMENT OF PATIENTS WITH OPERABLE HIGH-GRADE GLIOMA**

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There are 29,000 cases of high-grade glioma per year in Europe, of which 18,000 are operable. Patients with operable high-grade glioma are susceptible to early death due to tumor recurrence. In a phase II clinical study, Cerepro™, an adenoviral vector containing the Herpes simplex virus–thymidine kinase gene in conjunction with ganciclovir (GCV), has been shown to almost double survival time, providing on average an extra seven months of life with an acceptable safety profile that meets this high clinical need.

Cerepro™ is administered by multiple injections into the normal brain tissue in the wound bed following resection of the tumor. Infected cells subsequently express the Herpes simplex–thymidine kinase transgene. This enzyme phosphorylates GCV, resulting in the production of a cytotoxic nucleotide that the pro-drug incorporates into DNA of proliferating cells inducing cell death. Normal neurons surrounding the tumor are nonproliferative and are therefore not affected by Cerepro™. The therapeutic effect of Cerepro™/GCV is further enhanced by the “bystander effect,” in which the cytotoxic nucleotide analogue spreads to the neighboring noninfected cells and induces apoptosis.

This communication describes the design of a confirmatory phase III, multicenter, controlled, randomized parallel group study to determine if Cerepro™/GCV is superior to standard care for the treatment of operable primary glioblastoma. A maximum of 250 patients will be randomized in a 1:1 ratio to either the active group, receiving Cerepro™ followed by Cerepro™/GCV is superior to standard care for the treatment of operable primary glioblastoma. A maximum of 250 patients will be randomized in a 1:1 ratio to either the active group, receiving Cerepro™ followed by Cerepro™/GCV is superior to standard care for the treatment of operable primary glioblastoma. A maximum of 250 patients will be randomized in a 1:1 ratio to either the active group, receiving Cerepro™ followed by
O43. MONITORING THE EFFECTS OF INTRATUMORAL PACLI TAXEL DELIVERY BY DWI AND FET PET

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Objectives: Convection-enhanced delivery (CED) of various drugs and toxins represents a new locoregional therapeutic approach in patients with malignant glioma. This study evaluated the therapeutic effects of paclitaxel (Taxol®) delivered by CED with O-2-[F-18]fluoroethyl]-L-tyrosine (FET) PET and MRI including diffusion weighted imaging (DWI).

Methods: Nine patients (six men and three women; mean age, 53 ± 10 years) with recurrent glioblastoma were treated with CED of paclitaxel. Paclitaxel was infused over stereotactically placed catheters into the recurrent solid tumor for five days (0.3 ml/h). FET PET (Siemens, ECAT EXACT HR+, cold transmission, 185 MBq FEI) and MRI scans (Siemens, Magnetom Vision, 1.5 T) were acquired before as well as four weeks after paclitaxel CED, followed by three-month intervals. For quantitative evaluation of PET, the maximal standardized uptake value (SUVmax) in the area of the recurrent tumor was determined and the ratio to the background (BG) was calculated. PET data were compared to contrast uptake in T1 weighted MRI (T1w) and to the distribution volume (Vd) of convective calculated with DWI data.

Results: At baseline, all patients showed pathological contrast enhancement on MRI as well as high FET uptake of recurrent glioblastoma with a mean ratio, SUVmax/BG of 3.32 ± 0.77. However, there was a mismatch of contrast enhancement and FET uptake in six of nine patients with larger pathological areas in PET than in T1w. After therapy, necrosis (hypointensity in T1w) induced by paclitaxel corresponded well with Vd documented by DWI over the area of decreased FET uptake. In three of nine patients with early progression, FET uptake only slightly decreased immediately after therapy (decrease of SUVmax/BG < 12%), whereas six of nine patients with a clinically long-term stable course experienced a stable decrease of ~26%. Recurrences were detected by FET PET in all cases. In contrast, T1w showed unchanged or even increasing areas of contrast enhancement after therapy in all patients without the ability to assess disease progression reliably.

Conclusions: The distribution volume of CED can be determined by DWI. However, changes of contrast enhancement in T1w are unspecific after CED. FET PET imaging sufficiently enables pathological changes to be differentiated from those induced by tumor regression. Therefore, only the combination of both methods is sufficient for monitoring the effects of local therapies.

Disclosure: This study represents the first documented presence of pluripotent, highly proliferative CD133+ cancer stem cells in low-grade gliomas. Cellular costaining with Musashi-I indicates a neural origin of CD133+ cells in all gliomas. These cells can differentiate into cells expressing glial, neuronal, or endothelial markers. The role of these cells during stepwise glioma progression still has to be evaluated.

O44. CD133+/MUSASHI-I+/CD34+ NEURAL STEM CELLS IN GLIOMAS OF DIFFERENT GRADES

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Introduction: Within glioblastoma multiforme (GBM) specimens, CD133+ positive cells with ability for self-renewal and tumor generation could be isolated, suggesting the presence of cancer stem cells in GBM. Aim of this study was (1) to assess the presence of CD133+ cells in grade II, III, and IV gliomas, (2) to shed some light on the origin, and (3) to investigate the differentiation potential of these cells.

Methods: Samples of GBM, glioma WHO III and II (each n = 10) were investigated immunohistochemically using these antibodies: AC133/1x2, which binds to two different epitopes of CD133; Musashi-1, a neural stem cell marker; and CD34, a progenitor marker in hematopoiesis. Additionally, CD133, Musashi-I, and CD34 protein expression was quantified by ELISA. CD133+ cells were isolated from freshly resected grade II, III, and IV gliomas (each n = 6) by using a modified magnetic bead protocol. These cells were assayed for neural stem cell and glioma characteristics. Differentiation of cultured cells was assessed immunohistochemically by using glial, neuronal, and endothelial differentiation markers.

Results: CD133+ positive cells could be detected in 8 of 10 WHO II gliomas, 5 of 10 WHO III gliomas, and 6 of 10 WHO IV gliomas. These cells arranged in clusters, mostly associated with intratumoral vessels, rarely located diffusely within the tumor parenchyma. CD133 expression correlated with WHO grade being low (~5% of total cell count) in glioma grade II and high (10%–20% of total cell count) in glioma grade IV. ELISA data confirmed the correlation with tumor grade. CD133+ cells from specimens of all tumor grades stained positive for Musashi-I but lack CD34 expression, suggesting a neural origin of these cells. Under different culture conditions, CD133+ cells proliferated rapidly. After several passages, cells lost CD133 expression and became positive for GFAP, NSE, or CD31/CD105/VE cadherin.

Conclusions: The management of WHO grade II gliomas is still under discussion, essentially because of limitations regarding our knowledge of their natural (dynamic) history and our evaluation methods. The collaboration of French centers has allowed for study of a collection of 1000 eligible adult cases for which tumor volume was available and growth rate in 30% (mean/median age at clinical onset/discovery is 37 years, and 38 years at treatment (84% of the cases). Median tumoral volume was 62.5 cc at treatment, and 76% of the lesions showed no contrast enhancement. The mean follow-up is seven years after one/stereodiagnosis and six years after eventual treatment. Multivariate analysis of clinical (gender, age, symptomatology, and WHO PS), radiological (location, volume, and contrast enhancement), pathobiological (histology, Ki-67 index, and IP/19q status in 30% of the cases), and prognostic factors enabled us to isolate that which was of major importance in the tumor volume. In a subgroup with a mean tumoral diameter of 6 cm or larger, only age and location retain statistical significance with a significant differential influence of treatments. For tumors smaller than 6 cm, gender, age, and tumor volume and growth rate must be taken in account, as well as other markers awaiting validation (IP/19q status, spectro-MR, and perfusion MR). In this subgroup, treatment can delay, if
not avert, anaplastic transformation and hence prolong survival: surgery, if radiologically complete or leaving a residue of <10 cm3, and chemotherapy. These results led us to a common therapeutic attitude. After discovery of a lesion looking like a grade II glioma, a stereotactic radiosurgery. Follow-up images were available after a short phase of follow-up allowing an oncological (volume, growth rate) and functional [neuropsychological, fMRI] evaluation. Surgery is considered first when a (subtotal) resection seems feasible. Otherwise, chemotherapy is discussed (before radiotherapy), with less urgency, that can moreover in some cases optimize a resection without altering the brain plasticity potential. Last, but not least, a sequential therapeutic strategy during the “grade II phase” of the glioma appears equally important to delay the patient’s fate.

O47. EXPRESSION OF ENDOTHELIN-1 (ET-1) AND CYCLOOXYGENASE-2 (COX-2) IN INTRACRANIAL Meningiomas

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Much of the morbidity of intracranial meningiomas is related to the degree of tumor vascularity and the extent of peritumoral vasogenic edema that occurs in approximately 60% of the cases, adversely affecting the clinical course. COX-2 is an enzyme, universally expressed in meningiomas, that catalyzes the synthesis of the eicosanoid prostaglandin metabolites. ET-1 has been shown to constitute a potent growth-regulatory peptide in various tissues; it may have a role in neovascularization, tumor blood flow, and/or function of the blood-tumor barrier in meningioma tissues by interacting with specific receptors present on the surface of the endothelium.

Our aim was to evaluate the COX-2 and ET-1 expression and their potential relationship in a series of intracranial meningiomas, relating this molecule to histologic grade and development of vasogenic brain edema associated with meningiomas. A total of 62 tumors were evaluated with RT-PCR analyses and immunohistochemistry for COX-2 and ET-1 expression. COX-2 expression was detected by immunohistochemistry in 51 of 62 meningiomas (82.5%), ranging from 0 to 80% (mean, 33.5%; median, 30%); COX-2 mRNA expression agreed with the immunohistochemical data (χ² test, P < 0.02).

By IHC, ET-1 protein expression was observed in 35 of 62 meningiomas (56.4%), ranging from 0 to 90% (mean, 20%; median, 10%). The molecular results agreed with the immunohistochemical results (χ² test, P < 0.0094). Both molecules were significantly related to histologic grade (P = 0.0048 and P = 0.0020, respectively). Moreover, COX-2 expression and ET-1 expression were strictly related each other (χ² test, P = 0.0318).

The value of the edema index was calculated in 34 of 62 cases, and was >1 in 16 cases (45.7%). PTE was significantly associated with histologic grade (P < 0.05), and the edema index was significantly associated with histologic grade (P = 0.0318). A significant statistical value was found between COX-2 expression and histologic grade (χ² test, P = 0.0093). Thus, the more aggressive meningiomas more expressed COX-2 and ET-1, which could be an important determinant of malignant phenotype. Moreover, they were strictly related each other and seemed to contribute to the development of meningioma-associated vasogenic edema.

In conclusion, our study focused attention on a possible role of ET-1 and COX-2 expression as a predicting factor and a novel target for adjuvant therapies in edema treatment in meningiomas.

O48. FIRST CLINICAL TRIAL OF BORON NEUTRON CAPTURE THERAPY (BNCT) FOR MALIGNANT Meningiomas

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We administered boron neutron capture therapy (BNCT) using sodium borocapate (BSH) and boronophenylalanine (BPA) not only for malignant gliomas but also for malignant meningiomas. These compounds have different accumulation mechanisms. BSH does not enter into tissue with a normal blood-brain barrier and enters into tumor tissue selectively while accumulation occurs passively. BPA can accumulate tumor tissue more actively while it accumulates into normal brain somewhat. Malignant meningioma is a more difficult pathohistological entity to control and cure, as well as glioblastoma. Since 2005, we administered BNC in seven cases of malignant meningioma related to meningiomas with 13 times neutron irradiation. Three cases were anaplastic meningiomas (WHO grade III), two were papillary meningiomas (WHO grade II), and one was a sacroma transformed from a meningioma with cervical lymph node metastasis. All cases were introduced to us after the patients had undergone repetitive surgeries and external fractionated x-ray radiotherapy (XRT) treatment. Follow-up images were available after six cases with an observation period of 2 to 9 months. We administered F-BPA-PET before BNCT in six of seven cases. One case was administered methionine-PET instead of it. In some cases, BPA-PET was administered even after BNCT. Five of six patients who received BPA-PET showed good BPA uptake, with a tumor–normal brain (T/N) ratio of >3. One atypical meningioma case showed a T/N ratio of 2.2. Original tumor sizes were 9.2 to 92.7 cm3. Two of five malignant meningiomas showed complete response after BNCT, and all six cases showed radiographic improvement. Clinical symptoms before BNCT, such as hemiparesis and facial pain, improved after BNCT in malignant meningiomas. However, in an atypical meningioma case that arose from tentonium and extended bilateral occipital lobes and brain stem, visual problems worsened after repetitive BNCT administration, with an increase in peritumoral edema, and only 20% mass reduction was observed. In BPA-PET, after treatment, there was a marked decrease in BPA uptake, which showed the biological effects of this treatment. Three cases showed increased edema 2 to 3 months after BNCT, which seemed to be radiation damage, which seems to be correlated with target volume and previous radiation dose. Malignant meningiomas seem to be good candidates for BNCT. This is the first report of BNCT administered for treatment of malignant meningiomas.

O49. PODOPLANIN EXPRESSION IN PRIMARY CENTRAL NERVOUS SYSTEM GERM CELL TUMORS: A USEFUL HISTOLOGICAL MARKER FOR THE DIAGNOSIS OF GERMINOMA

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Podoplanin, a mucinlike transmembrane sialoglycoprotein, promotes platelet aggregation and may be involved in cancer cell migration, invasion, metastasis, and malignant progression. Previously we reported that podoplanin is expressed in testicular seminoma but not in embryonal carcinoma, suggesting that it may be a sensitive marker for seminomas. Here we investigated the expression of podoplanin in central nervous system (CNS) germ cell tumors (GCTs) by immunohistochemical staining of tumor samples from 62 patients. In 40 of 41 (98%) germinomas (including germinomatous components in mixed GCTs), podoplanin was diffusely expressed on the surface of germinoma cells; lymphocytes, interstitial cells, and syncytiotrophoblastic giant cells were negative for podoplanin. Except for immature teratomas (12 of 17 [71%]), podoplanin expression was absent, including seven teratomas, seven embryonal carcinoma, seven yolk sac tumors, and seven choriocarcinomas. In immature teratomas, focal podoplanin staining was observed in fewer than 10% of immature squamous and columnar epithelial cells. Thus, podoplanin expression may be a sensitive immunohistochemical marker for germinoma in CNS GCTs. As such, it may be useful for diagnosis, for monitoring the efficacy of treatment, and as a potential target for antibody-based therapy.

O50. RESPONSE IN PEDIATRIC HIGH-GRADE GLIOMA: WHAT DOES IT MEAN?

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Introduction: The relevance of response to judge experimental treatment protocols is debatable.

Materials and methods: We used the HIT-GBM registry, which required either histologically confirmed high-grade glioma (HGG) or radiologically confirmed diffuse invasive pontine glioma (DIPG).

Results: A total of 310 patients (173 male; median age, 9.9 years) were analyzed. Tumor locations were cerebral cortex, 83; basal ganglia, 37; pons, 132; brain stem outside of pons, 15; cerebellum, 13; spine, 8; and orbit, 22. The resection was complete, 49; subtotal, 35; partial, 58; or biopsy, 33; or no surgery, 69. WHO grading was IV, 123; III, 101; no histology, 71; and grade I/II, 11. Response after 8 weeks of treatment was documented in 219 patients with incompletely resected tumors: CR, 8 (3.4%); PR, 32 (14.6%); SD, 116 (53.0%); and PD, 63 (28.8%). The median overall survival time (mOS) was 1.02 years (+SD 0.05), and the...
median event-free survival time was 0.54 (±0.03). Location, grading, and surgery determined OS. DIPG had the worst outcome (mOS 0.86 years [±0.08], and completely resected neocortical grade III tumors the best outcome (81.5% 5-year OS). Chemotherapy (P = 0.001) and radiation therapy (P = 0.0001) appeared of prognostic relevance on univariate analysis. The influence of response on OS was limited: Only the eight CR patients had OS comparable to those with complete resection; four of those responders had subsequent RT failures.

Conclusion: The relevance of response is very limited in pediatric HGG.

O51. INTENSITY-MODULATED RADIOTHERAPY (IMRT) AND FRACTIONATED STEREOTACTIC RADIOTHERAPY (FSRT) FOR CHILDREN WITH HEAD-AND-NECK RHABDOMYOSARCOMA

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Purpose: To evaluate intensity-modulated radiotherapy (IMRT) and fractionated stereotactic radiotherapy (FSRT) for children with head-and-neck rhabdomyosarcoma (RMS) with special regard to radiation induced toxicity.

Materials and methods: From 1995 to 2005, we treated 19 children with head-and-neck RMS with FSRT (n = 14) or IMRT (n = 5) as a part of multimodal therapy. The median age of the patients at the time of RT was 62.5 months. All children received systemic chemotherapy (vincristine, actinomycin D, ifosfamide, adriamycin) according to the German Soft Tissue Sarcoma Study protocols (CWS). Patients were classified according to the surgical-pathological grouping system used in Intergroup Rhabdomyosarcoma Study Group (IRSG) trials. Patients were immobilized using an individual mask fixation system made of Scotch-CastTM. For treatment planning, contrast-enhanced MRI and CT scans were performed using a noninvasive stereotactic localization frame. Median size of PTV was 93.4 ml. We administered a median total dose of 45 Gy in a median fractionation of 5 x 1.8 Gy/week. Deep sedation or general anesthesia was required for children under 5 years of age not tolerating precision head mask fixation after a training period.

Results: Radiotherapy was well tolerated and could be completed without interruptions > 4 days. No toxicities greater than CTC grade II developed. The median follow-up period after precision RT was 17 months. Until now, no secondary malignancies have developed. After RT, the 3- and 5-year survival rate was 94%. The 5-year actuarial local control rate after RT was 89%. With respect to tumor localization, the 5-year local control rate was 100% for patients with RMS of the orbit and 91% for patients with parameningeal tumors. The actuarial freedom of distant metastases rate at 5 years was 85% for all patients. For patients with RMS of the orbit only, the actuarial distant progression-free survival rate was 100% at 5 years. One patient with parameningeal RMS developed distant metastases in both lungs 6 months after completion of RT and 1 month after local tumor progression. The actuarial 5-year distant metastases-free survival rate was 91% in children with parameningeal RMS.

Conclusion: High-precision RT techniques such as IMRT and FSRT offer good coverage of complexly shaped target volumes in critical locations while adhering to the tolerance limits of nearby critical normal tissue structures. Local and distant control rates are not reduced.

O52. DEVELOPMENT OF HIGHER CNS FUNCTIONS IN CHILDREN TREATED FOR POSTERIOR FOSSA TUMORS

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The aim of the study is assessment of functional neurodevelopment in children who were treated because of posterior fossa tumors, and especially examination of whether the tumor location in particular cerebellar structures—that is, in the vermis, or the left or the right hemisphere—determines various neuropsychological deficits. The examined group consists of 32 children treated between 1999 and 2003 at the Division of Pediatric Neuroradiology, Silesian University Medical School, in Katowice, Poland. Eleven girls and 21 boys aged between 3 and 21 years were examined. The average age was 12.3 years. There were 21 total and 8 subtotal resections of tumor. Internal hydrocephalus coexisting in 19 patients was fixed surgically. Histopathological diagnoses of tumors were as follows: 4 medulloblastomas, 8 pilocytic astrocytomas, 6 fibrillary astrocytomas, 1 anaplastic astrocytoma, 2 oligodendrogliomas, 4 anaplastic ependymomas, 1 choroidplexus papilloma, and 3 arachnoid cysts. The children were assessed by means suitable to their age tests, those examining higher mental functions such as cognitive processes, visual-spatial functions, verbal fluency, planning, sequential memory, and emotions. The clinical state of all patients was also evaluated, including full neurological examination. We found significant correlation between age at the time of surgery and spatial perceptual tasks. Children under the age of 8 years at the time of surgery significantly more often present neurobehavioral disturbances. On the other hand, older children, at the time of surgery, less frequently present disturbances in freedom of distraction (memory function) and concentration. Children thought to arise from undifferentiated NSCs present in the external granule layer of the cerebellum. However, the mechanism of tumorigenesis remains unknown for the majority of medulloblastomas. Using human medulloblastoma tumor samples and mouse models, we found that abnormal expression of REST/NR3F and Myc in NSCs causes cerebellum-specific, medulloblastoma-like tumors by blocking differentiation and thus maintaining the “stemness” of these cells. Furthermore, these results suggest that such a mechanism plays a role in the formation of human medulloblastoma.

O53. TRANSITIONAL REMODELING IN NEURAL STEM/PROGENITOR CELL: ROLES IN NEUROREGENERATION AND NEURAL TUMORS

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REST/NR3F is a transcriptional repressor that can repress the transcription of several neuronal genes by binding to a specific DNA sequence (RE1/NR3F) present in the genes’ regulatory regions. We constructed a recombinant transcription factor, REST-VP16, which binds to the same RE1/NR3F but instead activates these REST/NR3F target genes. We found that activation of REST/NR3F target genes with REST-VP16 is sufficient to convert neural stem/progenitor myc-positive cells into an embryologically active neuronal phenotype, which can survive in mouse brain without forming tumors. This is the first instance in which mycoblasts were converted to a neuronal phenotype. Our results provide an efficient way of triggering neuronal differentiation in myoblasts and possibly other stem cells through transcriptional remodeling and suggest that the experiments have the potential to have an impact in cell therapy for neuronal injury and diseases.

Many brain tumors represent a less differentiated or progenitor-like phenotype, but a direct mechanism for this process is still unknown. Medulloblastoma, one of the most malignant brain tumors in children, is thought to arise from undifferentiated NSCs present in the external granule layer of the cerebellum. However, the mechanism of tumorigenesis remains unknown for the majority of medulloblastomas. Using human medulloblastoma tumor samples and mouse models, we found that abnormal expression of REST/NR3F and Myc in NSCs causes cerebellum-specific, medulloblastoma-like tumors by blocking differentiation and thus maintaining the “stemness” of these cells. Furthermore, these results suggest that such a mechanism plays a role in the formation of human medulloblastoma.

O54. QUALITY CONTROL AND HIGH-GRADE GLIOMA TREATMENT FOR CHILDREN: WHAT IS NECESSARY?

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Background: Measuring the quality of medical care may use direct measures such as survival or less complicated indirect measures. Using indirect measures such as time from diagnosis to treatment is easier but requires a correlation to direct measures.

Materials and methods: The HIT-GBM database registered diffuse intrinsic pontine glioma (DIPG) and high-grade glioma (HGG) in pediatrics. The date of diagnosis was defined as the date of the MRI for DIPG and the date of histology for HGG.

Results: A total of 310 patients (137 female; age range, 3.3–18 years) were registered in 1995–2003 in this database: 4 medulloblastomas, 8 pilocytic astrocytomas, 6 fibrillary astrocytomas, 1 anaplastic astrocytoma, 2 oligodendrogliomas, 4 anaplastic ependymomas, 1 choroid plexus papilloma, and 3 arachnoid cysts. The children were assessed by means suitable to their age tests, those examining higher mental functions such as cognitive processes, visual-spatial functions, verbal fluency, planning, sequential memory, and emotions. The clinical state of all patients was also evaluated, including full neurological examination. We found significant correlation between age at the time of surgery and spatial perceptual tasks. Children under the age of 8 years at the time of surgery significantly more often present neurobehavioral disturbances. On the other hand, older children, at the time of surgery, less frequently present disturbances in freedom of distraction (memory function) and concentration. Children thought to arise from undifferentiated NSCs present in the external granule layer of the cerebellum. However, the mechanism of tumorigenesis remains unknown for the majority of medulloblastomas. Using human medulloblastoma tumor samples and mouse models, we found that abnormal expression of REST/NR3F and Myc in NSCs causes cerebellum-specific, medulloblastoma-like tumors by blocking differentiation and thus maintaining the “stemness” of these cells. Furthermore, these results suggest that such a mechanism plays a role in the formation of human medulloblastoma.

Abstracts for the Seventh Congress of the European Association for Neuro-Oncology (EANO)
OS5. ATYPICAL TERATOID/RHABDOID TUMOR OF THE CNS: A RETROSPECTIVE ANALYSIS OF OUTCOME DEPENDING ON INITIAL DIAGNOSIS AND TREATMENT RECEIVED IN 12 CONSECUTIVE PATIENTS

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Atypical teratoid/rhabdoid tumor (AT/RT) of the CNS is a rare and aggressive tumor of early childhood. Before immunohistochemistry with an antibody to INI1 was described as a specific means to distinguish AT/RT from other CNS tumors, these tumors were often misdiagnosed as medulloblastoma or supratentorial PNETs because of the sometimes indeterminate histologic features. We report on the outcome of 12 consecutive patients with AT/RT of the CNS, depending on initial diagnosis and treatment received.

Patients: A retrospective analysis applying the INI1 antibody to all highly malignant pediatric brain tumors treated at the University of Vienna between 1992 and 2005 disclosed 12 patients with AT/RT. Only the last five patients were originally diagnosed correctly. Diagnoses of the first seven patients included medulloblastoma, supratentorial PNETs, ependymoblastoma, and Ewing sarcoma. Metastatic disease was present at diagnosis in four, and not known in two.

Results: The five patients diagnosed initially as AT/RT were treated with an intensive, multilagent chemotherapy including cyclophosphamide, vincristine, cisplatin, ifosfamide, etoposide, mitotane, and Adriamycin, followed by high-dose chemotherapy with autologous stem cell rescue (modified Finlay protocol) and local radiotherapy. Four of the patients are in CCR 9 months and 3, 4, and 8 years after diagnosis, respectively. The seven patients originally misdiagnosed were treated according to the HIT SKK 92 or HIT 91, and Ewing sarcoma protocols of the German Society of Pediatric Hematology and Oncology. Of this earlier cohort, only one 11-year-old patient who was assumed to have a supratentorial PNET and had received extended chemotherapy including intraventricular therapy and a Gamma Knife boost became a long-term survivor.

Conclusions: Because of the poor outcome and a median survival of 6 to 19 months with conventional infant brain tumor therapy, proper diagnosis of AT/RT is essential.

OS6. OUTCOME OF THE TREATMENT OF ADULTS WITH MEDULLOBLASTOMA (MB) AT A LONG-TERM FOLLOW-UP

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Background: Retrospective studies available in the literature on adults with medulloblastoma (MB) use different chemotherapy and radiotherapy regimens, and have small patient series with a short follow-up, thus making it difficult to assess survival rates and prognostic factors in these patients. In the present study, we therefore report on the treatment outcome of MB patients in a trial initiated in 1989 and terminated in 2001.

Patients and methods: Patients (>18 years of age) with a histologically confirmed diagnosis of MB were staged according to the classification of Chang et al. Low-risk (M-) patients received 36 Gy radiotherapy to the craniospinal axis, supplemented by a local tumor dose of 18.8 Gy (total dose, 54.8 Gy). High-risk (M+) patients had two cycles of “up-front” chemotherapy followed by the same radiotherapy and by maintenance chemotherapy with the same regimen.

Results: Thirty-six evaluable patients were enrolled. With a median follow-up of 3.7 years, 19 of 27 patients were free of disease at the time of last follow-up, and the percentage of survivors at 5 years was 56%. In M+ patients, the EFS at 5 years and the percentage of survivors at 5 years were 43% and 56%, respectively. With a median follow-up of 7.6 years, in the same M- cases, the EFS at 5 and at 10 years was 80% and 31%, respectively, and the percentage of survivors at 5 and 10 years was 80% and 72%, respectively. In the same M+ cases, the EFS at 5 and at 10 years was 60% and 40%, respectively, and the percentage of survivors at 5 and 10 years was 65% and 45%, respectively.

Conclusions: The findings made in the present study on adults with MB demonstrate that, in M- patients treated with radiotherapy alone, MD recurred in any case, even if later than in M+ patients. Radiotherapy alone does not provide a permanent cure, the recurrence rate at 10 years in M+ patients being similar to that in M+ patients. Further studies should be conducted to evaluate whether the outcome in M- patients might be improved by chemotherapy. Moreover, M+ patients who are disease free at 5 years are highly unlikely to have recurrences at 10 years.
Follow-up was 80 months (range, 4–227 months). All patient follow-up data were updated as of December 2005.

Results: The particularity of our series consists of a very long-term follow-up of fractionated RT given to selected patients with general contraindications for surgery and/or large tumors for most of them. Nineteen patients died: two with progressive disease, and seventeen of non-AN causes. Three were lost to follow-up. A serviceable level of hearing was preserved in seven of nine hearing patients. No patient experienced facial or trigeminal neuropathy. Among the 46 ANs, tumor shrinkage was observed in 27 (59%) and stable disease in 16 (35%). Tumor progression occurred in three patients, 12–15 months after RT. Furthermore, two additional tumors recurred after shrinkage 20 and 216 months after treatment and were operated on. Actuarial local tumor control rate at 5, 10, and 15 years after RT were 86%. For the patient who had a tumor recurrence at 216 months, histological examination revealed a low-grade malignant peripheral nerve sheath tumor.

Conclusions: The long-term efficacy of fractionated RT is well documented in this series. Acute and delayed tolerance was good and hearing was preserved for a long time. Malignant transformation can occur many years after RT, so we advocate caution when using this treatment for benign tumors.
The aim of this study was to evaluate the importance of various elements of normal murine brain anatomy showed 11C-methionine And 18F-fluorodeoxyglucose (FDG) PET seems to have no value in the workup of recurrent tumor biopsy And radiotherapy with computer-assisted conventional imaging.

Results: Various elements of normal murine brain anatomy showed characteristic multiphoton-excited intensity and fluorescent lifetime profiles, which could be clearly differentiated from experimental gliomas and normal brain tissue. Fluorescent lifetime imaging of human ex vivo brain tumor specimens demonstrated visualization of the cellular composition of solid tumor, allowing the discrimination of individual tumor cells, tumor cell clusters, and vasculature. Low-grade gliomas and malignant gliomas showed distinct fluorescent lifetime profiles. Acquisition of 3-dimensional data arrays obtained from solid tumor and the wall of the resection cavity showed that this technology may be used to quantify the density of tumor cells per native tissue volume.

Conclusions: We have demonstrated that multiphoton microscopy and fluorescent lifetime imaging can discriminate tumor and normal brain. Fluorescent lifetime profiles may contribute to identification of malignant tumor biopsies.


SIP Lab Innsbruck, Innsbruck, Austria, Neurosurgery, Medical University, Innsbruck, Austria, Radiotherapy, Medical University, Innsbruck, Austria, Neurology, Medical University, Innsbruck, Austria, Nuclear Medicine, Medical University, Innsbruck, Austria, and Radiology I, Medical University, Innsbruck, Austria

Objectives: To apply the universal Innsbruck SIP lab frame attached to the VBH vacuum mouthpiece for unified, multidisciplinary diagnosis and therapy by allowing image fusion of CT/MR/SPECT/PET and fixation for radiotherapy and neurosurgery. We report the concept and our clinical experience with this new device in various disciplines over the last five years.

Methods and methods: The VBH mouthpiece is an individualized vacuum dental cast that is attached under pressure to the upper palate. For immobilization of the patient head, the VBH mouthpiece (MP) is secured to a base plate by hydraulic arms. The frame contains multimodal markers and provides external reference points for image fusion, computer-assisted surgery, and radiotherapy. The diagnostic scans are performed with the VBH mouthpiece. Image fusion is based on reference points on the frame. Biopsy is performed with frameless stereotactic navigation. If surgical intervention is necessary, the image-guided surgery relies on the fiducials on the frame, thus necessitating an additional scan. For radiotherapy, the patient is repositioned with the original VBH mouthpiece, thus necessitating the fabrication of a mask. For follow-up, subsequent image acquisitions are performed with the frame.

Results: The VBH head holder was successfully used for fractionated radiotherapy, brachytherapy, SPECT acquisition, and computer-assisted surgery. The VBH head holder offered rigid, accurate, and reproducible fixation of accurate external reference points for image-to-image and image-to-patient registration.

Conclusion: Application of the mouthpiece is an important step toward unified, multidisciplinary diagnosis and therapy by allowing image fusion of CT/MR/SPECT/PET and use of imaging data for neurosurgical intervention and radiotherapy.


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Aim: The aim of this study was to evaluate the importance of [11C]methionine (MET) and [18F]fluorodeoxyglucose (FDG) PET in the follow-up setting of glioblastoma multiforme (GBM) suspicious of recurrence or progression. Both radioligands have been successfully applied in diagnosis and therapy evaluation of brain tumors.

Methods: After surgical and/or conservative treatment, 28 patients with GBM underwent FDG and MET PET 12.7 months on average after diagnosis. Scans were evaluated visually and by calculating a maximal tumor SUV and a ratio of tumor vs. contralateral region (RTU). Degree of tracer uptake was compared with survival time, disease duration, and MRI findings.

Results: The mean survival of the patients was 12.7 months; two patients groups with survival of more or less than 12 months were established. MET PET showed focally increased uptake in 24 patients, whereas FDG PET showed this in two patients. In MRI scans, viable tumor tissue was suspected in 18 patients. Tumor SUVs of MET ranged from 1.1 to 8.3 and RTUs from 0.8 to 4.2, for FDG, tumor SUV values lay between 3.3 and 9.4, and RTUs between 0.2 and 1.5. No correlations were found between MET and FDG uptake and survival time or disease duration, respectively. Kaplan-Meier estimations were negative. With respect to survival groups, positive MET PET revealed a sensitivity of 86% and specificity of 84% SUV and RTU values did not differ between patients with positive or negative MRI results.

Conclusions: FDG PET seems to have no value in the workup of recurrent glioblastomas, MET PET visualizes viable tumor tissue without adding any prognostic information, and offers only questionable advantage over conventional imaging.

O64. STRUCTURAL AND PHOTOCHEMICAL IMAGING OF BRAIN TUMOR TISSUE BY TIME-RESOLVED MULTIPHOTON EXCITATION MICROSCOPY

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Introduction: Multiphoton-excited in vivo fluorescent microscopy is a label-based technology that enables subcellular resolution of native tissues in situ. We have recently demonstrated that multiphoton microscopy allows a discrimination of different cell types, neurons, glia, or tumor cells and visualization of organelles. In addition, selective excitation of endogenous biomolecules offers means of imaging cellular metabolism or cellular functions in situ. Here we demonstrate that the excitation profiles and lifetimes of endogenous fluorophores may be used to discriminate tumor cells and elements of normal brain.

Methods: Invasive and noninvasive experimental gliomas were analyzed by multiphoton microscopy, and corresponding samples were processed for conventional histology. Biopsy samples of human brain tumors were obtained during resection of glial tumors, and biopsy sites were documented using neuronavigation. Fluorescence intensity and fluorescence lifetime imaging were used to detect the tumor cell density in biopsy specimens.

Results: Various elements of normal murine brain anatomy showed characteristic multiphoton-excited intensity and fluorescent lifetime profiles, which could be clearly differentiated from experimental gliomas and normal brain tissue. Fluorescent lifetime imaging of human ex vivo brain tumor specimens demonstrated visualization of the cellular composition of solid tumor, allowing the discrimination of individual tumor cells, tumor cell clusters, and vasculature. Low-grade gliomas and malignant gliomas showed distinct fluorescent lifetime profiles. Acquisition of 3-dimensional data arrays obtained from solid tumor and the wall of the resection cavity showed that this technology may be used to quantify the density of tumor cells per native tissue volume.

Conclusions: We have demonstrated that multiphoton microscopy and fluorescent lifetime imaging can discriminate tumor and normal brain. Fluorescent lifetime profiles may contribute to identification of malignant tumor biopsies.
in any other location and sharp border (HR = 0.55; 95% CI, 0.37–0.80; P = 0.01).

Conclusions: 1p/19q deleted oligodendroglial tumors, including mixed oligoastrocytomas, are preferentially located in the frontal lobe and have more likely indistinct tumor border on T1–wi. In addition, the association of frontal location with indistinct border is independently related to longer overall survival. Larger studies are needed to validate these results.

O66. CORRELATION OF METHIONINE (MET) AND GLUCOSE (FDG) PET WITH MRI IMAGES AND HISTOLOGY IN GLIOMA SURGERY USING NEURONAVIGATION AND IMAGE FUSION

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This study was investigated the histological correlate of [18F]fluorodeoxyglucose (FDG) and [11C]methionine (MET) PET uptake of brain gliomas by image fusion for navigated surgery. Of 29 patients (19 male and 10 female; mean age, 42 years; range, 10–77 years; 8 low grade and 12 high grade astrocytomas or mixed gliomas, and 9 oligodendrogliomas), 26 underwent FDG PET, 27 underwent MET and 26 underwent both FDG/MET PET studies preoperatively. MIE and PET images were coregistered within a neuronavigation system, and radiological and metabolic tumor borders were outlined for surgical intervention and histological correlation (10 biopsies, 15 subtotal and 12 gross total resections). Among 26 patients, FDG PET tumor uptake was demonstrated in 31.1% (26); two low-grade glioma and one high-grade glioma), while MET PET tumor uptake was detected in 25 of 27 patients (92.6%). The quantitative MET tumor standardized uptake value (SUV) ratio was significantly higher in malignant gliomas and oligodendrogliomas than in low-grade gliomas (2.76 and 2.62, respectively, vs. 1.67, P = 0.003). Qualitative visual grading of MET uptake in 26 tumors revealed two main patterns: focal MET uptake in 24 and uniform global MET uptake in 11. Focal uptake corresponded to malignant glioma histology in 66.7%, and uniform global uptake to oligodendroglioma histology in 72.7%. The three patients revealed a mixed pattern (global/local, all malignant gliomas). In oligodendrogliomas, global MET uptake constituted 81.5% (range, 53.8% and 135%) of the MRI T1 tumor volume on average and was limited to the MRI FLAIR tumor volume in seven of eight (86%) patients. Tissue samples of focal MET uptake areas correlated with histological anaplasia in 8 of 12 (66.6%) glioma patients, verified by navigated tumor tissue sampling, although five (62.5%) of eight patients had no MRI contrast enhancement. In conclusion, MET PET image coregistration for neuronavigated glioma surgery demarcated anaplastic tumor even in homogeneous MRI nonenhancing gliomas, as well as characterized global homogenous MET uptake within MRI T1/FLAIR tumor areas, with a high probability of oligodendroglial histology.

O67. OPERATING MICROSCOPE INTEGRATED OPTICAL COHERENCE TOMOGRAPHY FOR SURGERY OF INTRINSIC BRAIN TUMORS

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Introduction: Optical coherence tomography (OCT) is a noninvasive imaging technique with a micrometer resolution. It allows noncontact analysis of CNS tissues with a penetration depth of 2 mm, reaching a spatial resolution of approximately 8 μm. We have integrated a spectral-domain OCT microscope into a neurosurgical microscope for intraoperative detection of residual tumor during brain tumor surgery.

Methods: Human brain tumor tissue and areas of the resection cavity were analyzed during the resection of gliomas. The sites of analyses were registered using the neuronavigation integration of the operating microscope and biopsy specimens were taken for routine histology. Post–image acquisition processing was used to compensate for movements of the brain and to realign A-scan images for calculation of a light attenuation factors for semiautomated detection of tumor tissue.

Results: Microscope integrated OCT imaging of normal cortex and white matter showed a typical light attenuation profile. Depending on the cellularity of the specimen, tumor tissue showed a loss of the normal light attenuation profile, resulting in altered light attenuation coefficients compared to normal brain. Based on this parameter and the microstructure of the tumor tissue, which was entirely absent in normal tissue, OCT analysis allowed the discrimination of normal brain tissue, invaded brain, solid tumor tissue, and necrosis. Following macroscopically complete resections, OCT analysis of the resection cavity displayed the typical microstructure and light attenuation profile of tumor tissue in some specimens, which in routine histology contained microscopically residual tumor tissue.

O68. THE ROLE OF NEUROENDOSCOPY IN NEURO-ONCOLOGY

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Advances have been made in endoscopic technology and optical guided stereotaxy. Consequently, the indications for endoscopic neurosurgery especially for ventricular or paraventricular tumors have increased. The simultaneous treatment of hydrocephalus is an important advantage in the treatment of brain tumors. Between 2003 and 2005, we used neuroendoscopy for 14 patients. Six underwent endoscopic biopsy of tumor. In all cases, a histopathological diagnosis was established. We illustrate the advantage of guided stereotactic neuroendoscopy by means of two videos of selected cases.

Case 1: A 21-year-old man presented with a recent diplopia. An MRI study of the brain revealed a mass lesion in the pineal region without hydrocephalus. A PET scan with methionine showed a increased uptake in this tumor. A tumor biopsy guided by neuronavigation was performed. The histological diagnosis was a germinoma. The patient was subsequently treated with multimodality therapy, including chemotherapy and radiotherapy.

Case 2: A 16-year-old girl presented with three weeks of progressive headaches. Her examination revealed Parinaud’s syndrome. An MRI indicated an obstructive hydrocephalus with a mass lesion in the left thalamus. An ETV and biopsy guided by neuronavigation was performed by a left precoronal approach. The histological diagnosis was a astrocytoma III. Conventional surgery was performed a second time.

Discussion: The management of hydrocephalus related to posterior fossa tumors in adults could be resolved by ETV. During the same period, eight patients presented with a hydrocephalus with a posterior fossa tumor. A preoperative ETV was performed in seven cases, and a postoperative ETV was performed in one case. In all cases, the problem of hydrocephalus was efficiently resolved without the need of ventricular drainage. No patient required a shunt, and no CSF fistula was deployed.

Conclusions: Neuroendoscopy in the management of brain tumors is safe and efficient, with low morbidity. It is an additional tool in the multimodal management of selected brain tumors.

O69. ENDOSCOPIC TRANSSPHENOIDAL TREATMENT IN RECURRENT AND RESIDUAL PITUITARY ADENOMAS: OUR EXPERIENCES

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The aim of the study has been the assessment of the endoscopic method in the surgical management of recurrent and residual pituitary adenomas, as regards treatment efficiency, substantial complications, and its possible advantages for patients and operating surgeons.

In the Department of Neurosurgery, Silesian University School of Medicine, in Katowice, between October 2001 and June 2004, 125 patients underwent endoscopic surgery because of pituitary adenoma. The analysis comprised 20 patients who were operated on because of recurrent adenomas or residual tumor not completely removed during the first surgical procedure. The group of patients was composed of 9 women and 11 men (age range, 32–79 years; mean, 53.9 years). In this group were 14 nonfunctioning adenomas, four GH-secreting adenomas, one PRL-secreting adenoma, and one ACTH-secreting adenoma; 19 tumors were macroadenomas and one was microadenoma. Eleven tumors infiltrated cavernous sinuses. The surgical procedures were performed by a stable team of two neurosurgeons, a laryngologist, and an anesthesiologist. The surgical method was based on the technique developed by Jho and Carrau, with our own modification. The follow-up period after surgery was 12 to 42 months (mean, 24.2 months).
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Of the 20 cases, complete recovery was achieved in 40% of patients undergoing secondary surgical procedures. In the group of 11 patients with adenomas not infiltrating cavernous sinuses, recovery was reported for eight (72.7%) to occur. Seven cases of liquefactive occurrence following operation, requiring reconstruction and sealing of the sella by means of tissue glue and artificial dura or freeze-dried human dura. In one case, despite the application of postoperative lumbar drainage, rhinorrhea occurred one month after the procedure, which required endoscopic reconstruction of the treat- ment. In the same patient, pneumoencephalocele was observed. The average time of repeated surgical procedure using endoscopic technique was shorter by 18 min than the repeated procedure using the microscopic technique.

Conclusions: The endoscopic method is a safe, hardly invasive, and efficient surgical technique in the treatment of recurrent and residual pitui- tary adenomas. Advantages that add to its attractiveness are reduction of the procedure duration, very good visualization of the operative field, absence of serious complications, and less pain after the surgery.

O70. ITALIAN STUDY ON NEUROENDOSCOPIC BIOPSYs: A RETROSPECTIVE SURVEY
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Objective: Although neuroendoscopic biopsies (NEB) are routinely performed, the safety and validity of NEB has been studied only for a small number of patients in single-center reports. The aim of this study is to col- lect data in a large number of patients and to compare the results of centers known to perform neuroendoscopic procedures on a regular basis.

Materials and methods: Retrospective data was collected from seven centers routinely performing NEB over a period of 11 years. The essential patient data focused on all biopsy attempts. Feedback from the neuropa- thologist on the study form was essential.

Results: We received 60 patient data forms from seven medical centers in Italy. Patients’ age ranged from 5 to 78 years (median, 43.1 years). Tumor location was pineal (38%), thalamic (20%), mesencephalon (18%), or other locations (24%). Tumor size was less than 10 mm (11%), 10 to 20 mm (33%), and 20 mm (49%). In addition to the NEB, 54% of patients had endoscopic third ventriculostomy (ETV), and 12% had septum pel- lucidotomy. The major complication was hemorrhage (14%). Of the patients, 33% had bleeding during the procedure: mild, 20%; moderate, 10%; and severe, 3%. Infection occurred in one case (1.7%), and other complications, mostly reversible, in 10%. Tumor types ranged across the spectrum, includ- ing 27% glioma (low grade and high grade), 15% germinoma, 12% pineal tumor, 4% PNET, 9% lymphoma, 4% metastasis, 6% craniopharyngioma, two cases of nonneoplastic lesions, and 13% other tumor types; 10% had nonconclusive pathology. According to diagnosis, specific therapy in 35% of the patients was performed: 15% microsurgical removal, 9% chemother- apy, and 9% radiotherapy.

Conclusions: This is one of the largest series confirming the safety and validity of NEB. NEB had a relatively low, and mostly reversible, complica- tion rate of below 14%. Neuroendoscopic biopsy provided meaningful pathological data for 90% of the patients by allowing complementary therapy when possible. By ETV or septum pellucidotomy (66%) to control intracranial hypertension, CSF pathways can be restored. Based on these results, NEB should be considered a safe and efficient procedure for diag- nosis and therapy of periventricular or ventricular tumors.

O71. MYXOPAPILLARY EPENDYMOMAS OF THE CAUDA EQUINA REGION: PRESENTATION OF NINE CASES IN THE MIRI ERA
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Myxopapillary ependymomas are located essentially in the cauda equina region, forming a histologically distinct entity. They are rare tumors with a generally good prognosis if totally excised. Delay in making the proper diagnosis is the rule because of a blunt and late clinical presentation.

Over the last 16 years, nine cases of myxopapillary ependymoma have been reviewed. The patients’ mean age was 30.44, and the mean time between the first symptom and the diagnosis was 25.9 months. Clinical symptoms were often nonspecific, with low-back pain or radioculopathy present in all patients. At the time of operation, clinical signs were essentially motor defects, usually moderate (six cases), sphincter disturbances (five cases), and sensory loss (two cases). Total removal of the tumor was possible in four cases, subtotal removal in four, and partial removal in one. Postop- erative complications included one patient who developed aspetic meningi- tis. Additional radiotherapy was advocated for all patients who underwent a subtotal/partial resection. Overall, six patients (66.6%) had an excellent outcome, two (22.2%) were left with some degree of deficit, and was lost to follow-up. All four patients with total tumor removal had good functional recovery, and no recurrence has been observed in this group. Only one tumor subtotally excised recurred, four years later. Prognosis seems to be related to spasticity of the extremities, since two of five patients who presented with bowel or bladder dysfunction had a less favorable outcome.

Younger patients with recurrent episodes of low-back/radicular pain should raise the physician’s suspicion of a tumor in the cauda equina or the filum terminale, and early MRI scan should be obtained. Thus, an early diagnosis can be achieved, providing a better chance for effective treatment. When appropriate, can yield a good prognosis.

O72. INTRAOPERATIVE SUBCORTICAL LANGUAGE TRACT MAPPING GUIDES SURGICAL REMOVAL OF GLIOMAS INVOLVING SPEECH AREAS
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Subcortical stimulation can be used to identify functional language tracts during resection of gliomas located very close or in proximity to areas or pathways. The objective of the present study was to investigate the feasibility of the routine use of subcortical stimulation for identification of language tracts in a large series of patients with gliomas, and to determine the large influence that identification of subcortical language tracts exerted on the extent of surgery and the appearance of immediate and definitive post- operative deficits.

Subcortical stimulation for the identification of language tracts was sys- tematically used during surgical removal of 88 gliomas (44 high grade and 44 low grade) involving language pathways. Procedures were performed during asleep-awake craniotomy. Subcortical stimulation was continuously alternated with surgical resection in a back-and-forth fashion. Language performance was tested by neuropsychological language evaluation pro- peratively and at 3, 30, and 90 days after surgery.

Language tracts were identified in 59% of patients, with differences according to tumor location but not according to histological grade. Identification of language tracts influenced the ability to achieve complete tumor removal in low-grade gliomas, where tracts were documented inside the peripheral mass of the tumor. Identification of language tracts was asso- ciated with a higher occurrence of transient postoperative deficits (69.2%), but a low definitive morbidity (2.3%). A pattern of typical language distur- bances related to the phonological and semantic system can be identified based on tumor location, the preservation of which is important for the maintenance of language integrity.

Our study supports the routine use of subcortical stimulation for identification of language tracts as a reliable tool for guiding surgical removal of gliomas in, or in close proximity to, language areas or path- ways.

POSTERS
P1. MULTIPLE BRAIN METASTASES: ROLE OF THE SURGERY
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Over recent years, the treatment of metastatic brain cancer has progres- sively evolved toward multimodal and more aggressive approaches: radio- therapy, surgery, stereotactic radiosurgery, and chemotherapy may grant a survival benefit of more than one year. Such results have been obtained also in patients with multiple brain metastases.

We retrospectively analyzed a series of 14 patients affected by multiple brain metastases treated at our institute between January 2003 and January 2006. Primary tumor sites were lung (seven cases), prostate (three), kidney (one), breast (one), and gross bowel (one); neuroendocrinal carcinoma, with unknown primitive location was found in one case. The mean age of patients was 58.3 years, the KPS was >70 in all patients, and mean number of lesions was three (range, 2–8). In all these cases, only symptomatic and/or life-threatening lesions were operated on; 12 supratentorial and two suboccipital craniotomies were performed. Twelve patients underwent post- operative conventional radiotherapy and chemotherapy; in two patients, radiosurgery was performed on the one residual lesion.
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P2. ONE-STAGE IMAGE-GUIDED POLYMETASTASECTOMY IN PATIENTS WITH MULTIPLE AND RECURRENT PARENCHYMAL BRAIN METASTASES
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Summary: One-stage image-guided polymetastectomy allows fast tumor cytoreduction and control of the neuro-oncological disease. Using intraoperative neuronavigation does not change the progression of the disease but can influence operative results.

Materials and methods: Since January 2004, 32 patients (20 men and 12 women) with multiple and recurrent brain metastases were operated on with the neuronavigation system Vector Vision 2 (Brain Lab, Germany) in the Department of Neurosurgery. All patients were classified in prognostic class II according to RTOG classification.

Results: Eight patients undergone one-stage bimetastectomies, and one-stage thremetastectomy was performed in one patient. Three concurrent craniotomies were performed, and two of them completed with bimetastectomy and one with tritetametastic. The postoperative complications were divided into systemic, neurological, and surgical. The postoperative outcome was assessed using Karnofsky score and GL index of Spitzer.

Conclusions: One-stage image-guided polymetastectomy is related to good surgical outcome and prolonged and saved quality of life in oligo- and asymptomatic patients with good performance status and controlled systemic disease.

P3. PROMOTER CPG ISLAND METHYLATION OF DAP KINASE IN BRAIN METASTASES OF SOLID TUMORS
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Death-associated protein kinase (DAPK) is a gene regulating apoptosis by IFN that is frequently inactivated by aberrant promoter CpG island methylation in cancer. Loss of DAPK expression has been found to confer a relative advantage on tumor cells resistant to apoptotic stimuli, following their detachment from the original tumor and their transport in the circulation; accordingly, DAPK may be considered as a metastatic suppressor gene. To determine the potential involvement of DAPK silencing in brain invasion, we analyzed the promoter methylation status of DAPK in a series of 28 samples derived from brain metastases of solid tumors by methylation-specific PCR and sequencing. The metastases originated from lung carcinoma (eight cases), malignant melanoma (seven cases), myosarcoma (four cases), breast carcinoma (three cases), ovarian carcinoma (two cases), and one each from colon, kidney, bladder, and undifferentiated carcinoma. Abrupt DAPK promoter methylation was identified in 15 metastases (54%) and in peripheral blood lymphocyte DNA from 5 of 18 cases (44%) in which this nontumoral DNA was available. Our data suggest an important role of the DAPK promoter hypermethylation in brain metastases from solid tumors. The detection of aberrant methylation of this gene in serum may represent a potential clinical application as a diagnostic marker for cancer cells. Financial support: PI 03/0353 and PI 05/0829.

P4. CLINICAL AND IMAGING PROFILE OF BRAIN METASTASES FROM A HOSPITAL IN INDIA
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Brain metastases pose a unique specter over cancer care in developing countries. Most patients encounter cerebral invasive disease after they have exhausted their socioeconomic and emotional reserves. Optimal treatment therefore is rarely delivered. Radiation continues to be the mainstay. Thirteen patients with cerebral metastases were diagnosed over the last nine months.

The male–female ratio was 4:9; median age, 51 years (range, 25–75 years). Five patients had lung cancer, four breast cancer, two colon, one pancreas, and one renal carcinoma. Patients with lung cancer developed cerebral mets after a median of seven months (range, 1–58 months) and a median of two regimens of chemotherapy. The primary was controlled in four patients.

Three patients were asymptomatic at presentation and were diagnosed during routine screening. Ten had neurological deficit and symptoms of raised intracranial tension. The remaining two patients, altered behavior (one patient) vomiting (three patients), headache (one patient) giddiness (two patients) and convulsions (three patients). Five of the latter had brain metastases at presentation, and three had bone and liver mets. Six patients underwent MR imaging, and the others were diagnosed on contrast-enhanced CT scan. Contrast-enhanced magnetization transfer preparation sequence was used to better detect metastases on MRI (12 multiple and one solitary). Two patients with breast cancer had leptomeningeal metastases, and the patient with renal cell carcinoma had diplocic metastases with intracranial extension. Radiation planned was 30 Gy/10 for 11 patients and 20 Gy/5 for two patients. Two patients with breast cancer with leptomeningeal metastases also received intrathecal methotrexate. Two asymptomatic patients died during treatment because of progression of disease, whereas the remaining eight patients reported improvement in the symptoms. Of these patients one died of myocardial infarction one month after conclusion of radiation, and one patient died of lung metastases three months after conclusion of radiation.

In our setting, radiation therapy plays an important role in the palliation of these patients. Temozolomide and other effective therapies are not feasible for economic reasons. Brain metastases among lung cancer are on the increase as a result of effective systemic treatment.

P5. A1 ADENOSINE RECEPTOR DEFICIENCY IN THE HOST BRAIN PROMOTES THE GROWTH OF INOCULATED GIOBLASTOMA CELLS
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Objective: In the present study, we have addressed the question whether deletion of A1 adenosine receptors affects glioblastoma-host interaction observed in A1AR-deficient mice. Methods: Stably expressing G261 glioblastoma cells were inoculated into A1AR+/− mice and A1AR−/− littermate controls. With this approach, we deleted the A1AR in the host cells, but not in the inoculated G261 glioblastoma cells. Animals were sacrificed 14 days after GL261 inoculation, and the tumor area was determined double-blinded in axial section at the maximal diameter. Immunofluorescent triple labeling was carried out on 4-μm free-floating sections by using a spectral confocal microscope.

Results: The tumor size in A1AR+/− mice was significantly larger as compared to A1AR−/− mice (mean ± SE, 0.96 ± 0.09 mm for control [n = 7] and 1.69 ± 0.03 mm for A1AR+/− mice [n = 9]), whereas the remaining eight patients reported improvement in the symptoms. Of these patients one died of myocardial infarction one month after conclusion of radiation, and one patient died of lung metastases three months after conclusion of radiation.

Conclusion: These results imply that A1AR modulates tumor growth and that microglial cells are the cellular candidates for mediating this effect.

P6. EXPRESSION OF ABC-TRANSPORTER PROTEINS IN HUMAN MALIGNANT GLIOMAS
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Background: The problem of drug resistance as it pertains to cancer mimics that of antibiotic resistance. Limitations in drug delivery via poor absorption, increased metabolism, environmental changes, and poor penetration to certain sites are recognized. In the central nervous system (CNS), one of the noteworthy problems in cancer chemotherapy is the blood-brain barrier (BBB). This BBB forms a very effective barrier to the free diffusion of many polar solutes into the brain. Many metabolites that are polar have their brain entry facilitated by specific inwardly directed transport mechanisms. These molecules are substrates for the ABC (ATP-binding cassette)
All 18 of 18 glioblastomas showed a strong expression of angiogenin. In all cell cultures analyzed, the angiogenin tumor grade mRNA in three established glioma cell lines, two endothelial cell cultures, and nonneoplastic brain were negative for angiogenin. It is expressed in cancer cells, binds to the surface of endothelial cells, and promotes their invasion. Since there is not much known about the role of angiogenin in gliomas, we assessed its expression pattern and function in these tumors.

**Results:** We investigated the expression of angiogenin mRNA in three established glioma cell lines, two endothelial cell cultures, and nonneoplastic brain. In all of them, we observed a high expression of angiogenin, revealing a correlation with tumor grade. Moreover, we have further analyzed the immunoreactivity of individual sera. The first two proteins assayed were identified as eef1a1 and mark3. Immunoreactivity directed to the second antigen was detected only when patients had gliomas. The mRNA corresponding to this protein was significantly decreased in patients with a tumor nonresponsive to chemotherapy. A negative correlation was observed with eef1a1 mRNA content was significantly decreased in patients with a tumor nonresponsive to chemotherapy. A negative correlation was observed with eef1a1 mRNA content. The mRNA of eef1a1 was identified as eef1a1 and mark3. Immunoreactivity directed to the second antigen was detected only when patients had gliomas. The mRNA corresponding to this protein was significantly decreased in patients with a tumor nonresponsive to chemotherapy. A negative correlation was observed with eef1a1 mRNA content was significantly decreased in patients with a tumor nonresponsive to chemotherapy. A negative correlation was observed with eef1a1 mRNA content.

**Conclusions:** To achieve a good response to the drugs, we chose anticancer drugs for each patient individually, based on the results of the drug-resistant gene expression. Measuring the expression of the drug-resistant genes facilitates rapid determination of the drug sensitivity to chemotherapy in patients with malignant gliomas.

**P7. EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 3 IN GLIOMA CORRELATES WITH TUMOR GRADE**

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**Introduction:** Tumor-induced angiogenesis as a key feature of malignant gliomas represents a novel target for neoadjuvant therapies. Among various angiogenic signaling systems, the vascular endothelial growth factor receptor 2 (VEGFR2) and its ligand VEGF-A are considered the most important. VEGFR3 is a receptor tyrosine kinase binding VEGF-C and VEGF-D which has an essential role in embryonic development and lymphangiogenesis. Furthermore, VEGFR3 recently gained further importance in tumor biology and metastatic spread. In this study, we show the strong expression of VEGFR3 as well as its ligands VEGF-C and VEGF-D in gliomas, revealing a correlation with tumor grade.

**Materials and methods:** Expression of VEGFR3, VEGF-C, and VEGF-D was investigated in human glioblastomas (n = 18), low-grade gliomas (n = 6), and nonneoplastic brain (n = 3) at the protein level by immunohistochemistry and Western blotting. mRNA level was measured by real-time PCR.

**Results:** All 18 of 18 glioblastomas showed a strong expression of VEGFR3 in tumor vessel endothelium. VEGF-C and VEGF-D were highly expressed in 18 of 18 glioblastomas in areas of high angiogenic activity. Two of six low-grade gliomas showed single endothelial cells positive for VEGFR3. VEGF-C and VEGF-D expression in nonneoplastic brain was negative for VEGFR3 and its ligands. On mRNA level, glioblastomas showed a significant upregulation of VEGFR3 compared to low-grade gliomas and nonneoplastic brain.

**Conclusions:** VEGFR3 as well as its ligands are highly expressed in malignant gliomas, indicating a functional significance of this receptor system in tumor angiogenesis. Expression of VEGFR3 is related to tumor grade in gliomas and may therefore represent a significant factor in malignant transformation.
LPA receptors are not only expressed in GBM but also in astrocytes, oligodendrocytes (ODGs), and microglia. Activation of LPA receptors through LPA has cell-specific effects, which depend on the type of downstream axis coupled to the receptor.

ATX activity stimulates invasion of glioma cells. U87 spheroids respond to LFC treatment with increased invasion into a collagen I matrix, whereas endogenous ATX depletion with siRNA results in an abrogated response to LFC, an effect that was reversed with the addition of LFC. Inhibiting the LPA1 receptor with K16425 results in decreased invasion. Similarly, ATX activity increased invasiveness of U251 and SNB19 cells in an orthotopic rat brain-slice assay as compared to control cells. Taken together, this shows that, in GBM, ATX functions as an autocrine invasion factor.

To investigate possible paracrine effects, media containing wild-type or a catalytically inactive (H316Q) of ATX was used to examine a dependence of GBM cells. ATX (but not H316Q) enhances adhesion of T98G and U251 cells in a manner similar to LPA. This effect is reversed by the LPA receptor inhibitor Ki616245, suggesting that ATX is pro-adhesive in GBM. Interestingly, the murine ODG cell line N19 expressed to ATX and LFC showed reduced adhesion, signifying that the effect of ATX-mediated signaling is cell-type specific. N19 monolayers were used to assess the influence of ATX on cross talk between GBM and ODG. U87 cells expressing ATX invaded through a N19 monolayer, whereas ATX knockdown reduced invasion. Conversely, U251 cells overexpressing ATX invaded the monolayer more than did cells expressing H316Q. These data suggest that GBM cells secreting ATX and ODGs less of a barrier than do cells that do not express ATX, indicating autocrine and paracrine roles in GBM invasion.

P11. CD133+ AND CD133 – CANCER STEM CELLS IN GliOBlastoma

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Introduction: According to the cancer stem cell (CSC) hypothesis, tumors are maintained by a small population of stem cell–like cells. Although this concept was already proposed in the late 1960s and is established for hematological malignancies, CSCs have only recently been isolated from solid cancers such as breast cancer, ependymomas, medulloblastomas, and glioblastomas.

Methods: We prospectively analyzed 25 tumor samples from glioblastomas for CD133+ cancer stem cells. Tumor samples were dissociated mechanically and enzymatically within 12 h after resection. Single cell suspension was cultured in stem cell–permissive medium. Cancer stem cells were detected by flow cytometry, and stem cell properties were investigated by minimal dilution assay and immunocytochemistry.

Results: Sixteen tumor samples (64%) showed a CD133+ subpopulation (range, 3% – 40%). In all glioblastoma samples with CD133+ cancer stem cells, formation of tumor spheres was observed within 10 to 28 days. Notably, only a small population of the CD133+ sphere-forming cells showed stem cell properties. In contrast, in all CD133– tumors samples except one, formation of tumor spheres were not noted. In a CD133– sample of a recurrent glioblastoma, a small population of cells with stem cell properties not showing CD133 positivity was detected.

Conclusion: Our data suggest that CD133 is a specific marker for cancer stem cells in most glioblastomas. CD133– stem cells are rare but must be taken into consideration in CD133– gliomas.

P12. EXPRESSION OF CXC CHEMOKINE RECEPTORS AND LIGANDS IN HUMAN MENINGIOMA: ROLE OF SDF1 ACTIVATION OF CXCR4 ACTIVATES ERK1/2 AND STIMULATES MENINGIOMA CELL PROLIFERATION

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The epidermal growth factor receptor (EGFR) regulates important cellular processes and is frequently implicated in cancer. Alterations in this tyrosine kinase receptor influence several mechanisms of malignant tumor progression (e.g., proliferation, apoptosis, angiogenesis, and metastasis) and are common in brain tumors, namely, gliomas. Two single nucleotide polymorphisms (SNPs) were found in the essential promoter region (-216G/T and -191C/A) of EGFR gene. The -216G allele is found in about 10% of the general population, while the -191C allele is present in about 20% of the general population. Furthermore, no associations were detected when the whole genome was stratified by biological types (e.g., astrocytoma and oligodendroglioma) and grade (WHO low grade and high grade).

In conclusion, our findings suggest that –216G allele and -191C allele polymorphisms in the EGFR promoter region do not seem to be biomarkers.
P15. GFAS6 EXPRESSION IN GLIOMAS AND GNAS6 LEVELS IN SERUM AND CSF OF PATIENTS WITH NEOPLASTIC AND VARIOUS NONNEOPLASTIC NEUROLOGICAL DISEASES

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Background: Gas6 is the product of the growth arrest–specific gene 6 and the natural ligand of the Axl tyrosine kinase family. Axl and Gas6 are expressed in a variety of tumors. In a previous set of experiments, we showed that Axl is strongly expressed in gliomas. We therefore hypothesized that Gas6 may also be expressed in gliomas, and Gas6 in CSF or serum could serve as biological tumor marker in primary and/or metastatic CNS tumors.

Methods: Formalin-fixed paraffin-embedded tissue from 80 surgically treated patients, including those with WHO grade II–IV gliomas and epilepsy, was used for immunohistochemistry. Gas6 expression was determined by using an anti-Gas6 polyclonal antibody and analyzed semiquantitatively. Gas6 concentrations in serum and CSF were measured by a sandwich ELISA. Paired samples of serum and CSF from a total of 100 patients with various neurological diseases were studied, including gliomas, extracerebral tumors with and without leptomeningeal metastases, neurodegenerative diseases, multiple sclerosis, and viral/bacterial meningitis. For statistical analysis, the Kruskal-Wallis test was used and visualized by box plots.

Results: Gas6 was strongly expressed in gliomas of various grades of malignancy and various histopathological subtypes. Gas6-positive cells could be identified as tumor cells and endothelial cells of tumor vessels. However, only patients with viral and bacterial meningitis showed elevated Gas6 levels in serum. In CSF, Gas6 concentrations were increased in patients with viral/bacterial meningitis and leptomeningeal metastases, which was not detectable in other neurological diseases. After calculation of the Gas6 CSF–serum ratio in relation to the albumin ratio among the various subgroups, no significant differences were detectable, indicating that elevated CSF Gas6 levels result from leakage of the blood-brain barrier rather than intrathecal synthesis in patients with viral/bacterial meningitis and leptomeningeal metastases.

Conclusions: The immunohistochemical findings may indicate a possible role of Gas6 for tumor cell proliferation and neangiogenesis in gliomas. However, Gas6 is a nonspecific inflammatory marker in serum and has no value as a tumor marker in serum or CSF for brain tumors. This work was supported by grants of the MFF (Medizinische Forschungsfond Tirol) and the Austrian Cancer Society (Krebshilfe Tirol).

P16. FUNCTIONAL INTERACTION BETWEEN TUMOR-SUPPRESSOR P53 AND PROTO-ONCOGENE ETS-1 DETERMINES THE INVASIVE POTENTIAL OF GLIAL TUMORS

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A high invasive potential and resistance to apoptosis is a hallmark of glioblastoma multiforme (GBM), the most aggressive type of intrinsic brain tumor. The proto-oncogenic factor etv1 associated with tumor progression in different types of human cancers is frequently found overexpressed in GBM. Although the impact of ets-1 in glioma invasion has been firmly established, the molecular mechanisms underlying oncogenic activities of ets-1 remain poorly understood. We have found previously that expression of p53 in glioma cells to the two PBR ligands tested strongly protects against ErPC3-induced apoptosis, and that the PBR and hence the MPTP may be involved in this process. This work was supported by a grant from the Volkswagen-Stiftung to W.K. and L.V. (Joint Lower Saxony–Israeli Research Projects).

P17. ERUFOSINE-INDUCED APOPTOSIS IS BLOCKED BY PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR (PBR) LIGANDS IN HUMAN GLIOMA CELLS

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Unlike most chemotherapeutic drugs which target nuclear DNA, alkylphosphocholines (APCs) interact with the cell membrane. Erufosine (ErPC3) is a member of a promising class of APCs for parenteral administration. It has potent antineoplastic activity on various malignant tumors of different origin. The peripheral-type benzodiazepine receptor (PBR), an 18-kDa protein of the outer mitochondrial membrane, is involved in a functional structure designated as the mitochondrial permeability transition pore (MPTP), which plays a critical role during early events of apoptosis. High PBR expression has been associated with tumor expansion and was noted in rapidly growing breast and glioma tumors. Several studies have demonstrated increased binding of PBR ligands in cancer cells, including glioma.

Here we determine the interaction between PBR ligands and ErPC3 effects on apoptosis. The specific PBR ligands, PK11195 and Ro5-4864, were analyzed for their ability to interfere with cell survival in human glioma cell lines expressing PBR. Both specific ligands inhibited viability in a dose-dependent and time-dependent manner. To evaluate the effect of PBR ligands further, we analyzed their influence on proliferation by measuring bromodeoxyuridine (BrdU) incorporation into newly synthesized DNA. We have reported previously that erufosine (ErPC3) and its congener ErPC3 induce apoptosis proceeding through the mitochondrial pathway. Therefore, we studied the effects of coadministration of PK11195 and Ro5-4864 with ErPC3 on apoptotic levels of the glioma cell lines. The PBR ligands, which showed minor pro-apoptotic action themselves, blocked ErPC3-induced apoptosis. To obtain further insight into the ErPC3-induced apoptotic mechanisms affected by PK11195 and Ro5-4864, we measured the release of cytochrome c and the processing of caspase 3, which was blocked in both cases. From these findings, we conclude that exposure of glioma cells to the two PBR ligands tested strongly protects against ErPC3-induced apoptosis, and that the PBR and hence the MPTP may be involved in this process. This work was supported by a grant from the Volkswagen-Stiftung to W.K. and L.V. (Joint Lower Saxony–Israel Research Projects).

P18. PROTEASOME INHIBITORS EFFICIENTLY REACTIVATE TRAIL-INDUCED APOPTOSIS IN MALIGNANT GLIOMA

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Glioblastoma multiforme (GBM) is characterized by potent resistance against antineoplastic treatment. The purpose of this study was to elucidate dysfunctions in apoptotic signaling cascades and to evaluate the efficiency of novel therapeutic approaches to reactivate apoptosis in GBM. Here, we investigated the sensitivity of a panel of human GBM cell lines (established GBM cell lines A172, U87, U251, U343, and U373, and a set of 15 cell lines derived from patients with grade IV astrocytomas) to apoptosis induced by anoixia (<0.1%, 48 h), the death receptor ligand TRAIL (250 ng/ml), and TRAIL in combination with proteosome inhibitors (2.5 μM MG132, 30 μM epoxomicin) or Bcl-2/Bcl-xL inhibitors (30 μM HA14-1, 30 μM BH3-12). In depth analysis of six of the GBM cell lines revealed drastic differences in their sensitivity to these distinct apoptotic stimuli, with two of the six cell lines revealing no significant induction of cell death in response to either anoixia and TRAIL. The combinatory treatments with TRAIL revealed that apoptosis could be efficiently potentiated with the Bcl-2/Bcl-xL inhibitors BH3-12 and HA14-1. Interestingly, our data show that apoptosis could be potentiated in the TRAIL-resistant cell lines with the proteosome inhibitors MG132 and epoxomicin. New analyses employing RNA interference techniques were carried out to identify the molecular signaling pathways leading to enhanced TRAIL sensitivity induced by proteosome inhibitors, with a major focus on the roles of the pro-apoptotic transcription factor GADD153/CHOP and its transcriptional regulation of the TRAIL receptor DR5. These novel therapeutic approaches with TRAIL and agonistic TRAIL receptor antibodies in combination with proteosome inhibitors will be additionally analyzed in an immunocompetent, transplantable mouse model, and might be a promising therapy approach to reactivate apoptosis efficiently in therapy-resistant GBMs in the future.
P19. ALPHA-B-CRYSTALLIN: A NOVEL PROTEIN ASSOCIATED WITH INVASIVE GLIOMA PHENOTYPE

By xenotransplantation of biopsy specimens from human glioblastomas in nude rats, a highly invasive nonangiogenic phenotype was established. Serial animal passages of the tumors led to less invasive, angiogenesis-dependent tumors. By 2-D electrophoresis, we observed differentially expressed proteins between these two phenotypes and used mass spectrometry and bioinformatics to identify their amino acid composition. By MALDI-TOF analysis, several proteins were identified to be overexpressed by the invasive glioma phenotype. One of them was α-B-crystallin, which is a small heat-shock protein of 175 amino acids. It has autokinase activity and inhibits apoptosis. By immunohistochemistry, α-B-crystallin was found to be expressed on the invasive glioma cells in the xenografts. Also in human GBM biopsy specimens, α-B-crystallin was expressed by cells in the invasive tumor rim. In contrast, in the developing rat fetus and the newborn rat, α-B-crystallin was expressed only in the lens and the developing heart.

Immunofluorescence staining showed α-B-crystallin–positive migrating glioma cells in vitro. Immunofluorescence showed α-B-crystallin to be strongly expressed in the lamellipodia. Double staining for α-B-crystallin and β-catenin showed that, in the migrating glioma cells, both proteins were expressed in the leading edge of the lamellipodia. To determine the role of α-B-crystallin in glioma cell migration in vitro, we downregulated α-B-crystallin by siRNA. Three days after transfection with α-B-crystallin siRNA, a strong reduction in cell migration was observed that lasted for several days. In the periphery of the α-B-crystallin synthesis was inhibited by siRNA, no colocalization of α-B-crystallin and β-catenin was seen.

We conclude that α-B-crystallin is associated with a highly invasive glioblastoma phenotype, and inhibition of α-B-crystallin synthesis reduces the malignant cell migration in vitro.

P20. INHIBITION OF CEREBRAL ENDOTHELIAL CELL PROLIFERATION AND MORTALITY BY RAF-1 SMALL INHIBITORY RNA
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Objective: Malignant gliomas are highly vascularized tumors. Therefore, antangiogenic therapies emerge as a promising approach to improve the prognosis in these patients. The Ras-Raf signaling is activated by various growth factors and may enhance tumor and endothelial cell growth and motility. Aim of our study is to evaluate the effects of small inhibitory RNA (siRNA) directed against Raf-1 on glioma and endothelial cell viability, proliferation, and motility.

Methods: Three different Raf-1 siRNA sequences and nonfunctional control siRNA were obtained from Dharmacon and Invitrogen. Human U173 glioma cells as well as human cerebral microvascular endothelial cells (HCMECs) were transfected with Raf-1 siRNA by using a modified lipofectamine protocol. RT-PCR and Western blotting were used to control siRNA-mediated Raf-1 knockdown on mRNA and protein level, respectively. Functional effects on viability and proliferation were evaluated in both cell types by the MTT assay and cell counting. The effects on cell-specific motility were investigated by a spheroid-based migration assay (U173) and a tube formation assay on Matrigel (HCMECs). For all assays, pure lipofectamine and nonfunctional siRNA served as controls.

Results: Half-quantitative RT-PCR and Western blotting revealed pronounced siRNA efficacy in U173 and HCMECs, with a significant reduction of Raf-1 transcript and protein in both cell types. In U173 glioma cells, Raf-1 downregulation did not affect proliferation or glioma cell migration at various siRNA concentrations. In HCMECs, however, a highly significant decrease of cell proliferation and a significant inhibition of tube-forming ability was achieved by Raf-1 siRNA as compared to nonfunctional siRNA or vehicle controls.

Conclusions: Inhibition of the Ras-Raf pathway by Raf-1 siRNA caused a significant reduction of endothelial cell growth and motility. If this can be confirmed in transformed endothelial cells derived from malignant gliomas, this strategy appears suitable for future antangiogenic approaches in the treatment of malignant gliomas.

P21. SCHEDULE-DEPENDENT EFFECTS WHEN COMBINING GEFTINIB WITH IRRADIATION OF GLIOMA AND ENDOTHELIAL CELLS IN VITRO
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Alterations in epidermal growth factor receptor (EGFR) signaling contribute to clinical radiation resistance of glioblastoma multiforme (GBM). Therefore, inhibition of EGFR signaling pathways by the selective EGFR tyrosine kinase inhibitor, gefitinib (ZD1839, Iressa), may increase the therapeutic effects of radiotherapy. The effects of different schedules for administration of gefitinib on sensitivity to irradiation of the human glioma cell lines (251MG and SF-767), a rat glioma cell line (BT4C), and an immortalized rat brain endothelial cell line (RBE4) are reported. Differences in effects of the combined treatment on cell toxicity were determined by a fluorometric cytotoxicity assay, and nuclear DNA fragmentation was used for quantification of apoptosis. Preadministration with gefitinib for 30 min prior to irradiation followed by continuous incubation with gefitinib significantly increased the cytotoxicity of SF-767, BT4C, and RBE4 cells. However, the human glioma cell line 251MG was protected against radiation-induced damage by this treatment schedule, at lower concentrations of gefitinib. Preadministration with gefitinib for 24 h prior to irradiation without follow-up incubation with gefitinib increased the cytotoxicity of SF-767 and BT4C cells. Postirradiation treatment with gefitinib significantly increased the cytotoxicity in all cell lines except for 251MG. We demonstrated heterogeneity in the cytotoxic effects of gefitinib between cell lines. Response to gefitinib might be due to other mechanisms than through the EGF receptor, as some of the cell lines showed sensitivity to gefitinib despite no low expression of EGFR. This study also demonstrates the importance of timing of gefitinib administration when this agent is combined with irradiation.

P22. GALECTIN-1: EXPRESSION AND EFFECT ON PROLIFERATION AND MIGRATION OF GLIOMA CELLS
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Galectins are evolutionarily highly conserved, glycoprotein-recognizing lectins involved in numerous physiological and pathological processes. Galectin-1 has been associated with invasion and migration in malignant gliomas. In the present study, we examined the expression of galectin-1 in glioma cell lines and its influence on proliferation and migration. We detected galectin-1 in all 12 tested cell lines by Western blot and FACS. Levels of galectin-1 were higher in the tumor cells than in normal brain and higher in cell lines with wild-type as compared with mutated p53 status. Irradiation induced galectin-1 expression. Recombinant galectin-1 moderately enhanced proliferation in U188 cells and migration in A172 and U118 glioma cells. Downregulation with RNA interference resulted in reduced proliferation in A172 cells and migratory capacity in both glioma cell lines tested. Our data with intracellular downregulation confirm previous studies on extracellular application that point toward an impact of galectin-1 on the migration of glioma cells. Therefore, galectin-1 may be an interesting target to modulate migration and invasion in human gliomas.

P23. ISOLATION, CHARACTERIZATION, AND IN VIVO APPLICATION OF PUTATIVE GBM-DERIVED TUMOR STEM CELLS
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Objective: Despite recent advances in diagnosis and treatment, high-grade gliomas pose one of the major challenges in cancer research. Recently, evidence has mounted suggesting that the clinical properties of gliomas are largely determined by a small subpopulation of cancer cells that can continuously self-renew and regenerate the tumor and therefore might have escaped standard therapeutic strategies so far. In the present study, we sought to establish primary cultures of putative glioblastoma stem cells and to study their properties in a preclinical model.

Methods: Putative tumor stem cells were isolated from human glioblastoma tissues by enzymatic digestion and grown as spheres in stem cell–optimized culture media. Tumor cells were immunohistochemically characterized for the expression of different markers, including the stem cell markers nestin and CD133. DNA was isolated from single spheres and subjected to matrix-CGH analysis. To test their tumorigenic potential, cells were implanted stereotactically in NOD/SCID mice.
Results: Primary cultures of putative stem cells derived from human glioblastomas formed spheres and could be kept in continuous culture. Spheres were analyzed for surface marker expression and found to be positive for nestin and CD133. Matrix-CGH revealed aberrations frequently found in glioblastoma, such as PDGFRα amplification and loss of 10q, demonstrating that the spheres were derived from tumor cells. Tumor-gene expression of our primary cultures was demonstrated by xenograft experiments in NOD-SCID mice, where tumors presented with a highly invasive phenotype.

Conclusions: These data not only support the present tumor stem cell concept but also provide a suitable tool to develop new strategies for the treatment of high-grade gliomas. The thorough characterization of tumor stem cells will greatly improve our understanding of tumor pathomechanisms and provide keys for major improvements in cancer therapy.

P24. RELEVANCE OF COMBINATORIAL PROFILES OF TRANSCRIPTION FACTORS FOR GLIOMA HISTOGENESIS

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To define specific markers for histogenesis of three well-characterized subgroups of human gliomas (pilocytic astrocytomas, glioblastomas, and oligodendrogliomas), we used immunohistochemistry and in situ hybridization to study the expression of relevant markers that characterize glioma genesis. They include the intermediate filament proteins GFAP, vimentin, and nestin; the transcription factors Olig2, Nkx2.2, and Sox10; and the proteolipid protein transcripts pIPl2dm20. We show that the three major categories of human gliomas express a combinatorial profile of markers that gives new insights to their histogenesis and may help diagnosis. Pilocytic astrocytomas strongly express GFAP, vimentin, Olig2, Nkx2.2, and Sox10, but not nestin. In contrast, glioblastomas strongly express GFAP, vimentin, and nestin, but these tumors are heterogeneous regarding the expression of the transcription factors studied. Finally, in oligodendrogliomas, intermediate filament proteins are generally not expressed, whereas Olig2 was found in almost all tumor cells nuclei while only a subpopulation of tumor cells expressed Nkx2.2 and Sox10.

P25. ABERRANT EXPRESSION OF REGENERATION AND TOLERANCE FACTOR (RTF) CONTRIBUTES TO GliOBlastoma-ASSOCIATED IMMUNOSUPPRESSION


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RTF was originally identified in placenta, where it is thought to be essential for fetal allograft survival. Soluble RTF upregulates the production of interleukin 10 and interferes with interleukin 2 signaling in stimulated peripheral blood mononuclear cells. Since glioblastoma is a paradoxical tumor for tumor-dependent immunosuppression, we assessed a possible role for RTF in glioblastoma-associated immunosuppression. We here report that RTF mRNA and protein are expressed in human glioma cells in vitro. In vivo, RTF expression is hardly detectable in normal human brain specimens, but strongly upregulated in glioblastoma tissue samples. Suppression of RTF expression in the human glioma cell line LNT-229 by RNA interference promotes the lysis of these cells by NK and T cells in vitro. Moreover, RTF-depleted glioma cells are less tumorigenic than are control cells in nude mice in vivo. Depletion of NK cells nullifies this difference. RTF is thus a novel aberrantly expressed molecule that may confer immune privilege to human malignant gliomas.

P26. IDENTIFICATION AND CHARACTERIZATION OF GALECTIN-1 EXPRESSION IN GLIOBLASTOMA INVASION


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Despite the many therapeutic strategies used in the treatment of malignant gliomas, the prognosis for patients afflicted with this cerebral malignancy remains poor, in large part due to the highly infiltrative nature of these tumor cells. Our group has been focused on identifying and evaluating genes that are uniquely expressed by tumor cells at the invasive margin, because such genes may be novel/keys mediators of invasiveness and hence may constitute potential novel therapeutic targets aimed at arresting tumor motility. In pursuit of this postulate, we have taken advantage of a unique xenograft mouse model developed by our group (CD1) in which the intra-cranially implanted human glioblastoma cells (derived from operative specimens) maintain their invasiveness. RNA was isolated from tumor core and periphery via laser-capture microscopy, followed by microarray analysis for differential gene expression. To that end, we have identified galectin-1 (Gal-1) as being preferentially overexpressed at the invasive margin. Immunohistochemical analysis of xenograft tumors demonstrated preferential labeling at the tumor periphery and other areas of invasion, corroborating the microarray data that Gal-1 expression is increased at tumor periphery. A relatively noninvasive glioma cell line, U87MG, was then stably transfected for Gal-1 overexpression to determine if Gal-1 overexpression may transform this otherwise relatively noninvasive cell line to invade—indeed, Gal-1 transfectants demonstrated increased invasion in vitro. Given the encouraging in vitro experiments that demonstrate increased invasion with Gal-1 overexpression, we have proceeded to in vivo experiments in which immunocompromised mice are intracranially injected with nontransfected versus Gal-1-transfected U87 glioma cells. In preliminary experiments, animals implanted with Gal-1 transfecants had significantly worse overall survival than those implanted with control U87 cells. Experiments are currently under way to determine if the Gal-1–overexpressing transfectants demonstrate invasion of adjacent brain parenchyma. Taken together, our data support a role for Gal-1 in the invasive phenotype of malignant glioma.

P27. LG PROTEIN EXPRESSION IN OLIGODENDROGLIOMA

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Oligodendrogliomas are diffusively infiltrating tumors of the central nervous system. Allelic loss of chromosome 1p is observed in the majority of cases and serves as a predictor for chemotherapeutic response and long survival. However, prognosis and prediction of oligodendroglomas are suboptimal and new molecular markers are needed. The LG proteins encode integral membrane proteins that have been suggested to inhibit tumor growth by antagonizing growth factor signaling. LGRI1 – 3 mRNA and protein are expressed in a variety of human tissues, including the brain. LGRI2 is located at chromosome 1p33 and is, thus, anticipated to be subject to LOH and reduction of gene copy number in the majority of oligodendroglomas. In this study, we used immunohistochemistry to analyze the expression of LGRI2 in normal brain, and of LGRI1 – 3 in 65 oligodendrogliomas collected in a tissue microarray. We also sequenced LGRI2 cDNA from six oligodendroglomas. LGRI2 was expressed by oligodendrocytes in the normal brain, and the expression of LGRI1 – 3 proteins correlated to various clinical parameters in oligodendroglial tumors. No LGRI2 somatic mutations were found in the oligodendroglial cDNA. Importantly, LGRI2 was an independent prognostic factor for oligodendrogloma patients. This shows that LGRI2 may have a physiological function in normal oligodendrocytes and a role in the development of oligodendrogliomas with impact on patient survival.

P28. MGMT FINGERPRINTING IN GLIAL TUMORS

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Epigenetic methylation of the O6-methylguanine DNA methyltransferase (MGMT) DNA repair gene promoter in tumor tissue from glioblastoma multiforme patients is associated with improved survival after treatment with radiotherapy plus concomitant and adjuvant temozolomide. Glial...
tumors are pathologically heterogeneous and display tumor mosaicism associated with numerous tumor markers and proteins in different subpopulations of cells. We hypothesized that MGMT promoter methylation mosaicism would be characteristic of glial tumors and potentially could result in problems with tumor sampling and patient response to temozolomide. To assess MGMT promoter methylation mosaicism of a series of glial tumors, we sampled multiple regions of each tumor intraoperatively using neuronavigation techniques to localize the regions assessed along with peritumoral and blood samples. To prevent cross contamination, completely separate instruments were used for each biopsied region. Tissue specimens were immediately used for DNA, RNA, and protein extractions and methylation-specific PCR, RT-PCR, and MGMT activity assays. Part of each sample was embedded in paraffin, and immunohistochemistry studies used the specific MT 3.1 antibody for human MGMT. Our results confirm that individual tumors have a spectrum of the M/G promoter methylation fingerprint that can vary substantially from other tumors carrying the same pathological diagnosis. Low-grade oligodendrogial and astrocytic tumors tend to have intratumoral methylated–unmethylated ratios that are close to 1, but some areas may have substantial differences in this ratio. Malignant glial tumors and glioblastoma multiforme have widely varying MGMT fingerprints when specific areas of their tumors are biopsied and contain methylated–unmethylated ratios ranging from 0 to very high values. Cell lines derived from these tumors demonstrate different MGMT fingerprints from the original tumor. These results suggest that an assessment of glial tumor MGMT methylation fingerprinting using multiple intraoperative tissue sampling may provide a more accurate assessment of that individual tumor’s response to radiation and alkylating agents such as temozolomide.

P30. INVOLVEMENT OF NFKB IN THE REGULATION OF MGMT GENE TRANSCRIPTION: NOVEL MECHANISM THROUGH WHICH NFKB MEDIATES DNA DAMAGE REPAIR

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Introduction: Drug resistance to anticancer agents is an important mechanism related to treatment failure. Alkylating agents cause induction of NFKB. It was related to its role in protecting cells from apoptosis-induced death. Thus, the involvement of NFKB in alkylating-based DNA damage may play a crucial role in the development of drug resistance. MGMT is the only known gene that is directly involved in reversal repair of DNA affected by O6-methylguanine. Therefore, a tumor’s resistance to alkylating agents often correlates with MGMT expression level. MGMT induction following exposure to a variety of DNA damaging treatments is regulated at the transcriptional level. The potential function of the transcription-factor recognition sequences GRE (glucocorticoid-responsive elements) and AP-1 within the MGMT promoter region was previously investigated. Using the Genomax software, we have found two unpublished putative NFKB sites within the MGMT promoter. It may suggest that NFKB may induce drug resistance by an additional, yet unknown, mechanism.

Objective: To explore whether NFKB plays a role in MGMT gene regulation.

Methods and results: Using the EMSA (electrophoretic mobility shift assay) technique, we have demonstrated a specific and distinct palindrome between the two NFKB sites within the MGMT promoter and the NFKB subunit p65. Moreover, by real-time RT PCR, we have demonstrated a 15- and 270-fold induction of MGMT RNA expression after 24 h and 48 h, respectively, following transfection of CVM-p65 to HEK293. The addition of NFKB super-repressor ΔNFKB abrogated this expression completely. We also demonstrated a significant correlation between the extent of constitutive NFKB activation and MGMT expression level in both glioblastoma cell lines and solid tumors. These findings are of potential clinical significance because we showed that these cell lines with high constitutive expression of NFKB are less sensitive to BCNU treatment. Treatment with the proteasome inhibitor MG-132 that reduces NFKB activation sensitized these cells to BCNU treatment.

Discussion: Our findings strongly suggest that NFKB plays a major role in MGMT regulation. Together, these observations shed light on a novel role of NFKB in the regulation of DNA-damage mechanisms and its involve- ment in chemoresistance. Treatments with inhibitors of NFKB activation might offer a new approach to overcome resistance to alkylating agents because it will inhibit MGMT expression, as well.

P31. THE RECEPTOR TYROSINE KINASE AXL IS WIDELY EXPRESSED IN GLIOMAS OF VARIOUS HISTOLOGY

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Background: The receptor tyrosine kinase family Axl, Sky, and Mer and their ligand Gas6 function as neurotrophic and migratory factors in the developing and adult central nervous system. Previous studies have identified Axl as also playing an important role in tumorigenesis—including tumor cell survival, migration, and angiogenesis—in a variety of cancers. However, its role in primary brain tumors is unknown.

Methods: A tissue microarray (TMA) was constructed from 118 gliomas of different WHO grades, including both newly diagnosed and recurrent tumors (84 grade IV glioblastomas, 6 grade II astrocytomas, 6 grade III anaplastic astrocytomas, 7 grade II oligoastrocytomas, 3 grade III anaplastic oligoastrocytomas, 3 grade II oligodendrogliomas, 4 grade III anaplastic oligodendrogliomas, and 3 grade II ependymomas). Sections of the TMA blocks were stained with a monoclonal mouse anti-Axl antibody following standardized immunohistochemical protocols. Brain tissue from epilepsy surgery was used as a nontumor control. The stainings were evaluated by a neuropathologist using a semiquantitative method.

Results: Axl staining was present in 94 of the 118 (80%) gliomas, including various histological grades ranging from WHO II to WHO IV. In contrast, Axl expression was absent in brain tissue from epilepsy surgery. The most frequent expression pattern included cytoplasmic and membrane-associated staining of tumor cells and endothelial cells of tumor vessels. A characteristic finding was the pronounced staining of perinecrotic areas. In addition, reactive astrocytes at the border zone of the tumor showed intensive cytoplasmatic staining.

Conclusions: Our findings suggest Axl is highly expressed in gliomas of various histology and malignancy grades, especially in pseudopali- sades of GBMs. The staining pattern indicates a possible role for glioma cell migration and invasiveness. In addition, the presence of Axl in tumor vessels may suggest a role in neovascularization. This work was...
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P32. ABBRENT PROMOTER METHYLLATION OF MULTIPLE GENES IN SCHWANNOMAS
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To determine the DNA methylation profile of schwannomas, we examined the methylation status of 16 tumor-related genes (RASSF1A, RARB, VHL, PTEN, bMLH1, RBL1, p16INK4a, CASP8, ER, TIMP3, MGMT, DAPK, p73, GSTP1, p14ARF, and THBS1) in 80 sporadic and/or hereditary schwannomas. Our findings indicate that abrrent methylation of certain tumor-related genes may represent secondary events that contribute to the development of schwannomas, and that epigenetic inactivation is not restricted to the NF2 gene (primay event) in these neoplasms. Financial support: PI 03/0235 and PI 03/0829.

P33. STAT-1 IMMUNOHISTOCHEMISTRY IN HUMAN GLIOBLASTOMA
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Glioblastoma is a very aggressive brain tumor with poor prognosis despite radical surgery or radiotherapy. STATs (signal transducers and activators of transcription) proteins are important elements in intracellular signaling pathways; namely, the JAK-STAT pathway. They are activated by several biochemical substances such as kinases or interleukin, which leads to activation or inhibition of cellular transcription.

In our recent immunohistochemically performed study of 46 glioblastomas, 22 cases (48%) showed strong positivity, nine (20%) had intermediate reactivity, eight (17%) showed low immunoreactivity, and seven (15%) showed complete negativity. Neoplastic tissue revealed different STAT-1 expression mostly localized in the cytoplasm, with a most suggestive reaction also in tumor giant cells. The peritumoral brain tissue partly showed strong positive reactive cells.

A very interesting observation in our study was the finding of a strong STAT-1 expression in the reactive astrocytes, the glial and especially the microglial components within the infiltration area.

In general, our findings indicate the presence of a mostly nonphosphorylated form of STAT-1 protein expression leading to a possible role in chemotherapeutic response. Therefore, further studies are necessary to elucidate the importance of STAT-1 expression in glioblastoma.

P34. EXPRESSION OF THE STEM CELL MARKERS HOF AND NESTIN IN GlioBLASTOMA
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Introduction: Glioblastoma is among the most malignant cancers. Similar to other types of cancer, glioblastoma seems to be maintained by a subpopulation of tumor stem cells. In this study, the expression of the stem cell markers HOF and nestin were investigated by double immunohistochemical staining of glioblastomas. The expression was compared to EGFR, P53, MDM2, and RB immunohistochemical stainings, which to some extent show a characteristic distribution between primary and secondary glioblastoma.

Methods: All tumors used were graded according to WHO criteria. Glioblastomas were obtained as fixed paraffin-embedded samples at Odense University Hospital from 1999 to 2002. Representative tumor areas were selected for preparation of three multiblocks containing tissue sections from 27 patients. Paraffin sections were stained by double immunohistochemical staining for HOF and nestin. The percentage of tumor cells coexpressing HOF and nestin and expressing HOF and nestin only were quantified by using unbiased stereology. EGFR, P53, MDM2, and RB immunohistochemical stainings were scored, and correlation analysis with the expression of HOF and nestin was performed.

Results: The results showed that, on average, 48% of the cells counted coexpressed HOF and nestin and that 31% expressed HOF and 12% nestin only. The expression pattern was variable, and the expression of HOF was negatively correlated to the coexpression of HOF and nestin and the expression of nestin alone. The expression of HOF correlated with the P53 score, whereas the expression of nestin correlated with the MDM2 score. Both the percentage of cells coexpressing HOF and nestin as well as those expressing HOF only correlated with the RB score, whereas the percentage of cells expressing HOF only showed a negative correlation with the RB score.

Discussion: The results suggest that glioblastoma contains three populations of tumor stem cells according to the expression profiles of HOF and nestin. The differences in expression profile may imply differences in the reaction to radiation and chemotherapy. The expression profile of stem cell markers does not appear to have a different distribution in primary and secondary glioblastoma, but the results suggest that the P53, MDM2, and RB scores may be related to subtypes of tumor stem cells. Future studies including other stem cell markers and clinical data are needed to elucidate the value of stem cell markers as prognostic and predictive factors.

P35. TELOMERASE ACTIVITY AND hTERT mRNA EXPRESSION IN GLIOMAS
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Human glioblastoma is a structurally complex ribonucleoprotein that is responsible for the maintenance of telomeric DNA at the ends of the chromosomes. The enzyme is proposed to have an important role in cell immortalization and oncogenesis. A limited number of studies have been performed on the telomerase system in brain tumors, and these studies are somewhat conflicting. The relative ineffectiveness of current therapies for malignant gliomas led to the need for novel targets for more promising approaches. In this study, we quantified the telomerase activity of 42 gliomas (32 multiform glioblastomas, four anaplastic astrocytomas, four differentiated astrocytomas, one oligoastrocytoma, and one oligosarcoma) by using the polymerase chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP) assay. We also compared these results with the expression of the messenger coding the telomerase catalytic subunit (hTERT mRNA). The goal of this work was to expand the present knowledge of telomerase activity in brain tumors to assess clearly whether telomerase may be a novel prognostic marker for brain tumors with a high grade of malignancy, such as glioblastomas.

High telomerase activity was detected in 21 (50%) of 42 gliomas. The levels of telomerase in terms of its messenger level expression overlapped the activity; in fact, a significant association between telomerase activity and hTERT mRNA expression was found (r², r < 0.001).

In our cases, clinical follow-up was available for all patients. At univariate analysis, advanced age as well as high telomerase activity and hTERT mRNA levels were seen to be significant predictors of a worse prognosis (both overall survival (P = 0.007) and disease-free interval (P = 0.008), respectively) and disease-free interval (P = 0.0008).

In conclusion, the overall picture emerging from our study is that telomerase activity may influence tumor prognosis in gliomas. Our results, with further elucidation on telomerase involvement in the mechanisms of tumor angiogenesis, apoptotic pathway and telomere elongation, may encourage research on novel therapeutic strategies for the treatment of patients with malignant gliomas.

P36. PARACRINE ROLE OF GLIOMA-SECRETED TENASCIN-C IN TUMOR-INDUCED ANGIOGENESIS
C. Dichter, G. Vassarini, C.A.W. Unterberg, and C. Herold-Mende; 1Department of Neuroursurgery, University Hospital, Heidelberg, Germany, and 2ENT Department, University Hospital, Heidelberg, Germany

Objective: Tenascin-C, an extracellular matrix protein, is predominantly expressed in high-grade gliomas but can rarely be found in normal brain. Perivascular expression increases from low-grade to high-grade gliomas and is significantly correlated with a poor outcome. In general, its expression does not appear to have a different distribution in primary and secondary glioblastoma, but the results suggest that the P53, MDM2, and RB scores may be related to subtypes of tumor stem cells. Future studies including other stem cell markers and clinical data are needed to elucidate the value of stem cell markers as prognostic and predictive factors.

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Our data indicate that GBM-secreted tenascin-C plays an important role in the functional organization of tumor-derived vasculature because of its significant influence on migration and tube-formation capacity of endothelial cells. Thus future antiangiogenic therapy of high-grade gliomas might also address the pro-angiogenic function of tenascin-C, for example, by intravenous or intralesional application of neutralizing antibodies.

### Patients and methods

To date, data on 1726 patients were collected, including 1294 with primary brain tumors and 432 with brain metastases. We also collected data on 159 patients (9.2%) with neurological complications of oncological treatment, 157 patients with a suspicion of genetic syndromes (9.1%), and 6 patients with neurological paraneoplastic syndromes (0.3%).

In 261 patients represented 432 cases (25%) of the study population, including 177 (41%) lung cancer, 78 (18%) breast cancer, and 36 (8.3%) primary unknown cancer. Age (median and range) and survival (median and 95% CI) were respectively estimated at about 2–19,100,000 and 1–12,100,000. We collected data prospectively on patients with brain tumors who were attending our multidisciplinary group. Demographic parameters, histological diagnosis, and surgical treatment were recorded.

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Our results showed that the Italian version of the CRA had a five-factor structure like the U.S. version, considering the double criteria of engine-valve higher than 1 and variance explained higher than 5%. The Cronbach coefficient alpha showed values ranging between different subscales. The different subscales showed significant correlations, as foreseen by our hypotheses. No incoherent correlations were found showing a divergent validity. In conclusion, our results showed the Italian version of the CRA to be a robust, reliable psychometric instrument. Further, it was showed its feasibility for use in a brain tumor patients.

P41. FTA TECHNOLOGY FOR A RAPID IDENTIFICATION OF 1P/19Q DELETIONS BY FLUORESCENT MICROSATellite ANALYSIS IN Gliomas: ADVANTAGES FOR ROUTINE DIAGNOSIS AND THERAPEUTIC DECISION
D. Fontaine, S. Montor, F. Vanderbrouck, P. Paquis, J. Michiels, S. Bannwarth, V. Paquis-Flucklinger, and the Nice Brain Tumor Study Group; CHU de Nice, Nice, France

A loss of heterozygosity on 1p and 19q chromosomes is frequently found in oligodendrogliomas. These genetic markers turned out to be associated with a favorable response to chemotherapy and better survival. Although deletion of 1p and 19q losses is useful in the diagnosis and treatment of gliomas in patients, genetic testing for 1p/19q is not performed routinely in most institutes. Different techniques can be used, but results must be obtained rapidly after stereotactic biopsy or neurosurgical resection in order to play a role in the therapeutic decision.

We have adapted FTA technology to simplify DNA extraction from both leukocytes and fresh or frozen tumors. This strategy involves four successive steps: (1) DNA samples are applied to the FTA card, cells lyse, and DNA becomes entangled in the paper; (2) a small disc is removed from the card and washed to remove proteins; (3) the disc is placed directly in the PCR mix and amplified in situ; and (4) loss of heterozygosity by microsatellite analysis is determined on an automated sequencer.

Results can be obtained in 24 h. Furthermore, this method requires few materials and is really suitable for stereotactic biopsies. The analysis of a series of 55 gliomas demonstrates the feasibility and the simplicity of the technique. The same results were obtained when DNA was extracted by FTA technology or by using classic phenol chloroform extraction. We show that this method can be used as well with paraffin-embedded gliomas in order to improve molecular analysis by selecting more representative tumoral regions or to perform retrospective studies.

In conclusion, we demonstrate that our approach of using the FTA technique provides a fast, sensitive, and specific assay for PCR-based microsatellite analysis using blood and tumorous tissues. Thus, this technique can be routinely used for detection of 1p/19q deletions that have prognostic and therapeutic significance in glial tumors.

P42. INTRATUMORAL GENETIC HETEROGENEITY OF 4Q12 AMPLIFICATION IN Glioblastoma CELLS
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The aim of this study was to characterize intratumoral genetic heterogeneity of human astrocytic brain tumors. A special focus was placed on chromosomal region 4q12, which harbors the gene loci for PDGFRα and c-kit. Overexpression of these receptors is common in glioblastomas, and the PDGFRα gene has been shown to be amplified in a glioblastoma sub-group. Importantly, both receptors are known targets of Glivec. Microdissected regions of glioblastomas (n = 37) and primary tumor cell cultures (n = 14) from 29 patients were analyzed by comparative genomic hybridization (CGH). From one to six regions per tumor were compared with each other and with the corresponding cell culture. FISH analysis of metaphase chromosomes was performed using a chromosome 4 paint and a BAC clone against the PDGFRα locus. Sensitivity of primary cell cultures to Glivec was assessed by cytotoxicity testing, and protein levels were detected by Western blot.

CGH detected 4q12 gains in 12 glioblastoma sections (32.4%), reaching the level of amplification in five cases (13.5%). The presence and intensity of the 4q12 amplicon within individual tumors varied strongly. The distribution of the 4q12 amplicon was widely independent of histomorphology. The intensity of the 4q12 amplicon within individual tumors varied strongly. The distribution of the 4q12 amplicon was widely independent of histomorphology. The intensity of the 4q12 amplicon within individual tumors varied strongly. The distribution of the 4q12 amplicon was widely independent of histomorphology.

The extent of surgery is limited by anatomical and functional factors in gliomas. Glioma patients constitute an important subgroup of our patients with a dismal prognosis.

P43. CLINICAL BIOPSY-ALONE COURSE IN HIGH-GRADE GliOMA: IMPLICATIONS FOR FUTURE TREATMENT STUDIES
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Background: The extent of surgery is limited by anatomical and functional factors in gliomas. Glioma patients constitute an important subgroup of our patients with a dismal prognosis.

Materials and methods: We revise retrospectively the clinical course of patients with high-grade glioma (HGG) with a biopsy-alone surgical approach diagnosed from June 1997 to December 2005.

Results: Only 9% (3/315) of patients who had suspicious imaging of HGG did not have surgical diagnosis of the lesion. After surgery, 29% of patients showed no histological glioma. In 92 (31%) of them, only a biopsy was performed. Seventy-four patients (80.4%) had HGG. Reasons for “biopsy alone” were the following: motor area, 16.4%; multicentricity 27.4%; language area, 24.7%; basal ganglia location, 8.2%; corpus callosum invasion, 17.8%; and visual area, 5.5%. Forty-seven patients had GBM, which represented 26% of all GBMs seen in our hospital in this period. Average age was 60.7 years; 40 patients (85.1%) were over age 50. Only 35.1% of these patients had a KPS under 70. Twenty patients were considered by RPA on group VI. Oncological treatment was initiated in 76.6% of them: chemotherapy (CHT) in 74.5% (in 31.5% of them as a pre-radiotherapy [RXT] treatment) and RXT in 64.3%. The median survival was only 23.1 weeks. Seventy-two patients had grade III tumors (AA, OD, OA) and constituted 49% of all patients with grade III tumors treated in our hospital. Average age was 58.3; KPS was under 70 in only 33.5% of patients. Oncological treatment was initiated in 81.3%: CHT in 74% and RXT in 75%. Median survival was 77.7 weeks. Reasons for no treatment were the following: low KPS, 3 patients; postoperative death, 4 patients; family decision, 3 patients; not sent for treatment, 5 patients; and over 80 years of age, 1 patient. Median survival was longer in patients with grade III tumors (P = 0.0000). Patients lived longer if treated in the two grades (P = 0.0000). Patients with GBM lived longer if chemotherapy based on temozolomide was administered even as adjuvant or pre-RXT. Radiotherapy improved survival in all cases.

Conclusions: Of all glioma patients treated in our hospital, 31% can also be considered a biopsy alone. Of GBM patients, only 26% have a biopsy and get the benefit of standard treatment (Gliadel wafers of temozolomide and RXT). Median survival for these patients is 23.1 weeks despite aggressive CHT and RXT. New treatments must be investigated in this group because more than 50% maintain a KPS over 70%, and measurable disease is present to evaluate new therapies.

P44. THE EFFECT OF CATECHOL-O-METHYLTRANSFERASE VAL105/185MET AND METHYLENETETRAHYDROFOLATE REDUCTASE C677T POLYMORPHISMS IN BRAIN TUMORS
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Cancer is a disease that begins with mutation of critical regulatory genes oncogenes and tumor suppressor genes. The etiology of most human cancers remains unknown, but we think that the major carcinogenic risk to humans for malignant transformation is represented by endogenous carcinogens such as catechol estrogens. The neutralization of the genotoxic effects of these metabolites is achieved by catechol-O-methyltransferase (COMT). The 2-hydroxylated catechol estrogen is metabolized by COMT into nongenotoxic methyl esters that are excreted from the body. Suggested mechanisms have focused on the key role of folate as the donor of one-carbon groups for nucleotide synthesis and methylation reactions. These two pathways of folate metabolism are separated by an irreversible reaction regulated by the enzyme methylenetetrahydrofolate reductase (MTHFR).
In this study, we investigated the effects of polymorphisms that led to the defects in the COMT enzyme that neutralizes genotoxic metabolites and the MTHFR enzyme, which is important in DNA repair in the etiology of brain tumors. Genomic DNA was isolated from the cultures of nine patients (four women and five men) that were diagnosed with brain tumors and 30 members of a healthy control group (age, 45–65 years). COMT and MTHFR were genotyped by using the relevant kits according to the manufacturer’s protocols (Promega). The compound heterozygosity analyses of COMT and MTHFR showed a significant difference with regard to the genotype frequencies of the patient group and the control group (χ² = 49.49, P < 0.05). The compound haplotype analysis revealed that the allele frequencies of the brain tumors were CG, 44.44%; TG, 5.56%; CA, 22.22%; and TA, 27.78%; and, in the control group, CG, 55.00%; TG, 43.33%; and CA, 1.67%. The compound haplotype analysis indicated a significant difference in haplotype frequencies between the patients with brain tumors and the control group (χ² = 71.00, P < 0.05). In brain tumor cases, increases of mutant alleles that decrease enzyme activity were found.

There is no reported literature about the association of COMT and MTHFR gene polymorphism analyses. A larger number of studies are needed to confirm our findings and unravel the underlying mechanisms, but we believe that the uniqueness of this study will ignite a new era in understanding the molecular genetics of brain tumors.

P45. NONRANDOM SELECTION OF GENETIC CO-EVENTS IN THE HUMAN GLIOMA GENOME

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The integration of genetic data with gene-expression data provides a promising avenue for cancer gene discovery. It particularly enables the prioritization of seemingly random gene copy number alterations. A tumors by assessing their effect on the transcriptomic level. Glomiogenesis is a complex process involving the accumulation of multiple supposedly independent genetic alterations leading to deregulation of signaling networks central to the control of cell growth and cell fate. We have previously reported nonrandom patterns of co-occurrence of distinct chromosomal aberrations in human gliomas. Based on reverse network engineering and in silico interaction mapping, we here show that such genetic coincidence facilitates coordinated patterns of pathway deregulation that promotes gliomiogenesis in a synergetic fashion. As a first-pass means, we have implemented a stringent model for interfacing genome-wide gene dosage and gene-expression maps in the human glioma genome. This model reveals the TOPORS candidate tumor suppressor, which we have previously mapped to a minimal common region (MCR) on chromosome 9p21.1 in glioblastomas, among genes with the greatest gene dosage–expression relationship. By means of linking this model to a reverse network engineering algorithm based on present interactome knowledge, we have limited the multitude of possible hypotheses to those that are biologically plausible. Such modeling has identified compelling target genes that are functionally interrelated but reside in MCR territories of independent, coincident chromosomal aberration (e.g., coincidences of +EGFR [7p11.2] and –ANXA7 [10q22.2] or of –BIF1 [1p22.3] and –BAX [1p13.33]). We have extended the gene–gene product relationship to the protein level for several targets, which we believe could drive the concurrence of distinct chromosomal alterations in certain glioma subtypes. In conclusion, our data lend support to the notion that the nonrandom concurrence of territorially autonomous genetic events in human gliomas may be selected for during their formation to enable cooperative effects of distinct target genes in a higher gated logic circuit, topologically and functionally organized in a global composite network of glioma progression. Our model for the first time provides mechanistic and functional explanation for the recurrent coincidence of distinct chromosomal alterations (e.g., +7f–10; +1p1–19g) in human gliomas.

P46. DETERMINATION OF EGFR, HER2/NEU, PTEN, AND P53 PROTEIN EXPRESSION AND GENES STATUS BY IMMUNOHISTOCHEMISTRY AND FLUORESCENCE IN SITU HYBRIDIZATION IN HIGH-GRADE GLIOMA

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Background: The EGFR, Her2/neu, PTEN, and p53 genes play an important role in the pathogenesis and biology of high-grade glioma (HGG, WHO grade III and IV). All of them are either involved through amplification, loss of heterozygosity, and/or mutation, Immunohistochemistry (IHC) and FISH tests are commercially available to determine the expression and copy number status of all four genes. This study aimed to determine the usefulness of IHC and FISH and to establish the correlation between both tests for these genes in an HGG cohort. This knowledge should be of help in stratifying glioma patients in clinical trials with targeted agents.

Materials and methods: Tumor tissues were collected from 72 patients from six Belgian hospitals. In addition to conventional histopathologic assessment, tumors were characterized by IHC for expression of the EGFR (DAKO, K1492), Her2/neu (DAKO, K1204), PTEN (Cascade Biosciences, ABM-2052), and p53 (Novocastra, NCL-p53–D07); and by FISH for the copy number status for EGFR (Vysis, 32–190105), Her2/neu (Vysis, 30–161060), PTEN (Vysis, 32–231010), and p53 (Vysis, 32–190008).

Results: The patient population (n = 72) consisted of 49 men and 23 women with a median age of 55 years at diagnosis. According to WHO classification, there were 23 anaplastic astrocytomas (AA) and 49 glioblastomas multiforme (GBMs). None of the gliomas in this series expressed Her2/neu. FISH has revealed no altered Her2/neu gene copy number in 16 of 16 gliomas examined so far. EGFR identified an amplification of the EGFR gene in 4 of 23 AAs (17%) and in 16 of 49 GBMs (33%). Interpretation of FISH results was straightforward. A positive EGFR IHC staining was observed in 18 of 23 AAs (78%) and 43 of 49 GBMs (88%) when a cutoff of 10% positive cells with membrane staining taken into account. EGFR gene amplification did not correlate with the level of expression assessed by IHC staining. Analysis of the PTEN (FISH and IHC) and P53 (FISH and IHC) genes is ongoing.

Conclusions: The Her2/neu proto-oncogene is not expressed in AA and GBM, and we found no alteration of the gene copy number so far. Therefore, we assume no important role for this gene in the biology of HGG. We confirmed that EGFR is expressed in most AAs and GBMs and that the EGFR gene is frequently amplified in these tumors. EGFR IHC results did not correlate with EGFR gene amplification status as determined by FISH. Stratification of glioma patients in clinical trials according to EGFR FISH results seems feasible and preferable as compared to IHC.

P47. PROTEOMIC ANALYSIS OF GLIOMAS DURING PF4–DLR ANTIANGIOGENESIS TREATMENT

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Angiogenesis, tumor cell proliferation, and migration are the hallmarks of solid tumors, such as gliomas. Recent study has shown that modified COOH-terminal PF4 peptide containing the sequence DLR (PF4–DLR) inhibits endothelial cell proliferation, migration, and microvessel assembly. Systemic administration of PF4–DLR to human glioma models in nude mice resulted in a significant inhibition of tumor growth. No data are available on the molecular determinants associated with the therapeutic response. Proteomics is a powerful technique which allows to identified group of proteins which expression change or is associated to treatment. In this study, we have used two-dimensional electrophoresis and mass spectrometry to directly analyze protein profile changes in gliomas during PF4–DLR treatment. Nude mice were intracranially inoculated with 50,000 U87 cells and implanted two weeks later with an osmotic minipump filled with PBS or 0.5 mg of PF4–DLR. Mice were sacrificed 10 and 20 days later, and protein analysis was performed by comparing at least seven 2–D gels for each treatment group. Thirty-seven significant spots have been analyzed by mass spectrometry, resulting in the identification of 28 proteins significantly upregulated and 9 proteins significantly downregulated after PF4–DLR treatment. Only three identified proteins originated from the mouse host.
Abstracts for the Seventh Congress of the European Association for Neuro-Oncology (EANO)

P48. MOLECULAR CYTODYNAMIC ANALYSIS OF MALIGNANT BRAIN TUMOR CELLS
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The most frequent tumors of the central nervous system include gliomas—a heterogeneous group of tumors with various histological subtypes that differ in response to treatment and in prognosis. Therefore, new diagnostic and prognostic indicators are sought to enable stratification of treatment and to help reduce morbidity and mortality of patients. One possibility is subclassification of patients according to findings of specific chromosomal aberrations.

For the detection of most frequent chromosomal changes in glial cells (deletion of tumor-suppressor genes TP53, CDKN2A, and RBI, deletion of Ip36 and/or 19q13.3, amplification of the EGFR gene, trisomy 7 and monosomy 10), we used FISH with locus-specific and/or α-satellite probes (Abbott-Vysis®). We examined 69 tissue specimens in 68 patients with different types of gliomas (2x pilocytic astrocytoma, 15x diffuse astrocytoma, 10x anaplastic astrocytoma, 2x glioblastoma, 9x anaplastic oligodendroglioma, and 2x anaplastic oligoastrocytoma). The results of molecular cytogenetic analyses were correlated with morphological and clinical findings. In two patients with an original diagnosis of anaplastic astrocytoma, we proved amplification of the EGFR gene—a typical aberration found in glioblastoma, which we proved in eight glioblastoma specimens. The most typical finding, monosomy of chromosome 10, was found in 25 of 28 glioblastomas, and amplification of the EGFR gene in 11 cases. In one case with oligodendroglioma, we proved deletion of the RFI1 gene—a typical finding in high-grade astrocytoma. In seven patients with anaplastic oligodendroglioma and two with anaplastic oligoastrocytoma, a combined deletion of Ip36 and 19q13.3 was found, which is considered to be a predictor of good response to chemotherapy. FISH is a powerful tool for surveying chromosomal aberrations in tumor cells. A systematic molecular cytogenetic analysis may advance diagnosis, grading, classification and, in some cases, also treatment of brain tumors. This work was supported by grant IGA MZ CR 1A/8237-3 and VZ 64165.

P49. METHYLATION PROFILES OF P16, RASSF1A, AND HMLH1 PROMOTER CGP ISLANDS IN BRAIN TUMORS
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Background: Association of an altered DNA methylation pattern of the promoter CpG islands with the expression profile of cancer-related genes has been found in many human tumors. In this pilot study, we aimed to determine whether the DNA methylation status of promoter CpG islands of three cancer-related genes might serve as an epigenetic biomarker in brain tumors.

Methods: After the isolation of DNA from the tissues of three low-grade gliomas, six high-grade astrocytomas and one oligodendroglioma, six meningiomas (two atypical meningiomas and four benign meningiomas), one metastatic adenocarcinoma, and 10 high-grade astrocytomas, the DNA was modified by using the CpGenome DNA modification kit and then modified DNA was amplified by using methylation-specific PCR for p16, RASSF1A, and hMLH1 genes. The products were analyzed on 3% agarose gel electrophoresis.

Results: The p16 and hMLH1 genes displayed a uniformly unmethylated pattern in all samples examined. Methylation of RASSF1A promoter CpG islands was found in samples of six high-grade astrocytomas, one atypical meningioma, one metastatic carcinoma, and one transitional meningioma. Thus, methylated promoter CpG islands of the RASSF1A gene were significant in 9 of 20 samples (45%), of which the majority were malignant in character histopathologically.

Conclusion: As a preliminary finding, methylation of RASSF1A promoter CpG islands might have an important role in the malignant transformation of brain tumors. The role of RASSF1A gene methylation as an epigenetic biomarker in brain tumors and prognosis of high-grade astrocytomas will be evaluated in larger series.

P50. THE PROGNOSTIC VALUE OF MGMT PROMOTER HYPERMETHYLATION AND OTHER GENETIC MARKERS IN MALIGNANT GLIOMA PATIENTS TREATED WITH TEMOZOLOMIDE
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Objective: The present study investigates the potential prognostic significance of various molecular parameters, including MGMT promoter methylation, and MGMT sequence polymorphisms in exons 3 and 5, as well as MGMT protein expression, for progression-free survival (PFS) and overall survival (OS) of patients with malignant gliomas. In addition, we determined the prognostic significance of loss of heterozygosity (LOH) on Ip and 19q, as well as aberrations in the TP53, CDKN2A, MDM2, PDGFR, and EGFR genes in the same patient series.

Methods: A total of 95 patients, including 23 with anaplastic gliomas of WHO grade III (AG) and 72 with glioblastomas of WHO grade IV (GBM), were treated with temozolomide (150 mg/m² daily for 5 days, followed by 7 days off). Tumor specimens were evaluated for the presence of MGMT promoter CpG islands, and for alteration of the tumor-suppressor genes TP53, MDM2, PDGFR, and EGFR in the same patient series.

Results: Mean OS was 53 months (AG) and 20 months (GBM). Statistical analyses revealed that MGMT hypermethylation was significantly associated with longer PFS and OS in AG (P = 0.02 and P < 0.001, respectively; log-rank test (LRT) and GBM (P = 0.02 and P = 0.001, respectively; LRT). MGMT promoter methylation correlated with MGMT hypermethylation (P<0.03). The six investigated MGMT polymorphisms were not associated with survival. In the AG group, LOH Ip was associated with longer OS (P = 0.026), while TP53 mutation was associated with shorter PFS and OS (P = 0.027 and P = 0.036, respectively). Interestingly, four GBMs showed LOH on Ip but shorter PFS and OS as compared to GBMs with intact Ip.

Conclusions: Our study corroborates MGMT promoter methylation as a clinically important prognostic marker that correlates with longer PFS and OS in both AG and GBM patients. In contrast, the presence or absence of MGMT polymorphisms does not appear to be related to patient prognosis. LOH Ip and TP53 mutation were associated with prognosis in only a minority of AG patients. All other investigated genetic alterations do not appear to be of prognostic value in malignant glioma patients.

P51. IDENTIFICATION OF KINASE THERAPEUTIC TARGETS FOR MALIGNANT GLIOMA BASED ON CHANGES IN DNA COPY NUMBER AND GENE EXPRESSION LEVELS IN 48 PATIENTS
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Protein kinase inhibition is a promising therapeutic strategy for the treatment of malignant glioma, because of relevant clinical responses that have been observed in several advanced solid tumors. However, the problem is that the protein kinase family is large, and an increasing number of inhibitors is available. Therefore, a rational strategy would be to identify the molecular drivers among the protein kinases, if existent in malignant glioma. Molecular drivers are molecules that dictate the malignant phenotype of cancer cells, on which the tumor is crucially dependent, and as such are high potential therapeutic targets. In general, potential molecular drivers can be identified by studying differential profiles of tumor versus normal tissue in the various genomic or proteomic domains (genome, RNA transcription, protein abundance, and protein activity). Several protein kinases, such as EGFR, PDGFR, and CDK4, have previously been suggested as a molecular driver for malignant glioma.

In the present study, a ranking of protein kinase involvement based on changes in DNA copy number and gene expression levels is determined by microarray analysis in 48 malignant glioma samples. Online available data sets from cDNA microarray platforms were used to define a DNA copy

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P52. MAGNETIC RESONANCE IMAGING AND MGMT METHYLATION STATUS: CORRELATIONS IN 70 PATIENTS WITH GlioBlASTOMA MULTIFORME

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Approximately 40% of glioblastomas (GBM) have very low levels of expression of the O6-methylguanine-DNA methyltransferase (MGMT), mostly due to hypermethylation of the MGMT promoter. In GBMs, MGMT inactivation by aberrant promoter methylation correlates with a longer survival following radiation and adjuvant chemotherapy. To improve therapeutic management of GBMs, we investigated the relationship between magnetic resonance imaging (MRI) features on the preoperative scan, the histopathological diagnosis, and the genetic signature of these tumors: methylation status of MGMT, TP53 mutations, EGFR amplification, and loss of heterozygosity (LOH) on chromosomes 1p and 19q.

In each MRI examination, the following parameters were analyzed: location of the tumors, presence of necrosis, and presence and features of enhancement (ring, nodular, or mixed).

In 20 GBMs, in 24, the MGMT promoter was hypermethylated (34%). Tumors with methylation of the MGMT promoter were more often located in parietal and occipital lobes, while tumors without methylation of the MGMT promoter were frequently in the temporal lobes (P = 0.01). All GBMs exhibited macroscopic necrosis. In two cases, presurgical MRI showed no enhancement. Ring enhancement was significantly associated with diagnosis of de novo GBMs (P = 0.0006) and with tumors with an unmethyalted MGMT promoter (P = 0.001). No correlations were found between MRI features and EGFR amplification, TP53 mutations and LOH 17p and 19q.

These findings indicate that molecular alterations associated with cancer may confer physical or biochemical characteristics to the tumor that can be imaged.

P53. GENOTYPICAL ANALYSIS IN MIXED OligoAstROCYtOMA USING FLUORESCENCE IN SITU HYBRIDIZATION

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Oligoastrocytomas (OAs) are phenotypically heterogeneous tumors. It remains unclear whether OAs have identical genetic alterations in oligodendroglial and astrocytic portions. To clarify this issue, we examined paraffin-embedded OAs differentially with separable oligodendroglial and astrocytic areas for 1p/19q status by using FISH (fluorescence in situ hybridization). Surgically resected tumors from eight patients (two OAs and astrocytic areas for 1p/19q status by using FISH (fluorescence in situ hybridization)).

R.T. Johnson). Two-dimensional gel electrophoresis (2-DGE) and MALDI-TOF-MS (matrix-assisted laser desorption ionization - time of flight) mass spectrometry-based method for peptide profiling of blood plasma/serum. Plasma samples were analyzed from eight high-grade and eight low-grade brain tumor patients. Peptide profiles were obtained with peptide capture by using solid-phase extraction coupled to MALDI-TOF-MS.

**Results:** Proteomics: The glioma spiedum proteome contained about 220 detectable proteins. Comparative analysis of the 2-D protein maps of irradiated versus nonirradiated spheroids revealed 67 significantly regulated proteins. Irradiation resulted in an approximately 100-fold upregulation of macrophage migration inhibitory factor (critical protein in neovascularization), as well as in an approximately 10-fold upregulation of peptidyl-prolyl cis-trans isomerase A (apoptosis-associated protein). Peptidomics: A pilot mass spectrometry-based proteomic profiling on plasma samples from high-grade versus low-grade brain tumor patients showed overlapping but differential patterns, and PCA analysis revealed (some) clustering in two groups.

**Conclusions:** Identification of peptide profiles from glioma patients through plasma or tumor tissue, correlation with tumor grade and clinical behavior, as well as response to (radio)therapy might elucidate signaling pathways involved in resistance to therapy and uncover candidate targets for treatment optimization.

P54. PROTEIN EXPRESSION IN BRAIN TUMOR PATIENTS: MONITORING OF RESPONSE TO RADIOTHERAPY

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Objective: To investigate the global peptide and protein expression patterns in blood samples from patients with low-grade and high-grade gliomas, and a series of primary and recurrent glioma specimens from the same patients, as well as of glioma spheroids, prior to and following radiotherapy.

**Materials and methods:** Two proteomic strategies were used for the analyses and identification of glioma treatment response markers: (1) A 2-D gel-based proteomic method coupled to mass spectrometry for protein expression profiling of glioma spheroids and tumor specimens. For this purpose, spheroids of the radioresistant U87 human glioma cell line were incubated with 20 Gy or sham treated. Proteome analyses were performed 24 h after irradiation. In addition, sets of primary and recurrent glioma specimens from 10 patients were analyzed. (2) A high-throughput mass spectrometry-based method for peptide profiling of blood plasma/serum. Plasma samples were analyzed from eight high-grade and eight low-grade brain tumor patients. Peptide profiles were obtained with peptide capture by using solid-phase extraction coupled to MALDI-TOF-MS.

**Results:** Proteomics: The glioma spiedum proteome contained about 220 detectable proteins. Comparative analysis of the 2-D protein maps of irradiated versus nonirradiated spheroids revealed 67 significantly regulated proteins. Irradiation resulted in an approximately 100-fold upregulation of macrophage migration inhibitory factor (critical protein in neovascularization), as well as in an approximately 10-fold upregulation of peptidyl-prolyl cis-trans isomerase A (apoptosis-associated protein). Peptidomics: A pilot mass spectrometry-based proteomic profiling on plasma samples from high-grade versus low-grade brain tumor patients showed overlapping but differential patterns, and PCA analysis revealed (some) clustering in two groups.

**Conclusions:** Identification of peptide profiles from glioma patients through plasma or tumor tissue, correlation with tumor grade and clinical behavior, as well as response to (radio)therapy might elucidate signaling pathways involved in resistance to therapy and uncover candidate targets for treatment optimization.

P55. COMPARATIVE PROTEOMICS OF MALIGNANT GLIOMA AND BRAIN TUMOR PATIENTS: MONITORING OF RESPONSE TO RADIOTHERAPY

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An evaluation of the qualitative and quantitative differences between the proteins present in malignant glioma and control brain is now possible using two-dimensional gel electrophoresis (2-DGE) and MALDI-TOF-MS (matrix-assisted laser desorption ionization—time of flight) mass spectrometry techniques. Such an analytical approach may provide insights into various pathophysiological mechanisms of malignant glioma. So far, only four studies have used proteomics to characterize human gliomas. The experimental design and analysis, classification of control brain, and size of these studies have been different, with limited quantification and very little consensus as a result. They suggest, however, that there are protein expression differences between glioma and brain. We have therefore undertaken a study to compare control brain and glioma tissue by using quantitative proteomic technology.

Patients undergoing craniotomy and resection of malignant glioma gave written informed consent for tumor tissue to be used in this study (approved by the local regional ethics committee). Fresh tumor and brain tissue is collected in the operating suite and transported directly to the laboratory, where it is homogenized in lysis buffer, centrifuged, and the total extracted soluble protein yield quantified. The whole protein extract is then separated by 2-DGE (by their isoelectric point (pI) of their molecular mass) and stained using SYPRO Ruby fluorescent. The captured gel image is analyzed using software that quantitates protein according to the normalized density of each protein. Gels are initially matched manually using recognizable collagen marker protein features and then by a computer. Comparative image analysis of the gels (control vs. tumor) is then assessed to identify statistical changes in protein levels. Protein spots of interest are identified by peptide mass fingerprinting using MALDI-TOF-MS analysis.
So far, 18 patient samples have been collected. The initial findings confirm that there are consistent and readily recognizable expression differences in tumor (malignant glioma) and normal brain. These proteins will be discussed in terms of biological function and possible therapeutic strategies.

Materials and methods: iodine-125 brachytherapy (2 mCi single seed) was combined with antibody against murine VEGF-R2 (DC101; 40 mg/kg i.p. every 3 days) in an orthotopic athymic nude mouse GBM (U251-NG2) model. Treatment started seven days after stereotactic injection of the cells into the right frontal lobe. Both treatment modalities were controlled with sham treatment, resulting in four groups. Mice were sacrificed if losing more than 30% of their weight or showing neurological signs, and 13 weeks after cell inoculation. Histology was performed at point of maximal tumor size. Scoring was done for necrosis, tumor satellites, mitosis (Nissl in 3 hpfs), apoptosis (caspase 3 in 3 hpfs), proliferation (Ki-67 in 3 hpfs), reactive gliosis (GFAP), and luminal vessels (CD34 and SMA).

Results: Tumor take in 96 mice was 90%. Four treatment groups were compared: (1) sham treatment, (2) DC101 alone, (3) irradiation alone, and (4) DC101 + irradiation. Median survivals were 46.1, 47.5, 62.4, and 38.7 days, respectively. Mean tumor size (% of total brain surface): 41%, 16%, 0.3%, and 0.1%. Apoptosis: 3.2, 3.4, 1.0, and 0.0. Mitosis: 40.5, 16.6, 3.0, and 0.0. Lumina: 90.9%, 87.3%, 40.0%, and 22.2%. Compared to controls, irradiation alone improved mouse survival statistically significantly with histologically high tumor responses. By combining DC101 with irradiation, the survival benefit of irradiation was lost, but tumor effects were enhanced.

Conclusions: In our orthotopic murine GBM model, the antitumor effect of irradiation is enhanced by VEGF-receptor blockade by DC101. This synergistic antitumor effect did not result in better mouse survival. Possibly, the beneficial antitumor effects are counteracted by toxic effects.

P59. AQAPORIN 1 IS EXPRESSED ON INVADING GLOBLASTOMA CELLS: A POSSIBLE ROLE IN CELL MIGRATION?
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Aim: This study investigated whether the transmembrane water channel aquaporin-1 (AQP1) is implicated in the progression of glioblastoma multiforme (GBM).

Methods: Glioblastoma spheroids, derived from human GBM biopsies, were stereotactically implanted into the cortex of nude rats. After 4 to 6 months, highly infiltrative, nonangiogenic tumors were observed on MRI as well as by histological examination (first-generation tumors). These tumors were serially transplanted into new animals, less invasive, more angiogenic tumors were established (high-generation tumors). Thus,
two distinct tumor phenotypes were observed, both characterizing human GBMs in situ. The distribution of AQPI was determined by immunohis-
tochemistry.

Results: In normal brain, AQPI was expressed only in the choroid
plexus. In brains from first-generation tumors, we observed a marked
expression of AQPI in the expanding periphery of the tumor, whereas the
tumor center was AQPI negative. In brains from high-generation tumors,
we observed expression of AQPI in the entire tumor, but with a marked
bandlike accentuation in the invading rim, as well as in single migrating
malignant glioma satellite cells.

Conclusion: The expression pattern indicates an important role for
AQPI in glioma growth and invasion.

P60. TUMORAL AND PERITUMORAL TISSUE O2, CO2, PH, HCO3, –, AND TEMPERATURE IN ArouSAW PATients WITH GLIOBLASTOMA: FINDINGs UsINg THE NEUROTRENDS MULTIPARAMETER MONITOR

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There is indirect evidence from neuroradiological and neuropathologi-
cal studies, and direct data from intraoperative recordings suggesting that
glioblastomas have foci of profoundly hypoxic tissue and peritumoral brain
has decreased cerebral blood flow and partial pressure O2 (pO2) compared
to normal tissue. We have used the Godman Neurotrrend multiparameter
monitor to measure brain tumor and peritumoral brain pO2, pCO2, pH,
temperature, and HCO3 in awake patients after they have had image-
directed brain tumor biopsies. The monitor was stabilized using a skull
bolt. Data were downloaded to a computer. The study was approved by the
local ethics committee.

Twelve patients were recruited, all had glioblastomas, monitoring was
done for 3 to 22 days after surgery, and none had any complications. Twenty-six
regions of tumor were monitored (range, 1–4 in each patient). Fifteen
regions had brain tissue pO2 > 15 mmHg; two had brain tissue pO2
between 5 and 10 mmHg; one was <5 mmHg; and four regions were 0.
Brain tumor temperature was always >34.5°C. pH ranged from 6.95 to
7.35. Tissue pHCO3 ranged from 40 to 75 mmHg. There was an inverse rela-
tionship between pH and tissue pCO2.

This study found little evidence for profound tumoral hypoxia in awake
patients with glioblastomas. Reasons for this will be discussed.

P61. HIGHLY TEMOZOLOMIDE-SENSITIVE GLIOBLASTOMA RECURRENCE AFTER RADIOThERAPY AND CHEMOThERAPY: A CASE REPORT

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Introduction: Glioblastoma is characterized by invasive growth and
almost general recurrence. Recently, chemotherapy has been shown to
improve patient survival significantly. Here we have analyzed chemosomal
timest, tumor cell aggressiveness, and chemosensitivity of three cell lines
determined from primary tumor and two consecutive recurrences of a long-
term surviving glioblastoma patient.

Materials and methods: Primary cell cultures were established from
all three surgery specimens (BTL1–BTL3). Chromosomal changes were
analyzed by comparative genomic hybridization (CGH). Chesomesivity
of BTL1–3 cell cultures against temozolomide was analyzed by MTT tests.
Gen expression was analyzed by RT/PCR and Western blot. Methylated
specific PCR (MSP) was performed with oligonucleotide primers against
either methylated or modified unmethylated MGMT promoter DNA.
Migratory potential was investigated for each cell culture by scratch assays.
Following surgery, progressive disease was observed under RT and chemo-
therapy with CCNU. After reoperation, the patient received seven cycles
every 28 days) at 5 × 300 mg/m2/day temozolomide, leading to partial
regression. Eight months after therapy, a second recurrence was confirmed
by MRI. After reoperation, the patient refused chemotherapy. Tumor progres-
sion led to disease-related death 27 months after surgery of the primary
tumor.

Results: CGH of BTL1 revealed chromosomal imbalances typical for
highly aggressive glioblastomas (e.g., gains of chromosome 7 and loss of
chromosomes 9p and 10). BTL2, established from the first recurrence under
therapy, showed signs of severe chromosomal instability, with numerous
additional chromosomal changes. In contrast, BTL3 from the second
recurrence resembled a less aggressive subtype of the primary tumor, with
reduced chromosomal alternations including lack of chromosome 7 amplification.
While BTL2 cells exhibited a highly aggressive phenotype,
BTL3 cells were characterized by a significantly lower proliferation and
migratory potential. All three cell lines were responsive to temozolomide,
as reflected by persistent methylation with the highest sensitivity detected in
BTL3 cells.

Summary: Our data suggest that even heavily treated glioblastoma
patients may relapse with a less aggressive, highly chemosensitive tumor
otype. This implies the feasibility of temozolomide treatment in glioblastoma
patients even in case of recurrent recurrence.

P62. LENTIVIRAL DELIVERY OF SHRNA IN GLIOBLASTOMA MULTIFORME SPHEROIDS

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Organotypic multicellular spheroids (OMS) represent the tumor biol-
ogy of glioblastoma multiforme (GBM) better than cell lines because the 3-D architecture preserves cell-cell interactions, the extracellular matrix,
and cellular heterogeneity. Therefore, we use OMS to validate new treatments for GBM. In the present study, we investigated whether spheroids can effectively be transduced by a lentiviral vector in comparison to monolayer cultures for RNA interference.

Three malignant glioma cell lines (U87, SKMG-3, and Gli-6) were cul-
tured under standard conditions and on 0.75% agar in overlying medium
to form spheroids. OMS were formed by culturing small fragments of
fresh GBM samples on 0.75% agar in overlying medium. Glioma cell
lines and spheroids were transduced with a lentiviral vector coding for
shRNAp53–GFP, which was chosen because of its high efficiency. Trans-
duction efficiency of cell lines was determined by FACS analysis, whereas
the transduction of spheroids in toto and sections was analyzed by confocal
microscopy. Spheroids were fixed in paraformaldehyde and embedded in
gelatin before cryosections were cut. Serial cryosections were hematoxylin-
and eosin (HE) stained to inspect tissue morphology.

Cell lines were transduced with a lentiviral vector coding for
shRNAp53–GFP up to 95% efficiency. We found GFP to be present only in
the outer ridge of the spheroids with confocal analysis of spheroids in toto.
To investigate this phenomenon in more detail, cryosections of the spher-
oids were prepared and analyzed by confocal microscopy. It was found that
in some spheroids the virus was shown to be present throughout the entire
spheroid, but, in the majority, only the layer outer of 1–2 cells was GFP
positive, indicating lentiviral transduction. The tissue morphology on HE
staining did not explain the different transduction patterns. There was no
difference in transduction efficiency between OMS and cell line spheroids.

In conclusion, whereas the high transduction efficiency is reproducible
in monolayer cell cultures, the distribution of lentiviral delivery varies between
spheroids, whether OMS or cell line spheroids. The reproducible transduc-
tion efficiency of the outer rim of spheroids may be useful to evaluate migration and
invasion response to silencing of potential target genes.

P63. TARGET-SPECIFIC GLIOMA THERAPY IN AN IMMUNOCOMPETENT MOUSE MODEL

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Main, Germany

We have established an immunocompetent mouse model representing
the typical progressive stages observed in human malignant gliomas for the
in vivo evaluation of novel target-specific regimens. Isolated clones from
tumors that arose spontaneously in GFAP-v-src transgenic mice were used to
develop a transplantable brain tumor model in syngenic B6C3F1 mice.
Striated implantation of 10,000 mouse tumor cells resulted in the robust
development of microscopically (2–3 mm) infiltrating malignant gliomas.

Immunohistochemically, the gliomas displayed the astroglial marker
GFAP and the oncocytic form of STAT3 (Tyr-705–phosphorylated), which
is found in many malignancies, including gliomas. Phosphorylated STAT3
was particularly prominent in the nucleus, but was also found at the plasma
membrane of peripherally infiltrating glioma cells. To evaluate the role
of STAT3 in tumor formation and therapy resistance, we stably expressed
shRNA against STAT3 in several murine glioma cell lines. STAT3 protein
was knocked down in tumor cells after infection with replication-defective
lentiviruses encoding STAT3–shRNA located in the coiled-coil domain.
Apoptosis is designed to be induced by recombinant TRAIL or the agonistic
TRAIL receptor antibody MD5–1. The antitumor effects of STAT3 deple-
tion on proliferation, migration, and resistance to proapoptotic stimuli
will be first assessed in vitro. Subsequently, after transplantation in vivo, the
synergistic antitumor effects of STAT3–shRNA and TRAIL treatment on
invasion and survival will be tested in vivo. Upstream and downstream
components of the TRAIL and of the STAT3 signaling pathway will be examined, too. In conclusion, its high rate of engraftment, its similarity to the malignant glioma of origin, and its rapid locally invasive growth should make this murine model useful in testing novel therapies for malignant gliomas.

P64. POTTENATION OF ANTIAGLIOMA EFFECT WITH COMBINED TEMOZOLOMIDE AND INTERFERON B

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Temozolomide (TMZ) is a DNA-methylating agent that has shown promising antitumor activity against high-grade glioma. Interferon β (IFN-β) is known to have antiproliferative and antiangiogenic activities. The aim of this study was to elucidate whether an antitumor effect could be potentiated by the combination of TMZ and IFN-β. In vitro, the combination of these drugs has been shown to suppress proliferative and migratory activities, as well as enhance apoptosis and cell cycle (S phase) arrest of U-87 cells more efficiently than TMZ or IFN-β alone. IFN-β has been shown to exert a potent inhibitory effect on the proliferation of human umbilical vein endothelial cells (HUVECs); however, no additive or synergistic effect was observed with the addition of TMZ. To determine in vivo effect, nude mice bearing intracerebral U-87 xenografts were treated with intraperitoneal administration of PBS, TMZ (15 mg/kg for 3 days), IFN-β (2 × 10^6 IU for 15 days), and a TMZ + IFN-β combination. The combination treatment (mean, 63.8 ± 5.1 days; P = 0.0005) was observed to increase significantly the survival of the animals compared to treatment with PBS (mean, 31.3 ± 1.6 days), TMZ (mean, 39.1 ± 8.3 days), or IFN-β (mean, 36.2 ± 1.3 days). These results suggest that antitumor activity can be enhanced by the combination of TMZ and IFN-β. These findings provide the possibility for a new strategy in the management of malignant glioma.

P65. HOMING OF HEMATOPOIETIC PROGENITOR CELLS TOWARD GLIOMAS IS ENHANCED BY IRRADIATION AND HYPOXIA

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Previously we defined a pathway of transforming growth factor beta (TGF-β)–derived and stromal cell–derived factor 1/CXC chemokine ligand 12 (SDF-1α/CXCL12)–dependent migration of adult hematopoietic stem and progenitor cells (HPCs) toward glioma cells in vitro and their homing to experimental gliomas in vivo. Hypoxia is a critical aspect of the microenvironment of gliomas, and irradiation is an essential part of the standard therapy. We therefore evaluated the impact of hypoxia and irradiation on the attraction of HPCs by glioma cells. Supernatants of irradiated or hypoxic LNT-229 glioma cells enhanced HPC migration in vitro. Reporter assays showed that the CXCL12 promoter activity is enhanced in LNT-229 cells at 24 h after irradiation at 8 Gy or after exposure to 1% oxygen for 12 h. The irradiation- and hypoxia-induced secretion of CXCL12 depended on hypoxia inducible factor 1 alpha (HIF-1α), but not on p53. An intact TGF-β signaling cascade is required for the transcriptional activity of HIF-1α by hypoxia or irradiation. These findings delineate a novel stress signaling cascade in glioma cells involving TGF-β, HIF-1α, and CXCL12. Stress stimuli can be irradiation, hypoxia, or temozolomide, but not hyperthermia. Cerebral irradiation of nude mice at 21 days after intracerebral implantation of LNT-229 glioma induces tumor satellite formation and enhances the glioma tropism of HPCs to the tumor bulk and even to these satellites in vivo. These data suggest that the use of HPCs as cellular vectors in the treatment of glioblastoma may well be combined with irradiation or other antiangiogenic therapies inducing tumor hypoxia.

P66. IN VITRO RESPONSE OF MALIGNANT GLIOMAS TO INHIBITORS OF THE AKT/PI3K PATHWAY IN RELATION TO GENOTYPE AND PROTEIN PHOSPHORYLATION STATUS

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A novel therapeutic strategy with enormous potential in the treatment of glioblastoma multiforme is based on protein kinase inhibition. A number of kinase targets have been identified that are important in malignant glioma. These include overexpression and/or activation of tyrosine kinase receptors and proteins of the PI3K/Akt signaling pathway. However, it is unclear what the incidence of their involvement and the effectiveness of their inhibition are.

With a panel of established cell lines (U87MG, U251MG, T98G, GA-MG, and CCF-STTG1) and six primary glioma cultures, the treatment response to inhibitors of Akt, PI3K, mTOR, FGF1R, PDGFR, and EGFR was determined as a single treatment and in various combinations with conventional therapy (temozolomide) and radiation. Quantification was performed with 96-well plate assays for cell toxicity (colorimetric MTS assay) and apoptosis (fluorescent caspase activity assay).

For correlation of response to inhibitors and protein status, the abundance of inhibitory effect on the proliferation of the same samples involved PTEN deletion, p53 deletion/mutation, EGFR and/or ERBB2 amplification, and MGMT methylation.

The small molecules used effectively inhibited the PI3K/Akt signaling cascade. Temozolomide was, as expected, most effective in those cell lines with methylation of the MGMT promoter. Cell growth reduction compared to untreated cells could be achieved with inhibitor alone or in combination therapy. However, the in vitro responses were markedly heterogeneous among cell lines and primary cultures. In some cell lines and primary cultures, growth reduction of more than 80% was observed, while the same inhibitors had had almost no effect on other cell lines and primary cultures. This heterogeneity was reflected in the genotype and protein phosphorylation status. The correlation between molecular and proteomic data and treatment responses is currently being investigated. Our initial results underscore the importance of an integrated approach as a basis for target validation and patient-tailored therapy in glioblastoma.

P67. VASCULAR ENDOTHELIAL GROWTH FACTOR A CONTRIBUTES TO GLIOMA-INDUCED MIGRATION OF HUMAN MARROW STROMAL CELLS (HMSCS)

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Objective: It has been demonstrated that murine neural stem cells (hMSCs) and human mesenchymal stromal cells migrate toward experimental gliomas, making stem cells a candidate for cellular carrier systems of antiangioma therapy. However, few data are available on the factors involved in regulating stem cell migration. The aim of our study was to characterize the migratory and invasive behavior of adult human marrow stromal cells (hMSCs) that interact with glioma cells, especially focusing on vascular endothelial growth factor A (VEGF-A)–mediated effects.

Methods: Human MSCs were isolated from bone marrow biopsies carried out for hematological indications. The chemokinetic activity of hMSCs in response to glioma-conditioned medium as well as VEGF-A was analyzed using a modified Boyden chamber assay. Invasion of hMSCs and glioma spheroids was investigated using confrontational cultures. VEGF-A secretion by glioma and the expression of VEGF-receptor 2 in hMSCs were assessed.

Results: Human MSCs showed an extensive invasion into glioma spheroids. Glioma-conditioned medium significantly increased hMSC migration and also invasion, driven by chemotaxis. VEGF-A also showed pro-invasive effects, but in a reduced fashion compared to glioma-conditioned glioma.

Conclusions: Human MSCs show intensive migratory and invasive behavior in the presence of glioma cells and glioma-conditioned medium. Among others, VEGF-A seems to be one important factor in enhancing and directing stem cell motility. Human MSCs appear to be promising candidates for a future role as treatment vectors.
The NDV strain V4 was propagated in the 250-250 family and in the genus 

Introduction: Newcastle disease virus (NDV) is a virus of the Para-

Objective: The objectives of this study were mainly to evaluate the cyto-

Methodology and results: The NDV strain V4 was propagated in the allantoc fluid of nine-day-old embryonated chicken eggs. The allantoc fluid was harvested, purified and stored at −20°C. In this study, we successfully deter-

P69. THE BINDING OF THE UNBS1450 CARDENOLIDE TO THE SODIUM PUMP IN HUMAN Glioblastoma (GBM) CELLS DRAMATICALLY IMPAIRS BOTH THEIR MIGRATION AND PROLIFERATION PROPERTIES

Malignant gliomas represent the most common primary brain tumor. With an incidence of five cases per 100,000 population per year, they are the fourth most common cause of cancer-related deaths and are among the most lethal cancers, with an overall survival time of less than two years. Many different adjuvant therapies have been developed. Among them, the most promising is photodynamic therapy (PDT), which is based on pho-

P70. CYTOTOXIC EFFECT OF NEWCASTLE DISEASE VIRUS (NDV) STRAIN V4 ON BRAIN CANCER CELL LINES

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Purpose: Malignant gliomas are the most common primary brain tumor. With an incidence of five cases per 100,000 population per year, they are the fourth most common cause of cancer-related deaths and are among the most lethal cancers, with an overall survival time of less than two years. Many different adjuvant therapies have been developed. Among them, the most promising is photodynamic therapy (PDT), which is based on pho-

Experimental design: We examined the distribution and retention of m-THPc in the rat C6 glioma brain tumor model. Thirty female Wistar rats received m-TPhC 12 days after tumor implantation. Tenoporfin was administered intrathecally in 24 rats at two different concentrations. Six rats constituted the control group and received m-TPhC by means of an intraperitoneal injection. The brains were extracted at 4, 24, and 96 h after temoporfin injection. The samples were examined with a confocal laser scanning microscope.

Results: All samples showed high fluorescence emission exclusively in tumor area, without appreciable differences between the samples taken at the different times of sacrifice and the two routes of administration. No fluorescence whatsoever was detected among normal brain tissue surrounding the tumour.

Conclusions: The intrathecal route appears to give comparable results to the systemic one, regarding transfection efficiency and tumor–normal tissue boundary. We are now planning to use a much shorter time needed to reach optimal intratumoral concentration—that is, just 4 h after m-THPc-injec-
tion. Our data suggest that the intrathecal route of m-THPC administration can represent a safe and efficient strategy to remarkably reduce hospitalization time and costs for patients that are eligible for PDT.

P72. THE EFFECTS OF A RHO KINASE INHIBITOR, FASUDIL, ON INVASION AND TUMOR-INDUCED ANGIOGENESIS OF HUMAN GLIOMA CELLS

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Background: Gliomas are the most common tumors of the central nervous system, and malignant gliomas are characterized by a high invasive potential and strong angiogenic ability. The control of tumor invasion and tumor-induced angiogenesis are the key problems for the improvement of treatment results of malignant gliomas. Much evidence has shown that constitutive activation of the small GTPase Rho and its downstream target Rho kinase (ROCK) is crucial for the invasion of tumor cells and the tumor angiogenesis. This study evaluated the effects of a selective ROCK inhibitor, fasudil, on the invasion of human malignant glioma cells and the glioma cell–induced angiogenesis.

Methods: Three human glioma cell lines (T98G, U87MG, and ONS12) were treated with fasudil, and the morphological change in glioma cells after the administration of fasudil was evaluated immunohistochemically by using TRITC-labeled phallolidin and an antipaxillin antibody. The anti-invasive effect of fasudil was analyzed by the Matrigel invasion assay. The effect of fasudil on glioma-induced angiogenesis was as investigated in vitro by using the culture insert method. The effect of fasudil on the migration of endothelial cells (HUVECs) was also assessed. The cytotoxicity of fasudil was determined by the MTT assay.

Results: Treatment of glioma cells with fasudil induced significant changes in cell morphology. Immunofluorescence microscopy showed disruption of actin stress fibers and focal adhesion plaques. The Matrigel invasion assay demonstrated that invasion of glioma cells was suppressed by fasudil. The angiogenesis assay showed that fasudil had a suppressive effect on glioma cell–induced angiogenesis. Further, fasudil inhibited migration of HUVECs. However, fasudil did not notably suppress the proliferation of the glioma cell lines in the MTT assay.

Conclusion: These results suggest that fasudil may have potentially suppressive effects on the invasion and angiogenesis of gliomas.

P73. SCREENING FOR TUMOR THERAPY SENSITIVITY USING AN EX VIVO INVASION ASSAY

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Background: Improved treatment for brain tumors is needed. Most models assessing chemotherapies fail to incorporate heterogeneity of patient responses because tumor progression is dependent on a tumor’s ability to invade and grow into surrounding tissue locally and at distant sites in the body. In this study, a surgical sample of each patient’s primary brain tumor was assessed while exposed to a panel of clinically relevant chemotherapies by using an ex vivo model of invasion and growth. The invasion and growth of the representative tissue fragments are thought to be reflective of clinical response to therapy.

Methods: Tissue specimens were placed into a nutrient-rich collagen matrix and monitored microscopically to measure actual distance tumors invaded in the presence of chemotherapies for five days following surgical removal. Each therapy was applied as an overlay to the tissue samples directly. Four samples were run for each condition. All samples were preserved for examination of markers related to tumor growth, invasion, and viability.

Results and conclusions: Twenty-two patients’ individual tumor therapy sensitivity was assessed. Each patient’s tumor displayed a unique and significant (P < 0.05) invasion and response profile. Nine patients’ tumors were not significantly sensitive to any therapy tested. Meningioma and ependymoma tumor samples from three patients did not migrate significantly into the matrix as was expected for benign tumors. Six malignant tumors responded to docetaxel, four responded to procarbazine, five responded to vincristine, and two responded to etoposide in glioma. Results from these data will continue to be compared to patient response, time to recurrence, and survival for up to two years. Individual response to chemotherapy is highly variable both clinically and in our ex vivo assessment. Prescreening each patient’s responsiveness to chemotherapies by using this methodology could lead to more individualized and therefore a more effective approach to the treatment of brain tumors.

P74. GLIOMA-INDUCED INTEGRATION OF ADULT HUMAN MENISCAL STEM CELLS INTO THE TUMOR VASCULARITY

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Objective: Much effort has been put into establishing human multipotent stem cells as candidates for malignant glioma therapy. We already described the EGFr-dependent interaction of adult human meniscal stem cells (hMSCs), which are easily available through bone marrow biopsy and glioma cells in vitro. The aim of our study was to characterize glioma-modulated invasive MSC behavior in vivo and distribution patterns in the glioma-induced brain.

Methods: Human MSCs were isolated from bone marrow biopsies carried out for hematological indications. Only early passages were used for the experiments. In an experimentally induced glioma (U373–GFP)-infiltrated brain (T-cell-deficient rats), hMSCs (DiI) were implanted simultaneously. All control cells served fluorescent and immunohistochemically. To exclude artificial attraction, a control incision was made. In a second setting, a murine MSC cell line (mMSC), transfected with a RFP/Tie2–promoter gene, was given intravenously. mMSCs that accumulated in the glioma (C6)-infiltrated brain were detected immunohistochemically. Tie-2-induced expression of RFP allowed detection of those mMSCs, which integrated into the endothelial lining of the vasculature.

Results: Confocal microscopy revealed a colonization of the hMSC and the infiltrating tumor. Fibroblasts as well as immortalized hMSCs did not show any localization in close vicinity to the tumor, thereby excluding a passive transportation phenomenon of the hMSCs within the glioma-infiltrated brain. Control incisions did not show any hMSC infiltration. Intravenously administered hMSCs showed extensive tropism to the glioma. The infiltrating borders of the tumor showed accumulation of hMSCs as well as enhanced RFP expression of the mMSCs, thereby indicating an integration into the tumor’s neovascularization. This phenomenon was confirmed immunohistochemically.

Conclusions: hMSCs show intensive tropism to invading glioma in vivo. Intravenously administered meniscal stem cells enrich within the tumor and seem to integrate into its vasculature. hMSCs proved to be hopefull candidates for a future role as glioma treatment vectors.

P75. ISOLATION AND CHARACTERIZATION OF HUMAN ADULT NEURAL PROGENITOR CELLS FROM SURGICAL SPECIMENS FOR THE DEVELOPMENT OF LOCAL BRAIN TUMOR THERAPIES

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Ethical and logistical difficulties are major obstacles to obtaining human tissue suitable for the isolation of neural progenitor cultures. Previous reports have demonstrated the extensive migratory and tumor-targeting capabilities of neural stem cells derived from fetal tissue. The aim of this study was to establish well-characterized long-term cultures of neural progenitor cells from human adult brain tissue and to explore their potential as drug delivery vehicles for targeting invasive glioma cells.

We modified previously reported protocols for establishing neural progenitor cultures from fetal and postmortem brain tissue. By using NeurobasalB27 medium and various combinations of growth factors, we obtained highly proliferative neuroectodermal cells from surgical specimens of amygdaohippocampocostomies (n = 8) and brain tissue adherent to resected arteriovenous malformations (n = 2).

We were able to isolate and propagate highly proliferative neural progenitor cultures from cortex, hippocampus, and amygdala, which were characterized by immunohistochemistry, RT-PCR, and FACS. Our cultures grew as homogeneous monolayers of progenitor cells expressing markers like nestin, A2B5, SOX2, BMI1 and, infrequently, CD133 and Musashi. Immunofluorescence microscopy showed dis...
we demonstrate that these progenitor cells are chemotactically attracted by human glioma cells, indicating their potential for cell-based local brain tumor therapies.

P76. ALTERED EXPRESSIONS OF WNT-BINDING PROTEINS IN TRANSGENIC MICE COEXPRESSION TETRACYCLINE-CONTROLLED TRANSACTION AND HUMAN MUTANT PRESENILIN 2

Nonregulatory promoters have mainly been used to produce transgenic mice that express the human genes for neurodegenerative pathogenesis and Alzheimer’s disease (AD). The aim of this study was to produce doubly transgenic mice expressing both the regulative tet promoter-controlled transactivator (tTA) and human mutant preseilin 2 (N141I, hPS2m) genes, which direct the genetic switches to a level up or down from the transgene. These transgenic mice were then used to examine several of the pheno-
types associated with AD and Wnt signal pathways. We concluded that the group that had doxycycline removed from their drinking water showed the induction of the transgene, Wnt signal defect, behavioral impairment, and elevated Aβ-42 and β-secretase activity compared with the group treated with doxycycline. In parallel, the expression levels of the hPS2m transgene gradually declined in the transgenic males, with clear changes being appar-
ent between two and four weeks of age. Therefore, tet-regulated transgenic mice can be used to increase the understanding of aspects of neurodegen-
erative pathologies and effect of the basal or inducible expression levels of hPS2m on the pathology of AD at the “on/off” states.

P77. DUAL ASSESSMENT OF HUMAN GLIOMA INVASION IN ORGANOTYPIC RAT BRAIN-SLICE CULTURES BY TIME-LAPSE FLUORESCENCE MICROSCOPY AND IMMUNOHISTOCHEMISTRY
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Introduction: Implantation of biopsy fragments from human gliomas into organotypic rat brain-slice cultures has been used to study glioma invasion. Many studies quantify glioma invasion based on fluorescence microscopy. In this study, we improve the method by performing time-
lapse recordings and by immunohistochemical identification of the invasive human glioma cells.

Methods: Organotypic rat brain-slice cultures and spheroids of freshly harvested human gliomas were prepared and cultured separately for one week. The spheroids were then incubated with the fluorescent dye Difl and imaged by time-lapse microscopy. This in vitro coculture system was monitored for up to two weeks with computerized time-lapse fluorescence microscopy using an inverted fluorescence microscope placed in a CO2 incubator. Photomicrographs were taken every 30 min. Larger series of slice cultures implanted with glioma spheroids were photographed by conventional inverted fluorescence microscopy at four- to six-day intervals. At the end of the experiment, the cultures were processed for immunohistochemical staining with an antibody specific to human nuclei.

Results: The invasion of human glioma cells into organotypic brain-
slice cultures was successfully monitored for up to 14 days by time-lapse and conventional fluorescence microscopy, demonstrating how glioma cell density increased with time. The glioma cell density was highest in the central area and decreased in the more distant zones. For the first time, we demonstrate the presence of invasive human glioma cells histologically by using the antihuman nuclei-specific antibody, confirming the findings of the photomicrographs.

Conclusions: Dual assessment of human glioma invasion in organotypic rat brain-slice cultures by time-lapse fluorescence microscopy followed by immunohistochemical identification of the invasive human glioma cells rep-
resents a powerful assay. This protocol allows detailed dynamic studies of glioma invasion over time, and we improve previously presented methods considerably by adding subsequent immunohistochemical detection of the invasive glioma cells, which enables future double-immunohistochemical studies.

P78. A NEW IN VITRO 3-D BRAIN TUMOR COCULTURE MODEL USED TO STUDY NANOPIRATE UPTAKE AND TUMOR INVASION
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Current in situ and in vivo brain tumor invasion models fail to pro-
vide the detailed information about the mechanisms underlying local invasion; therefore, numerous in vitro models have been developed to study these mechanisms. So far, there have been no in vitro models that take fully into account the structural organization and dimensionality of the host brain tis-
ue. Similarly, few investigations of drug delivery systems in an in vitro brain tumor invasion model have been found. Therefore, we aimed to develop a new 3-D coculture model by using brain tumor spheroids and organotypic brain slices to study the uptake of nanoparticles (NPs) and tumor invasion. For the uptake studies, we have used NPs prepared from a novel polymer, which were stabilized by Tween-80, and contained the fluorescent dye rhodo-
damine B isothiocyanate (RBITC) to represent the drug.

The 3-D model was established by coculturing tumor spheroids and organotypic rat brain slices. Cerebral slices were prepared from two-day- old neonatal rats, and tumor spheroids were formed from a medulloblas-
toma cell line (DAOY). The cocultures were grown for four days prior to
adding RBITC-labeled NPs into the culture medium and incubated for 24 h. Three fluorescent markers were used to label nuclei (DAPI), DAOY cells (FITC), and NPs (RBITC) and observed under the confocal microscope.

When DAOY spheroids were placed on the brain slices, DAOY cells migrated into brain slices after two-day coculture. Significant invasion of tumor cells was observed after four-day culture, DAOY cells not only reached the bottom of slices but moved up to 972 μm away from DAOY spheroids after six days in culture. Confocal micrographs illustrated that the NPs were preferentially taken up by the DAOY cells, with few NPs distributed in the host brain cells growing in the organotypic slice. These results were supported by a fivefold increase in NP uptake by DAOY spher-
oids in comparison with normal brain cell spheroids over a 24-h period.

The results suggest that this novel in-vitro model system, which mim-
ics the in vivo conditions of regional medulloblastoma invasion, could be used to effectively evaluate the selectivity of a drug delivery system between tumor cells and brain cells. Additionally, it will provide greater opportunity to evaluate antineurogenic therapeutic potential of drugs and drug delivery systems in a more realistic in vitro tumor/host setting.

P79. ANGIogenesis IN EXTRACRANIAL HUMAN GLIOMA IMPLANTED IN NUDE MICE: AN IMMUNOHISTOCHEMICAL, CORROSION CASTING–SCANNING ELECTRON MICROSCOPY STUDY
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Introduction: We applied corrosion casting–scanning electron micro-
scopic analysis (CC-SEM) and immunohistochemistry (IHC) to study the three-dimensional microvascular architecture of a malignant human extra-
cranial glioma implanted in the epicranic tissues of nude mice.

Materials and methods: A total of 50,000 U87–MG glioma human modified cells were implanted intracranially in 40 nude mice to obtain malignant gliomas. After 30 days of growth, the tumor infiltrated the dural sheet and the bone, exiting the cranium through the site of injection and diffusing into the subcutaneous connective and muscular tissues. The CC technique and IHC were performed to better understand the two-and three-dimensional microvascular architecture of both the tumor and the host epicranic tissues, along with their modifications.

Results: CC-SEM analysis and IHC analysis demonstrated an equalization both in orientation and shape of epicranic muscular vessels. In comparison to the control, the longitudinally oriented vascular trabeculae turn into enlarged disorganized vessels oriented toward the tumor. The intervascular dis-
tances, branching angles, and interbranching distances gradually decrease next to the tumor. On the vascular casts, it is possible to observe both dome sprouts and intussusceptive holes, clear signs of remodeling neangiogenic effects. Entering the tumors, these vessels end into vascular lacunae, some times delimited by the tumor cells themselves. The intratumoral vessels have a great variability in diameter, becoming tortuous, and losing any architectural organization. The endothelial cell lining is impaired, with a visible enlargement of intercellular junctions, and the consequent leakage of the resin in the interstitial spaces is documented.

Conclusions: CC-SEM and IHC analysis revealed a sharp method for focusing on the remodeling effects that infiltrating gliomas have on the microvascular structure of epicranic tissues and a good model for studying
both the spouting and intrususceptive angiogenic mechanisms that occur on host normal vasculature. It was also possible to picture well the timing of dynamic processes of infiltration and co-option, looking at the modification that occurred in the capillary net of epiptic tissue.

P80. VALPROIC ACID IS TOXIC TO MALIGNANT GLIOMA CELLS AND INCREASES SENSITIVITY TO IRRADIATION AND CHEMOTHERAPY

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Glioblastoma multiforme is the most frequent and the most aggressive of glial tumors, with a median survival of nine months, despite surgical resection, radiotherapy, and temozolomide treatment. Glioblastoma multiforme can be regarded as an abnormal “organ,” of which the growth is regulated by cancer stem cells rather than a homogenous mass of cells with unregulated growth capacity. If these cancer stem cells have a different therapeutic response as compared to their successive cancer cells, then the inefficacy of conventional therapy, the objective of which is to eliminate tumor mass, may be explained by remaining cancer stem cells that re-form the tumor. Valproic acid (VPA), a well-known anticonvulsant, inhibits histone deacetylate and has been shown to induce tumor differentiation, apoptosis, and growth arrest. In the present study, the therapeutic response to VPA was evaluated in a number of glioma cell lines (U87, U251, U373, U106, and T98G). The cytotoxicity of VPA was determined at clinically relevant concentrations in combination with irradiation and chemotherapy.

VPA treatment alone decreased the clonogenic potential of glioma cell lines. Glioma cell lines were sensitized to chemotherapy, such as cisplatinum and temozolomide, after 24 h of pretreatment with VPA, as measured by MTT assay. Pretreatment for 24 h with VPA prior to irradiation decreased the ability to form colonies in a clonogenic assay.

These results show that VPA as single treatment is toxic to glioma cells at clinically relevant concentrations and that its mechanism of action may not be the sensitization of cells to conventional therapy but rather a direct cytotoxic effect.

P81. VEROTOXIN 1 INHIBITS CELL VIABILITY AND INDUCES APOPTOSIS IN CD77-EXPRESSION HUMAN GLIOMA CELL LINES

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This study examined the toxicity and mechanism of apoptosis induction of verotoxin-1 (VT-1) on human glioma cell lines. VT-1 is a member of the Shiga-toxin family expressed by some serotypes of Escherichia coli and Shigella dysenteriae. Shiga toxins have been shown to induce apoptosis by binding to its membrane receptor CD77. The human glioma cell lines SF-767, U-343 MG, and U-251 MG were studied together with BT4C, a rat glioma cell line. Cells were first screened for CD77 expression by flow cytometry. Fluorescein diacetate was used to determine cell viability after VT-1 exposure, and apoptosis was studied by TUNEL staining, a mitochondrial membrane potential assay, and caspase-3, caspase-8, and caspase-9 activity assays. SF-767 and U-343 MG cells were found to express CD77 and were also sensitive to VT-1-induced cytotoxicity, whereas non-CD77-expressing U-251 MG and BT4C glioma cells were not. VT-1 depolarized the mitochondrial membrane and activated caspase-9 and caspase-3 of SF-767 and U-343 MG cells, and exposure to 5 μM/liter VT-1 for 72 h resulted in approximately 60% and 90% TUNEL-stained cells, respectively. D,L-Triose-1-phosphatase was used to block CD77 synthesis; 2 μM/pmol PPMP for 72 h abolished SF-767 and U-343 MG expression of CD77 and made the cells completely resistant to VT-1, with no caspase activation or TUNEL staining. A pancaspase inhibitor confirmed that caspases are required for VT-1-induced apoptosis in SF-767 and U-343 MG cells. The high specificity and apoptosis-inducing properties of VT-1 indicates that the toxin may be a potential antineoplastic agent for CD77-expressing gliomas.

P82. DTI FINDINGS ARE COMPLEMENTARY TO INTRAOPERATIVE SUBCORTICAL LANGUAGE MAPPING FOR SURGICAL REMOVAL OF LOW-GRADE GLIOMAS INVOLVING SPEECH AREAS

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Surgical removal of low-grade gliomas in, or in close proximity to, speech areas or pathways requires the localization of cortical and subcortical sites that mediate speech function. Intraoperative subcortical language mapping has been used to identify functional language tracts during resection of hemispheric low-grade gliomas invading language areas, minimizing the definitive morbidity and maximizing the quality of resection. DTI is a technique that allows the identification of functional tracts in the preoperative MRI and establishes their anatomical relationship with the tumor mass. In this work, we used DTI for visualization of language tracts in patients with low-grade gliomas in or involving speech areas or pathways, and we correlated DTI data with those obtained during intraoperative subcortical language or motor tract mapping. Thirty patients with low-grade gliomas were included in the study: 17 males and 13 females, with 17 gliomas located in the left frontal lobe, four in the left parietal lobe, and nine in left temporal lobe. DTI data were acquired by the use of 3 T MR scanner (Philips Intera) with a single-shot echo-planar sequence with gradients applied along 32 noncollinear directions. Fiber tracking was performed with a dedicated software.

Intraoperative subcortical language tract mapping was performed during awake craniotomy, by the use of a Ojemann stimulator, during object, famous people, and action naming, and word and sentence comprehension tasks. Data obtained during intraoperative mapping were correlated point by point with those identified with DTI by the aid of a neuronavigation system (Radionics). DTI allowed the reconstruction of fascicular arcuateus, fronto-occipitalis, uncus, subcallosus in the frontal lobe, longitudinalis and uncinatus in the temporal lobe, and helped in understanding the anatomical relationship between tracts and the tumor mass during the preoperative planning. Intraoperative language mapping identified functional subcortical sites in all patients. When DTI data were correlated with those from intraoperative mapping, sensitivity was 70% for motor tracts and 72% for language tracts. Our data support the use of DTI for language and motor tracts for surgical removal of low-grade gliomas involving speech areas or pathways.

P83. THE ANATOMOFUNCTIONAL CONNECTIVITY OF LANGUAGE REVISTED: APPLICATION TO SURGERY OF WHO GRADE II GLIOMAS

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Rationale: Despite extensive study of the cortical networks of language, due to the development of functional neuroimaging, the anatomofunctional connectivity of language remains poorly understood. Here, we used intra-surgical electrical cortico-subcortical stimulation in patients operated on for a low-grade glioma in the dominant hemisphere, with the double goal of studying the organization of the subcortical language pathways and applying this knowledge to optimize surgical management.

Methods: A total of 87 patients with a WHO grade II glioma within language areas were operated upon while awake, using direct electrostimulation to perform online cortico-subcortical language mapping all along the resection. Anatomofunctional correlations were possible by coupling the precise location where the stimulations have been applied using anatomical MRI and the transient language disorders elicited—accurately analyzed by a speech therapist.

Results: We were able to determine the anatomical trajectory and the functional role of the cortico-cortical, long-distance association and cortico-subcortical language pathways, as follows: (1) arcuates fasciculus, eliciting phonemic paraphasias when stimulated; (2) inferior fronto-occipital fasciculus, generating semantic paraphasias when stimulated; (3) subcallosal fasciculus, inducing transcortical motor aphasia during stimulation; (4) frontoparietal phonological loop, eliciting articulatory disorders during stimulation; and (5) striato-premotor loop, inducing perseverations when stimulated. These structures were preserved, representing the limits of the resection. Despite an immediate postoperative worsening, 85 patients (97.7%) recovered a normal neurological (especially language) examination within three months after surgery and returned to a normal professional life. The quality of resection, systematically evaluated by repeated MRIs, showed that 88% of removal was total or subtotal.
Conclusions: The use of direct cortical and subcortical electrostimulation offers a unique opportunity to perform a safe, reliable, accurate, and reproducible real-time anatomofunctional study of the language connectivity. Such knowledge allows (1) a better understanding of the language circuit, organized in parallel cortico-subcortical distributed networks; and (2) an optimization of the benefit–risk ratio of the surgery in low-grade glioma.

P84. TIME COURSE AND SIGN OF MALIGNANT TRANSFORMATION OF DIFFUSE ASTROCYTOMA AS SEEN BY PET
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Background and objective: Although the precise natural history of diffuse astrocytoma (DA; malignancy grade II) remains unclear, the majority of these tumors eventually transform to become malignant (malignancy grades III or IV) and are fatal within several years. For therapeutic planning, it is important to detect their malignant transformation as early as possible within their long biological history. The aim of this study was to evaluate positron emission tomography (PET) images of DA and anaplastic astrocytoma (AA), and to produce a hypothesis regarding the signs and the time course of malignant transformation of DA as seen by PET.

Materials and methods: Twelve patients with DA, eight patients with locally malignant transformed DA, and 10 patients with AA, who underwent [18F]-fluorodeoxy glucose ([18F]FDG), [11C]-choline, and [11C]-methionine [MET] immediately before surgery, were eligible for this study. In each tumor, the standard uptake value (SUV [<g/ml]) of FDG, choline, and MET were measured, and the uptake ratios (max. SUV of tumor/max. SUV of the contralateral normal occipital cortex) of these tracers were calculated.

Results: The uptake ratios of FDG in DA, locally malignant transformed DA, and AA were 0.82 ± 0.05, 0.94 ± 0.05, and 0.95 ± 0.05, respectively; those of choline were 1.76 ± 0.21, 2.87 ± 0.24, and 3.11 ± 0.31; and those of MET were 1.47 ± 0.14, 1.96 ± 0.1, and 2.0 ± 0.1.

Conclusions: The time course of malignant transformation of DA as seen by PET suggests that, first, MET uptake is increased, secondly choline, and lastly FDG, in that order. The following hypothesis was developed. A definitive sign of malignant transformation of DA is an increased uptake of FDG on PET. A probable sign is a locally increased choline uptake, even if there is not an increase in FDG uptake. An increased MET uptake is a possible sign of malignant transformation. MET uptake is inevitably increased in malignant transformed DA and is also increased even in some DAs without malignant transformation. Such DAs with increased MET uptake, but no other signs of malignant transformation, may be in an advanced stage of the biological history of malignancy grade II, just before transforming into a malignant stage. On the other hand, DA without an increased MET uptake is suggested to be far from malignant transformation.

P85. [18F]-FLUORODEXYOGLUCOSE UPTAKE IS ASSOCIATED WITH 1P AND 19Q LOSS IN WHO GRADE II GLIOMAS
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Objective: Oligodendrogial tumors harboring combined losses of heterozygosity on 1p and 19q (LOH1p/19q) are characterized by a favorable prognosis and response to chemotherapies and radiotherapy. Detection of LOH1p/19q losses relies on time-consuming postoperative procedures. We investigated the potential of [18F]-fluorodeoxy glucose (FDG) uptake in position emission tomography (PET) to predict LOH1p/19q in tumors consistent with low-grade gliomas and an intraoperative MRI scan.

Methods: We included 24 patients with operative FDG PET followed by tumor resection. Neuronavigation assured a precise match of FDG uptake with the site of biopsy. All tumor specimens were graded according to WHO criteria. LOH1p/19q was determined by microsatellite analysis.

Results: In this series, 15 of 24 gliomas corresponded to WHO grade II. LOH1p/19q was detected in six of these 15 WHO grade II gliomas. Raised glucose utilization within the tumor was seen in the five of six WHO grade II gliomas with LOH1p/19q and in none of the WHO grade II gliomas without this genetic alteration (P = 0.002).

Conclusion: These findings demonstrate the potential of FDG PET to predict LOH1p/19q in WHO grade II gliomas.

P86. AUSTRIAN NATIONAL LOW-GRADE GLIOMA STUDY
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Introduction: Postoperative policy for low-grade gliomas (LGGs) is still controversial. We present the results after long-term follow-up with a maximum of 33 years of 204 patients irradiated immediately after surgery.

Materials and methods: We retrospectively analyzed 204 patients with histologically proven LGG. All patients in the analysis were consecutively included, randomly unscreened patients from daily practices, who had been irradiated between 1964 and 1985 with megavoltage (range, 40–66 Gy). The overall survival curves were calculated for more than three decades of follow-up, and the prognostic factors that influence the outcome were identified.

Results: The overall survival curve shows a median follow-up of 50 months. The survival curves decreases further exponentially, with 28% survival at 10 years, and 14% survival at 20 years, followed by a stabilization of the survival curve at 12% without further decrease up to 33 years. There is a significant advantage in survival for patients with an irradiated volume of 500 cc or less compared to those with larger irradiated volumes: Five-year survival is, respectively, 86.4% and 45.3% (log-rank P = 0.019), and the 20-year survival is 32.3% and 15.4%, while the median survival is 147 and 50 months. Patients under 35 years of age show a significantly better survival than do patients between 35 and 45 years and less than 45 years, with a five-year survival of 64.4%, 53.3%, and 29%, respectively, while the 20-year survival is 23.7%, 7.7%, and 1.9% (log-rank P = 0.056).

Conclusions: This is the first analysis of a large series of megavoltage-irradiated low-grade gliomas with a follow-up of more than three decades. For the first time, it is shown that an irradiated volume of maximum 500 cc of the brain tissue is the most important prognostic factor, with a median patient...
survival of 147 months, compared to only 50 months for larger irradiated volumes, and still a 20-year survival of more than 30%. For the first time, it is shown that LGG can be a curable disease if surgery and irradiation are not delayed too long at the time of early diagnosis. This should encourage clinicians not to postpone treatment in these LGG patients.

Abstracts for the Seventh Congress of the European Association for Neuro-Oncology (EANO)

P89. PREOPERATIVE DIFFERENTIAL DIAGNOSIS OF LOW-GRADE GLIOMAS WITH MR SPECTROSCOPY (MRS)


Purpose: Prognosis and treatment of low-grade gliomas depends on differential diagnosis (oligodendroglioma vs. astrocytoma) and accurate grading. Preoperative MR spectroscopy provides valuable information regarding tumor grade, but lacks clarity in subtyping low-grade gliomas. Thus, new technical progress of MRS was investigated to improve the significance for preoperative glioma subtyping.

Methods: Eighty-two patients harboring histologically verified low-grade glioma (46 astrocytomas, 23 oligodendrogliomas, and 15 oligoastrocytomas) were retrospectively investigated. Forty-four patients underwent MRS by a clinical whole-body imager (Gyreson Intera 1.5T, Philips). Eight of them had an additional examination of the corresponding area or white matter in the nonpathological hemisphere. Difference metabolites (NAA, Cho, Cr, GABA, Asp, Tau, Lac, Glc2Glc, Glx, and mI) have been quantified, and ratios (NAA/Cho, NAA/Choc, and ChoCr) have been calculated. Metabolite changes and ratios were compared among each other and to control subjects.

Results: We found a good correlation between astrocytosis and oligodendroglioma or oligoastrocytoma in the number of metabolite changes referring to a control subject of the same gender and approximately the same age. There was a moderate increase of Cho in astrocytomas (52% ± 37%) versus a distinct increase in oligodendrogliomas (127% ± 56%). Cr mostly decreased in astrocytomas (-18% ± 38%) and increased in oligoastrocytomas (68% ± 60%). Due to the actual small number of control subjects, an expansion of these groups is proposed.

Conclusion: With further improvements in the MRS method, a preoperative MRI differential diagnosis of glioma subtypes seems possible.

P90. MRI GROWTH RATES CAN HELP DIFFERENTIATE "TRUE" AND "FAKE" WHO GRADE II GLIOMAS

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Introduction: WHO grade II gliomas are slow-growing infiltrative tumors. In a previous study, we showed that, before malignant transformation, their radiological tumor diameter increases at an average rate of 4 mm/year (range, 2.1 – 8.5 mm/year). The wide range of prognosis for grade II gliomas is probably due to their heterogeneous biological behavior. The aim of this study is to determine whether individual radiological tumor growth rates (IGRs) could be of prognostic value.

Methods: We reviewed a consecutive series of 143 unselected adult patients with histologically proven WHO grade II gliomas, for whom longitudinal follow-up was available before treatment. Repeated measurements of the mean tumor diameter were performed for each patient before malignant transformation (minimal interval of three months; at least two consecutive MRIs). Then, IGRs were fitted by linear regression. Survival by IGR was analyzed using Kaplan-Meier survival curves and log-rank tests to assess statistical significance.

Results: The median IGR of each patient was 4.4 mm/year (mean, 5.4; range, 1.0 – 15.8). A total of 121 patients (84.6%) had an IGR under 8 mm/year, and only 22 (15.4%) had an IGR at ≥ 8 mm/year or more. Between these two groups, there were no statistical difference regarding gender, age at radiological diagnosis, clinical presentation, tumor volume, surgical procedure used for histological diagnosis, or subsequent therapeutic strategy. There is an inverse correlation between IGRs and survival (P < 0.001), with a median survival of 5.16 years for an IGR of 8 mm/year or more and a median survival of longer than 15.0 years for an IGR under 8 mm/year. Neither histological diagnosis, tumor volume, nor the age at radiological diagnosis statistically influences survival.

Conclusions: In this study, we have shown that the prognoses of histologically proven grade II gliomas growing faster than 8 mm/year are similar to those of anaplastic gliomas. These results suggest performing a second MRI systematically three to six months after the first exam to detect IGRs of 8 mm/year or more. Thus, IGR, a dynamic macroscopic parameter easily available in clinical practice, should be incorporated along with the other "static" parameters to choose the most appropriate therapeutic strategy.

P91. O6-METHYLGUANINE-DNA METHYLTRANSFERASE (MGMT) PROMOTER METHYLATION AND RELATION TO IP/19q LOSS IN LOW-GRADE GLIOMAS: A GIGNO STUDY

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Background: 1p and 19q deletions have been associated with a favorable response to chemotherapy and a good prognosis in patients with oligoden- droglioma. MGMT promoter methylation has been associated with better survival in patients with glioblastoma who receive alkylating agents. As yet, there are no data on the expression of MGMT, and on the relationship between 1p/19q deletions and MGMT promoter methylation in low-grade glioma (LGG).

Methods: Patients who received a first-line chemotherapy regimen with temozolomide for progressive LGGs were enrolled in the study, which was designed to investigate the correlation between MGMT methylation status and 1p/19q deletions in this setting. 1p/19q deletions were analyzed by FISH, and MGMT promoter methylation by methylation-specific PCR (MSP).

Results: Seventy-five patients (26 women and 49 men; median age, 42 years; range, 22–68 years) were accrued. Of these, 48 (64%) had oligodendrogliomas (O), 19 (25.3%) had astrocytomas (A), and 8 (10.6%) had oligoastrocytomas (OA); 44 (58.7%) had a history of epilepsy, 41 (54.7%) had a frontal tumor localization, 27 (36%) had MRT contrast-enhancing lesions, and 35 (46.7%) had been pretreated with radiotherapy. 1p/19q deletions, evaluable in 38 patients (77.3%), were both present in 36 patients (94.7%), and one patient (2.6%) had 1p loss and 3 patients (5.2%) 19q loss. Combined 1p and 19q loss was not correlated with a frontal localization (P = 0.12), median age (0.47), and/or gender (0.62). MGMT promoter methylation, present in 17 (56.6%) of 30 assessable cases, was significantly associated with combined 1p/19q deletions (P = 0.03). MGMT promoter methylation was not significantly associated with age (P = 0.46), gender (P = 0.2), tumor localization (P = 0.12), and/or histology (P = 0.37).
Conclusions: 1p/19q deletions are strictly correlated to histology and to MGMT promoter methylation. Further prospective trials are required to clarify the impact of these molecular signatures on clinical outcome.

P92. TREATMENT OF LOW-GRADE OLGODENDROGLIOMAS WITH PCV CHEMOTHERAPY
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Background: Favorable prognostic factors have been reported for oligodendroglial tumors, including age younger than 40 years, low tumor grade, extent of resection, and molecular specificities such as 1p/19q deletion. We report the outcome of 57 patients with low-grade oligodendrogliomas diagnosed between 1995 and 2003 treated either with chemotherapy or radiotherapy as the front-line treatment.

Patients and methods: The tumors were rated histologically according to the WHO classification as low grade (grade II) or anaplastic (grade III), and transformation was documented. All patients were symptomatic at presentation and underwent neurosurgical procedure for histological diagnosis. Response was evaluated with clinical assessment, brain MRI, and MIBI scintigraphy.

Results: Patient characteristics were the following: 41 men and 16 women; and mean age at pathological diagnosis, 46.5 years. The most common first symptom was partial epileptic seizure (73.7%). Thirteen patients (22.2%) had initial gadolinium enhancement, generally associated with MIBI hypermetabolism (P < 0.0001). Twelve patients (19.3%) had total resection, 21.1% partial resection, and 59.6% had biopsy only. Of the patients, 78.9% had a second-line treatment after the initial surgery: 33 patients (73.3%) had PCV chemotherapy regimen, 8 patients (17.8%) had brain radiotherapy, 4 patients underwent surgery, and 12 patients with total resection had no second-line treatment. Twenty-one patients (36.8%) had a malignant transformation during the follow-up, with a median time to progression of 105 months. Twenty patients had third-line treatment (14 with chemotherapy and 6 with radiotherapy). Survival rates at 2, 5, and 10 years were, respectively, 85%, 75%, and 50%.

Conclusions: Up-front chemotherapy with PCV regimen is a good treatment for symptomatic low-grade oligodendroglioma. The results suggested that radiotherapy could be postponed until the malignant progression occurs.

P93. PROGRESSIVE HYPOTHALAMIC-CHIASMATIC GLIOMA: TUMOR STABILIZATION UNDER TREATMENT WITH IMATINIB
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Pilocytic astrocytomas of the hypothalamic-chiasmatic region account for up to 20% of tumors in patients younger than 2 years of age. While most children respond to chemotherapy, alternative treatment approaches are needed for those with refractory, progressive, and/or metastatic disease. Imatinib, a methylpiperazine derivative, inhibits the autophosphorylation of kinases such as BCR-ABL, c-KIT, and platelet-derived growth factor receptor (PDGFR). Imatinib is established as a safe and effective therapy in patients with neurofibromatosis. All six were treated with temozolomide. Four patients were treated with imatinib at a daily dose of 215 mg/m2. One of the patients showed regression in tumor size, four remained stable during treatment, and one is too early to evaluate.

We conclude that treatment with imatinib may warrant further study in children with refractory and progressive pilocytic astrocytoma.

P94. LOW-DOSE CHEMOTHERAPY PRODUCES PRONOUNCED METABOLIC RESPONSES IN MR STABLE DISEASE PATIENTS WITH LOW-GRADE GLIOMA (LGG)
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Background: LGG can respond to chemotherapy. Here we used positron emission tomography (PET) to investigate the effect of continuous low-dose temozolomide (TMZ) on tumor uptake of the amino acid F-18 fluoro-ethyl-tyrosine (FET). FET reflects tumor activity and represents a surrogate marker of microvascular density. We complemented our PET studies by PET measurements of tumor blood flow (CBF), using O-15 labeled water.

Methods: We prospectively enrolled 10 patients with progressive or recurrent WHO grade II LGG, and 75 mg/m2 TMZ was administered 2.5 days (one cycle). MR and PET measurements were performed before, and three, six, and 12 months after the initiation of chemotherapy. Region-of-interest analysis was used to quantify tumor tracer uptake which was normalized to cerebellar uptake (T:C). Active tumor was defined as a 110% cutoff of cerebellar activity. This provided measures of active tumor volume, global and peak tumor CBF, and FET uptake. MR FLAIR sequences were used for calculation of the tumor volume.

Results: A total of 97 TMZ cycles was administered. No patient showed tumor progression on MR or PET. Applying the Macdonald criteria, the MR tumor volume remained stable for six months, after which a partial response (>50% volume reduction) was achieved in two patients. The active tumor volume (PET uptake) was reduced by 31% already at three months. At six and 12 months, mean reductions of 42% and 61% were observed. One further patient showed complete tumor deactivation. Tumor PET uptake and CBF showed similar trends.

Conclusions: We demonstrate biochemical responders among patients otherwise classified as having stable disease by MR. Reduced FET uptake due to chemotherapy may serve as an early marker more closely associated with tumor growth inhibition than would MR FLAIR imaging. The impact of CBF reductions in terms of drug delivery, and with regard to the long-term course, has now to be addressed.

P95. ROSETTE-FORMING GLIONEURONAL TUMOR OF THE FOURTH VENTRICLE: EXPERIENCE WITH THREE PATIENTS
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Objective: Rosette-forming glioneuronal tumor of the fourth ventricle, first described in 2002, is generally regarded as benign. We report on three patients with this tumor treated at our department since March 2003.

Methods: Retrospective analysis of the clinical, radiological, and surgical data was performed. Biopsy specimens were embedded in paraffin and stained with hematoxylin-eosin and immunostaining.

Results: Two men, aged 49 years, 22 years, and 38 years, respectively, were operated on between March 2003 and November 2004. Signs and symptoms: In one patient, the tumor was an incidental finding, one patient presented with discrete cerebellar ataxia, and the third patient presented on an emergency basis with obstructive hydrocephalus. Neuroradiological findings: MRI showed a solid cystic tumor located in the cerebellar midline–fourth ventricle, with slight contrast enhancement in two patients and small calcifications in two patients. The tumor appeared relatively well circumscribed with no perifocal edema. Tiny non-contrast-enhancing nodular satellite lesions in the surrounding cerebellar cortex were noted. One patient had a large cyst with signs of bleeding and consecutive obstructive hydrocephalus. Histology: The typical two components with neurocytic rosettes and astrocytic cells dispersed in a fibrillary matrix were used for calculation of the tumor volume.

Conclusions: Our report supports the proposal of this tumor type as a separate histological entity. Until now, our patients have experienced the reported benign clinical course, although one patient had a life-threatening clinical presentation because of hydrocephalus. This tumor has to be considered in the differential diagnosis of cerebellar–fourth ventricular tumors.

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P96. ASSOCIATION OF TP53 CODON 72 POLYMORPHISM WITH GLIOMA TUMOR RISK

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TP53 is a key tumor-suppressor gene that is frequently inactivated by gene mutations in gliomas, where it is considered an early event. It encodes a transcriptional factor that is involved in several cellular mechanisms, including growth arrest, DNA repair, and induction of apoptosis. A common TP53 polymorphism, Arg72Pro, is located in exon 4, which is a proline-rich region important for pS3 protein function. The Pro72 variant has been associated with a slower induction of apoptosis that could therefore influence the risk of cancer development. The association of this Arg72Pro polymorphism to glioma susceptibility is not clear.

The aim of this study was to analyze the influence of TP53 Arg72Pro polymorphism as a predisposing factor for gliomas in a Portuguese population. A case-control study, including 159 patients with glioma and 326 healthy individuals, was performed. The genotype of Arg72Pro was assessed by PCR-RFLP technique.

The genotype and allelic frequencies of gliomas and control group did not show any statistical differences. The further stratification of gliomas by histological type showed a significant statistical difference in Pro/Pro genotype frequencies when patients with low-grade astrocytomas (n = 28) and healthy individuals (n = 326) were compared. Pro72Pro genotype carriers have approximately a fourfold increased risk of developing a low-grade astrocytoma, compared with the individuals with Arg72Arg (OR = 3.70; 95% CI, 1.24–11.06; P = 0.013) and Arg72Pro (OR = 3.47, 95% CI, 1.24–9.75; P = 0.012) genotypes. Furthermore, no significant statistical differences were observed in Arg72Pro genotype frequencies when patients with high-grade astrocytoma or oligodendroglioma and healthy groups were compared. In conclusion, our study shows an association of Arg72Pro TP53 polymorphism with increased risk of low-grade astrocytoma. Nevertheless, because of the small number of tumors analyzed, more studies are required to confirm these results. In addition, it would be important to evaluate the role of this polymorphism in the progression of low-grade astrocytoma.

P97. MICROVESSEL DENSITY (MVD) AND BLOOD FLOW (CBF) IN LOW-GRADE GLIOMAS (LGG): A PET STUDY

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Background: Histopathological studies reveal MVD as prognostic factor in LGGs. We used positron emission tomography (PET) to study in vivo spatial characteristics of tumor CBF (O15 H2O) and MVD (F18-fluor-ethyl-tyrosine, FET, as surrogate marker). These parameters may be critical for drug delivery.

Methods: Thirty patients with WHO grade II LGG were studied. Region of interest (ROI) analysis was used to quantify tumor tracer uptake, which was normalized to cerebellar uptake (ROI). Active tumor was defined as a 30% cut-off of cerebellar activity. This provided measures of active tumor volume, and global and peak tumor CBF and FET uptake. Race ROI across tumor created pixelwise profiles of CBF and FET uptake (MVD). Standard MR sequences were used for spatial correlations.

Results: Global and peak FET uptake corresponded to CBF increases (Spearman rank, P < 0.05). Also, the volumes of increased CBF and FET uptake were correlated (R = 0.01). Trace ROIs showed that, irrespective of increased MVD at the tumor periphery, CBF increases were confined to the tumor center. Tumors with contact to, or infiltration of, the corpus callosum showed reduced or even absent CBF and MVD in these particular regions. MVD and CBF patterns were not reflected in the activity of the tumors, which almost presented as homogeneous non-gadolinium-enhancing lesions.

Conclusions: LGGs are heterogeneous tumors with regard to regional vessel density and blood flow. At the tumor periphery, where tumor infiltration of surrounding brain occurs, blood flow may be low irrespective of increased vascular density. An ongoing study investigates the effect of chemotherapy on these measures.

P98. IMAGING GIOBLASTOMA USING IODINE-123-LABELED VASCULAR ENDOTHelial GROWTH FACTOR FACTOR 165 (123I-VEGF165)

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Aim: Glioblastoma is characterized by hypervascularity and invasive potential. Vascular endothelial growth factor (VEGF) is a major angiogenic factor, and its receptor has been shown to be overexpressed in human glioblastoma in vitro. In this study, we investigated the usefulness of scanning with VEGF-165 labeled with iodine 123 in patients with glioblastoma.

Materials and methods: Eight patients (three women and five men; mean age, 65 ± 11 years) with histopathologically verified glioblastoma and two male patients with benign glioma were included in the study. Dynamic acquisition was initiated immediately after intravenous administration of 123I-VEGF165 (190 ± 15 MBq) and carried out until 30 min after injection. SPECT was performed at various time points post injection (p.i.). Whole body images were acquired in anterior and posterior views at 1 h p.i.

Results: Glioblastoma lesions were visible shortly after intravenous injection of 123I-VEGF165 and were still demonstrated clearly 18 h p.i. All tumors were visualized in four patients with glioblastoma. 123I-VEGF165 was negative in four patients after receiving radiotherapy and chemotherapy. Negative scan results were also obtained in two patients with benign glioma. In vitro binding results confirmed high specific binding of 123I-VEGF165 to glioblastoma cells and tissues.

Conclusion: Our results suggest that 123I-VEGF165 scintigraphy may be useful for visualization of untreated glioblastoma, and its angiogenesis activity might be useful in monitoring treatment response.

P99. CERVICAL SPINE EPENDYMOMA: CASE REPORT

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This report presents the authors’ experience in this field, based on 42 cases operated on between January 2000 and February 2006 in Cluj-Napoca County Hospital, First Neurosurgical Department, Unive, Cluj-Napoca, Romania.

Clinical history: This 36-year-old woman presented with a two-year history of cervical-thoracic pain irradiating from her upper extremities. At clinical examination, tetraparesis, bilateral loss of temperature, pain sensation at the C5–D1 level, and urinary disturbances were present.

Preoperative MRI: Sagittal T1WI: There was focal enlargement of the cervical and thoracic spinal cord at the C3–D2 level, with hypointense signal above and below the tumor. Sagittal PD image: The tumor was hyperintense to the spinal cord, and the dark rim was better seen. Sagittal T2WI: Spinal cord edema was clearly visible. Sagittal Gd T1WI: No contrast enhancement was visible. Axial T1WI: The enlarged spinal cord filled the entire spinal canal. Axial and coronal T2WI: The tumor was hyperintense.

Surgery: C3–D1 laminectomy was performed, and an easy cleavage plane found between normal spinal cord and the tumor, which was completely removed by microsurgical techniques.

Histology: Grade II ependymoma.

Postoperative clinical evolution: Immediately after surgery, the patient’s clinical condition was good. After four months, the patient’s condition improved (McMorricon grade I), and the tetraparesis was in remission, although the deficits in pain and temperature sensation persisted at C6–C7 until eight months after surgery. One year after surgery, discrete spasticity was still present, but the urinary disturbances and the deficits in pain and temperature were in remission.

Follow-up MRI: Sagittal T1WI: The site of resection was clearly seen. Axial T1WI: The spinal cord was flattened at the level of the tumor resection.

P100. SURGICAL MANAGEMENT OF RARE MALIGNANT INTRASPINAL TUMORS

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Aim of the study: To stress the importance of early surgical management of rare intraspinal malignant tumors.

Materials and methods: During a five-year period, 10 patients who sustained rare malignant intraspinal tumors were surgically treated. There were six men and four women in the group. Mean age of those investigated
was 54.6 years. Neurological deficit ranging from discrete paresis to plegia was initially observed in 7 of 10 patients. Neuroradiological diagnosis consisted of CT and/or MR scans. All tumors were localized extramedullary and extracranially. A tumor was found at high cervical level in three patients, at the cervicothoracic junction in one, at thoracic level in five, and at lumbar level in one. Surgery was performed in all patients. The method of choice was laminectomy followed by total tumor removal in three patients, and resection of tumor masses in seven. Pathohistological tissue analysis and immunohistochemistry were performed in all cases. Vertebrosynthesis to stabilize the spine after laminectomy was carried out in four patients. Radiation therapy and/or chemotherapy was performed after surgery in all but one patient.

Results: At the six-month follow-up, considerable neurological improvement was observed in four of nine surviving patients, while one patient did not survive. No neurological improvement or additional neurological decline was recorded in the remaining five.

Conclusion: All patients sustaining rare malignant intraspinal tumors were adequately operated on, but no major neurological improvement occurred in majority of them. Nevertheless, early surgery in such cases is well required.

P010. MALIGNANT SPINAL EPIDURAL NERVE SHEATH TUMOR (MPNST) WITH GIANT ROSETTES

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The 24-year-old woman, with earlier signs of “forme-fruste” NF1 (cafe-au-lait spots and a mediastinal and cranial neurofibroma), developed an incomplete cauda syndrome because of a large tumor (L3–S2 vertebrae). The hemorrhagic and cystic, highly cellular neoplasm contained scattered fibrillary structures resembling fragments of a peripheral nerve. Hypercellular areas with elongated and at times regimented elements, wheels, and round-ovoid cells were abundant in hyalinized stroma. Antoni A and B separation was not seen, and Verocay bodies were missing. Striking, large, rosette-like or “Beurrette”-shaped eosinophilic structures were conspicuous, with a dark eosinophilic core and peripherally oriented, much lighter, lobularly arranged elements. Coalescent necrotic foci and abnormal mitoses frequently appeared. Vimentin was almost intensely positive. Large areas were almost diffusely reactive with S-100. GFAP decorated isolated cells or small groups of round cells with equivocal intensity. Smooth muscle actin (SMA) reacted with intramural, vascular elements. Antibodies against von Willebrand factor, CD31, and CD34 decorated the often abnormally thin endothelium. NSE was widely negative, but some areas with conspicuous fibrillary or felterlike-tissue pattern displayed intense and often coarsely granulated positivity. CD37 was negative. Some cells, scattered throughout the tumor and wrapped within either hyalinized or necrotic parenchyma, were choromagrin reactive. Small neurofilament-reactive fragments were detectable within the central and peripheral parts of the tumor. Cytokeratins and desmin were negative; scattered groups of cells were EMA positive. Oil-Ro staining appeared in isolated cells, mostly associated with small, necrotic foci (“degenerative” lipid). Mib-1 LI varied between 35% and 45%. Akan blue/toluidine blue stains did not indicate metachromasia, and in some cells moderate amounts of glycogen were demonstrated. The diagnosis was malignant peripheral nerve sheath tumor (MPNST) with giant rosettes. To our knowledge, MPNST with giant rosettes located in the spinal extradural space has not yet been described.

P011. SURGICAL TREATMENT OF CHILDHOOD MEDULLOBLASTOMA: CLINICAL STUDY OF 59 CASES

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Medulloblastoma comprises approximately 20% of all pediatric brain tumors. It is one type of PNET that is found near the midline of the cerebral hemispheres. The tumor expands most frequently along the midline, causing focal symptoms associated with increased ICP. The subjects of our study are 59 children diagnosed as having medulloblastoma who were surgically treated in the Department of Neurosurgery at University Hospital “St. Ivan Rilski,” Sofia, from 1993 to 2005. Data from available medical documentation were analyzed. The group consisted of 33 boys (55.9%) and 26 girls (44.1%).

Introduction: The generally accepted therapy of rare choroid plexus carcinoma (CPC) is comprised of maximal possible surgery and radiotherapy for those patients older than three years. In a literature analysis, we previously showed that chemotherapy improves survival in patients with incompletely resected CPC. However, choosing the best chemotherapeutic drug remains elusive.

Methods: A recently created database of all cases of choroid plexus tumors (CPTs) reported in the literature until 2004 was used. Patients have been treated with drug combinations, making analysis of single agents difficult. For any single drug, survival and response of those patients who had protocols including this drug were compared to patients treated without it, by using log-rank tests (survival) and Fischer’s exact tests (response).

Results: Information about chemotherapy was available for 233 of 347 patients with choroid plexus carcinoma (CPC). Etoposide (60 patients), vincristine (55), carboplatinum (47), cyclophosphamide (46), and cisplatinum (42) were used most often. Response was rarely reported. Ranking the drugs by response resulted in highest values for CCNU (all responders, n = 3), thiopeta (n = 2) and hydroxyurea (n = 2), but none of the response comparisons were significant. Survival was often reported, so statistical analysis was possible. The largest increase in survival was seen with protocols containing VP16 (improvement of 2YOS, 28.8%; P = 0.0081), cyclophosphamide (26.2% improvement; P = 0.0095), cisplatinum (17.4%; P = 0.0669) and carboplatinum (16.9%; P = 0.0481).

Conclusion: Patients treated with etoposide, vincristine, carboplatinum, or cyclophosphamide had the best survival. Those drugs are already included in the CPT-SIOP 2000 study. CCNU and thiopeta may offer an interesting option in treating relapsed CPC. The design of a relapse protocol using the information generated in this analysis is currently under way.

P102. PRIMARY SPINAL ANAPLASTIC GANGLIOGLIOMA: A CASE REPORT

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Spinal tumors are rare in childhood, with an approximate annual incidence of one per one million. Of these tumors, 21% to 33% occur in the spinal cord parenchyma. Spinal neoplasms in children differ greatly from those in adults with respect to histological features, location, and extent and degree of bony involvement. The 1993, the WHO tumor classification those in adults with respect to histological features, location, and extent.

Spinal tumors are rare in childhood, with an approximate annual incidence of one per one million. Of the tumors, 21% to 33% occur in the spinal cord parenchyma. Spinal neoplasms in children differ greatly from those in adults with respect to histological features, location, and extent and degree of bony involvement. The 1993, the WHO tumor classification those in adults with respect to histological features, location, and extent.
Patients with medulloblastoma uncommonly develop extraaxial metastases. We describe an adult patient with the unusual occurrence of bone marrow metastasis as well as subsequent treatment with chemotherapy and bone marrow transplantation. We review the literature regarding this rare presentation and association of metastatic manifestation. A man who presented with bone pain and pancytopenia had a history of medulloblastoma and had been treated with surgery, radiation, and chemotherapy about one year previously. Follow-up MRIs showed complete remission also at the time that symptoms arose. A bone marrow biopsy showed infiltration of a medulloblastoma. After an excellent complete response to chemotherapy with cyclophosphamide, etoposide, and carboplatin, an autologous stem cell transplantation was performed. The patient is still in complete remission. We conclude that adult medulloblastoma with bone marrow metastases is a rare and vicious complication, but can treated effectively.

P109. RARE METASTATIC MANIFESTATION OF ADULT MEDULLOBLASTOMA IN BONE MARROW: A CLINICAL CASE STUDY AND REVIEW OF THE LITERATURE

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We conclude that adult medulloblastoma with bone marrow metastases is a rare and vicious complication, but can treated effectively.

Our group had identified chromosome 8p deletion as one of the common events in the development of medulloblastomas (MB). Fine dissection mapping revealed a high frequency of deletion at b2p22–p23.1 and multiple interstitial deletions at b2p22–p221, suggesting the presence of at least one tumor-suppressor gene (TSG). The aim of our study was to identify the chromosome 8p-associated TSGs involved in MBs. We previously excluded PINX1 and DCL1 as tumor suppressors in MBs, and reported the molecular analysis of two other known TSGs, Lzt3 and N33, for their role in MBs. Quantitative real-time RT-PCR analysis revealed no significant downregulation of Lzt3 transcripts in a series of 30 primary tumors and four cell lines examined. Instead, we detected enhanced Lzt3 expression (more than fourfold above the normal level) in 53% of tumors. The significance of this upregulated Lzt3 expression in MBs is not known. Our results suggest that Lzt3 does not function as a tumor suppressor in MBs. For N33, expression analysis showed that two MBs (7%) had significantly reduced transcript levels (more than 10-fold downregulation). Such a low incidence of N33 expression suggests that this gene may be involved in a small fraction of MBs. To identify TSGs on other regions of chromosome 8p, we carried out global expression analysis by using the Affymetrix whole-genome oligonucleotide microarray (U133 Plus 2.0) in a series of eight MBs and two normal cerebella. Expression profiling revealed that a total of 45 genes, located on chromosome 8p, had decreased transcript levels in at least two of the eight MBs examined. We evaluated the expression patterns of four selected genes (DKFZp761P0423 [8p22.31], EFHAA2 [8p22], MTUSI [8p22], and KIAA0711 [8p22]) by real-time RT-PCR. DKFZp761P0423 expression was found reduced (more than 10-fold below the normal level) in 37% of 30 tumors, with eight of them showing more than 10-fold lower expression than normal cerebella. EFHAA2 expression was reduced (more than fivefold below the normal level) in nine (30%) tumors, with five of them showing a more than 10-fold decrease. MTUSI and KIAA0711 showed null expression. Decreased expression of MTUSI and KIAA0711 were seen in six MBs (20%) and five MBs (17%), respectively. The aberrant expression
patterns of these genes in MBs warrants further study of their role in the formation of this pediatric malignancy. In conclusion, our expression analysis has identified several candidate genes whose downregulated expression may be involved in the carcinogenesis of MBs.

P111. SOMATOSTATIN ANALOGUES AS SECOND-LINE TREATMENT IN ADULT PATIENTS WITH RECURRENT MEDULLOBLASTOMA: A CASE REPORT AND A CONCEPT FOR A MULTICENTRIC, OPEN-LABEL, PROOF-OF-CONCEPT, PHASE I STUDY
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Introduction: Adult medulloblastoma is a rare tumor with only few retrospective studies published so far, and the role of chemo/biotherapy has not been investigated under controlled conditions so far. Somatosta-tin analogues are widely used to treat endocrine tumors and thymomas, because these tumors express sstRs. Medulloblastomas also overexpress the somatostatin receptor subtype 2. Stimulatio of this and other recep-tors decreases the expression of genes promoting tumor survival. Further-more, somatostatin inhibits the proliferation of pPNET/medulloblastoma cell lines. A range of other antitumoral effects of somatostatin analogues has been described. This may be translated into clinical effects in medul-loblastoma.

Case report: We treated a 43-year-old woman presenting with a medulloblastoma relapse in the right optic nerve. Somatostatin receptor imaging with 111In-octreotide scintigraphy showed a positive receptor status. We initi-ated a treatment with SandostatinLAR (s.c., days 1–14, 3–0.1 mg/day) and Sandostatin LAR™ (i.m.; since day 3; cycles 1–2. 40 mg, and cycles 3–4: 60 mg/4 weeks). After 14 weeks of treatment, the patient presented with stable disease in MRI and a significant improvement of her hemianopsia in perimetry. Despite the high dose of Sandostatin LAR™, the treatment was well tolerated and no side effects have been reported thus far.

Discussion: As treatment options in advanced medulloblastomas are limited and as results with high-dose Sandostatin LAR™ have been promising in our center, we want to validate this new therapeutic option for recurrent medulloblastoma in a controlled setting to provide the basis for further studies. Therefore, we plan to conduct a single-arm, proof-of-concept study in which 15 adult patients with recurrent medulloblastoma and positive somatostatin receptor status (111In-octreotide scintigraphy), in whom therapy for primary disease (best possible resection, radiotherapy, and one chemotherapy regimen) failed, will be treated with high-dose Sand-ostatin LAR™. Biotherapy includes an initial period of Sandostatin™ s.c. daily and a following treatment with Sandostatin LAR™ i.m. every four weeks (proposed dose escalation: 60, 90, and 120 mg). Dose escalation will be continued until MTD is reached, and MTD will constitute the primary end point of the study. As secondary end points, objective tumor response and changes in the somatostatin receptor imaging under therapy will be investigated.

P112. NT-3/Trk-C REGULATED PROTEINS INVOLVED IN THE BIOLOGY OF MEDULLOBLASTOMA
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Medulloblastoma (MB) is the most common malignant childhood brain tumor. Because of its high risk of leptomeningeal dissemination, intensive treatment is mandatory. However, massive therapy-induced long-term sequelae demand a more sophisticated therapeutic approach. Recent reports identified high-neurotrophin receptor TrkC mRNA expression as a powerful independent predictor of a favorable survival outcome in MB patients. However, the role of activated TrkC receptors in the development and biology of MB remains unclear.

To determine downstream effector proteins of TrkC signaling, the MB cell line DAOY was stably transfected with a vector containing the full-length TrkC cDNA sequence or an empty vector control. Accounting for the complexity of ligand-induced changes in cellular pathways and effector proteins, we investigated proteomic changes at multiple time points for up to 48 h following neurotrophin 3–induced TrkC receptor activation. We identified 51 protein changes, including cathepsin D, metastasis inhibition factor nm23; multidrug resistance–associated protein Mgr1-Ag; vimentin; vinculin; superoxide dismutase (Mn); glutathione S-transferase P; stathmin, lamin A/C, valosin-containing protein, annexin A1; ULP protein; DJ-1 protein; fascin, minofitin; and heterogenous nuclear ribonucleoproteins H and K.

The proteins affected play substantial roles in differentiation, migration, invasion, proliferation, apoptosis, and drug resistance. Almost all of the proteins have been described as being essential in the pathogenesis of different solid tumors, but have not been related to MB pathogenesis so far.

P113. CISPLATIN-ETOPOSIDE (CDDP-VP16) IN THE TREATMENT OF ADULT PRIMITIVE NEUROECTODERMAL TUMORS (PNETs): A RETROSPECTIVE ANALYSIS
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Background: The CDDP-VP16 schedule showed promising activity in a prior phase II study on high-risk CNS PNETs. Based on these results, adult PNET patients have been treated at our center as an assistance protocol since 2001. The purpose of this study was to assess the activity of this schedule.

Patients and methods: Twelve patients with PNET were included in this retrospective analysis from 2001 to date. Inclusion criteria were age under 18 years, histologically confirmed PNET, KPS > 50, and one of these conditions: incomplete surgery, location out of cerebellum, leptomeningeal metastases, distant metastases, and relapse after radiotherapy (RT). Patients received CDDP 30 mg/m2/day, and VP16 120 mg/m2/day for 3 days every 21 days. Patients without previous radiation received chemo-therapy (CT) and RT (craniospinal, 30–36 Gy; and local boost, 24 Gy) in sandwich schedule.

Results: The median age of the 6 men and 6 women was 31 years (range, 21–44 years); and median KPS was 80 (range, 60–90). Primary tumor sites were cerebellum (7), supratentorial (3), pineal (1), and olfactory bulb (1). Ten patients had Chang stage 0, and 2 patients had Chang stage 3. The inclusion criteria for chemotherapy were incomplete surgery (4), location out of cerebellum (2), and relapse after RT (6). In 5 patients CT was adjuvant adjuvant to surgery, while in the other 6 cases CT was part of the treatment of the recurrence. The first 6 patients underwent surgery (1 complete resection, 3 partial resections, and 2 biopsy) and all of them received CT and RT. In the second group, those patients with recurrence, 2 patients received only CT, 3 patients received salvage surgery (2 complete resection preceding CT, and 1 patient received CT followed by stereotactic RT. The median of cycles for 12 patients was 6, range, 3–7. Toxicity was neutro-penia G-IV, 7 (all of them febrile neutropenia); G-III, 2; thrombocytopenia G-IV, 7; anemia G-III, 4; and asthenia G-III, 1. Only 2 patients had stopped treatment because of toxicity, and no deaths were caused by toxicity. Seven patients were valuable for response (relapses with measur-able disease or adjuvant treatment with persistent tumor): 2 CR, 4 PR, and 1 SD, for an overall response rate of 85.7%. Median follow-up was 40 months (range, 7–54). For all patients, the median time to progression was 20.5 months (range, 7–38); 11 months in relapsed patients and 25 months in newly diagnosed patients. At time of analysis, 3 patients have died (all of them of tumor progression), 4 are alive without tumor, and 3 are alive with tumor.

Conclusions: The CDDP-VP16 schedule is fairly well tolerated and appears to have activity against adult PNETs, producing a high response rate and lengthy time to tumor progression.

P114. SOMATOSTATIN-RECEPTOR SCAN FOR MALIGNANT EPENDYMAL AND EMBRYONAL BRAIN TUMORS
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In vitro data suggest that cell lines from primitive neuroectodermal brain tumors (PNETs) express somatostatin receptors (type2 =sst2) abun-dantly. Autoradiography reveals that most medulloblastomas express high levels of sst2. Using 111In-labeled Octreo-Scan in vivo, we investigated six patients with embryonal and ependymal malignant tumors.

Six patients (two female) aged 12 to 55 years (median, 31 years) with medulloblastoma (n = 4), anaplastic supratentorial PNET (n = 1), and anaplastic ependymoma (n = 1) were evaluated for restaging by Octreo-Scan. Whole-body imaging and brain SPECT were performed at 6 and 24 h after tracer application by a three-head gamma-camera. All cases were proven by histology obtained by neurosurgery. Radiation therapy had been adminis-tered to four patients and chemotherapy had been given to three patients at the time of scintigraphic examination.
Abstracts for the Seventh Congress of the European Association for Neuro-Oncology (EANO)

Viable tumor indicating recurrent disease and/or rest tumor tissue was detected by Octreo-Scan in five patients. Compared to a contralateral refer- ence region of interest, PNET tissue accumulated twofold to fourfold on the first day, with higher rates after 24 h. One patient was negative in respect to a suspected recurrence by MRI and is stable nine months later without therapy. Our limited data suggest that somatostatin-receptor scan may be useful for restaging of embryonal and ependymal malignant tumors and may select some patients for treatment with SST analogues or 99mTc-labeled radioimmunotherapy.

P115. PRIMARY INTRADURAL EXTRASSEOUS Ewing sarcoma (EES)/PERIPHERAL PRIMITIVE NEUROECTODERMAL TUMOR (PNET) WITH t(11;22)(q24;q12) TRANSLOCATION: CLINICAL, RADIOGRAPHIC, AND PATHOLOGIC FEATURES OF TWO CASES

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Background: Primary intradural EES/PNET has only rarely been reported in the literature. The EES/PNET family of tumors may present as a spinal or intracranial mass as these tumors often occur in the para-sellar region. Conversely, a primary intradural location is extremely rare. An intradural location of PNET requires explicit differentiation between central PNET and PNET, which historically look identical, although these tumors differ markedly in histogenesis, molecular characteristics, and clinical behavior. Almost all EES/PNETs have pathognomonic expression of the t(11;22)(q24;q12) translocation that results in the chimeric EWS/ FLI-1 fusion protein. We identified only seven cases in the literature (five intracranial and two spinal) with intradural EES/PNET verified by dem-onstration of the t(11;22)(q24;q12) translocation. We describe the clinical, radiographic, and histological features of two additional cases. Patient 1: A 59-year-old man developed severe low-back pain radiating down to his buttocks and hips that was followed by voiding dysfunction. Neurological status revealed mild proximal lower-extremity weakness, absent DTR, saddle anesthesia in the lower extremities, and mild hypoesthesia, and anal sphincter dysfunction. MRI showed an intradural L2–4, dural-based, enhancing lesion that was subtotally removed at surgery. The pathological diagnosis was EES/PNET with t(11;22)(q24;q12) translocation. Systemic evaluation was negative, and the patient is currently being treated with neuroaxis radiotherapy to be followed by systemic chemotherapy. Patient 2: A 29-year-old woman with a history of Hodgkin’s disease (of therapy for six years) developed severe headaches, diplopia, and vomiting. Imaging revealed a large, enhance meningioma. MRI showed a meningioma. Following gross total resection of the tumor, the diagnosis was of EES/PNET with t(11;22)(q24;q12) translocation. Systemic evaluation was negative, and the patient is currently being treated with neuroaxis radiotherapy to be followed by systemic chemotherapy. Conclusions: The diagnosis of primary CNS EES/PNET, although rare, should be considered in tumors that arise near the meningeal surface and have a pathologic appearance consistent with PNET. Demonstration of the pathognomonic t(11;22)(q24;q12) translocation confirms the diagnosis and enables one to choose the appropriate therapy, which is different from that for central PNET.

P106. PRIMARY ADULT INTRASELLAR EMBRYONAL RHABDOMYOSARCOMA: A CASE REPORT

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Objective: To present an unusual case of an isolated sellar/suprasellar embryonal rhabdomyosarcoma occurring in an adult. Primary sellar region rhabdomyosarcoma, without extension from paranasal, orbital, or middle-ear location, is quite rare. We report only the second such case, to our knowledge, both occurring in young adults.

Case presentation: A 35-year-old, previously healthy woman presented with a one-year history of amnorrhea, then developing subacute, mild left visual blurring over three months, with mild headache. A 2.0 x 2.0-cm globular mass involving both the sella and the suprasellar cistern, with encasement of the left internal carotid artery, was identified on MR imaging. No involvement of the paranasal sinuses or other parameningeal structures was noted. Extensive systemic imaging revealed no other tumors. Preoperative endocrine evaluation revealed elevated prolactin and decreased estradiol, FSH, and LH. Transsphenoidal biopsy of the mass revealed a hypercellular neoplasm with spindle cells and a few globoid cells invading the normal anterior pituitary gland. The tumor showed reactivity diffusely for vimentin, S-100, and BCL-2. The globoid cells were strongly positive for myoglobin and weakly positive for desmin, thereby diagnosed as embryonal rhabdomyosarcoma, with a low MIB-1 of 1.1%. Electron microscopy confirmed this diagnosis. Subsequent extensive, but subtotal, resection was achieved through a bifrontal approach followed. This was felt to be a group III or intermediate-risk lesion because of incomplete resection and stage II tumor characteristics. Postoperatively, the patient received VAC chemotherapy followed by fractionated stereotactic radiotherapy as per the Intergroup Rhabdomyosarcoma IV study. The patient has remained clinically quite stable with intact visual functioning despite optic atrophy and on medical management of panhypopituitarism. Serial MRI imaging has shown only partial response to treatment.

Discussion: This case demonstrates that the pituitary fossa may be another parameningeal locus for primary rhabdomyosarcoma in adults. Updated clinical follow-up will be presented. Rhabdomyosarcoma can be included in the differential diagnosis of sellar lesions.

P117. CRANIAL BASE MENINGIOMAS

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The cranial base is a common site for the origin of meningiomas. This report presents the author’s experience in this field, based on 42 cases oper- ated on between January 2000 and February 2006 in Cluj-Napoca County Hospital, First Neurosurgical Department, Iuliu Hatieganu University of Medicine. The case series female–male ratio was 1.2:1. The peak incidence was in the sixth decade. According to their site of origin or dural attach- ment, our case series includes meningiomas of the medial sphenoid wing, 13 cases (31%); posterior olfactory groove meningiomas, 10 cases (26%); planum sphenoidale meningiomas, 9 cases (21.5%); tuberculum sellae meningiomas, 7 cases (17%); cavernous sinus meningiomas, 2 cases (5%); and posterior clinoid process, 1 case (2.5%). We recorded six malignant and 36 benign meningiomas. Based on their site, we performed a peratinal approach in 29 cases and a bifrontal approach in 13 cases. Neurosurgical intervention was conducted for total removal (Simpson’s grade I and IIb) of the tumor in 40 (95%) of the cases and subtotal removal (Simpson’s grade III and IV) in two (5%) of the cases. Only one patient with giant cavernous sinus meningiomas died of pulmonary embolism 14 days after surgery.

P118. ROLE OF ENDOTHELIN-1 IN ANGIogenic PHENOMENON IN INTRACRANIAL MENINGIOMAS

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Recent clinical and molecular research provided new elements aimed at understanding the mechanisms deputed to meningioma development and malignant progression. A role of endothelin-1 (ET-1) in tumoral growth has been described in meningiomas. Endothelin is a well-known peptide with 21 amino acid residues, interacting with receptors ETA and ETB. In the physiological condition, it exerts a role as modulator of vasomotor tone, tissue differentiation and development, and hormone production. Our aim was to evaluate the role of ET-1 in the angiogenic phenomenon, considering that a tumoral microvesSEL network is essential for the survival and growth of neoplasms, and we investigated the possible interaction of ET-1 with vascular endothelial growth factor (VEGF).

We analyzed ET-1 expression by immunohistochemistry and also by RT-PCR in 56 cases of meningiomas (46 typical, 8 atypical, and 2 anaplastic). We confirmed the involvement of ET-1 in meningioma growth, demonstrating an association between high-grade meningiomas (II–III) and high ET-1 expression levels (P = 0.0022). Moreover, we found an elevated microvesSEL count in tumors with high endothelin expression levels (P = 0.0044). Of 55 cases, 51 (92.7%) showed that VEGF protein expression was significantly associated with MVD (P = 0.03) and with histologic grade (P = 0.01). The number of microvesSels ranged from 6 to 45 and was also significantly related to histologic grade (P = 0.000004). ET-1 expression/ upregulation may contribute to meningioma growth by inducing the for- mation of new blood vessels. The finding that ET-1 expression positively correlates with VEGF expression (P = 0.000004) suggests, as well as in other tumor models, also supports the hypothesized modulating effect of ET-1 on angiogenesis.

Our data highlighted a significant relationship between ET-1 and VEGF, as well as between ET-1 and vascular density, suggesting a putative role of ET-1 in the angiogenic phenomenon. In conclusion, considering the impor-
P119. UNIQUE CHROMOSOMAL ABERRATION IN A RECURRENT MALIGNANT MENINGIOMA

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Objective: Meningiomas are the second most common symptomatic adult central nervous system tumors. They account for approximately 20% of all primary intracranial tumors in adults, and approximately 20% display aggressive features. However, little known is about the genetic events associated with the aggressive phenotype in malignant meningiomas. Previous cytogenetic and molecular cytogenetic studies have shown complex numerical and structural aberrations, which are frequent findings in grade II and III meningiomas. The most commonly observed aberrations in decreasing frequency are monosomy 22 or deletion of 22q; deletions of 14q, 1p, and 10q; loss of a sex chromosome; and tetraploid karyotypes. We report a case of a recurrence anaplastic meningioma with unique additional chromosomal aberrations.

Methods: This 41-year-old man has been operated on seven times for a left parietal anaplastic meningioma within a period of four years. Histopathology revealed a malignant meningioma with high mitotic activity. Despite additional surgery, x-ray treatment, and systemic therapies with 5+ ACNU/VM-26 and hydroxyurea, postoperative follow-up consistently revealed tumor recurrence. Persistent symptomatic seizures are treated with Topinol (carbamazepine). For the present investigation, tumor cells derived from fresh surgical specimens were cultured long term in RPMI medium for conventional chromosome preparation and GTG banding.

Results: We detected three different karyotypes by conventional cytogenetic analysis (CCA): (1) hypoploid cells with a loss of the Y chromosome, (2) near-triploid cells with multiple chromosomal aberrations like dentricrome 2, deletion of 3p, additional materials on 4q and 8q, isochromosomes 9q and 17q, and a various number of marker chromosomes, and (3) near-tetraploid cells with additional materials on 1q and 9p, deletions of 3p and 14q, loss of chromosome 2, and a various number of marker chromosomes. Conclusions: The investigated malignant meningioma displayed karyotype alterations previously reported for malignant meningiomas, including the 14q aberration and a possible tetraploid karyotype. In addition, a near-triploid karyotype and alterations like 5p–, 8q+ are chromosomal aberrations that have not been reported before in these tumors. Our findings suggest that complex karyotype alterations might be a characteristic feature in malignant meningiomas.

P120. ATYPICAL AND MALIGNANT MENINGIOMAS: OUR EXPERIENCE WITH 38 PATIENTS

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The therapeutic goal in the treatment of atypical and malignant meningiomas is still under debate. The different options for treatment (surgery, radiotherapy combined or stand alone) lead us to be oriented to the best tailored choice for each patient. Between January 1999 and December 2004, 38 patients (22 female and 16 male) affected by WHO grade II and III meningiomas were treated at the neurosurgical unit of Varese Hospital. The mean age of the patients was 60.8 years. The symptoms at the onset were seizures, headache, and focal neurological deficits. The site of the lesion was the cerebral convexity in 15 patients, parasagittal in seven, anterior cranial base in five, middle cranial base in seven, occipital in two, posterior fossa in one, and the thoracic cord in one.

All patients underwent surgical treatment. As result of surgery, we used the Simpson scale: 20 patients were graded 1, 13 were graded 2, two were graded 3, and three were graded 4. Twenty patients underwent more than one surgical procedure (up to six). In nine of these patients, we observed a recurrence with a median time of 22 months from the first diagnosis. Three patients were reoperated upon for a lesion at a different site than in the first procedure. Among the 35 patients with grade II meningiomas, 16 patients underwent radiotherapy because they had several recurrences from the first surgical excision or they were graded Simpson 3 or 4. The patients with grade III meningiomas underwent radiotherapy in all cases. In each patient, conformational radiotherapy was performed on the surgical bed with a dose of 54 Gy fractioned in 27 or 30 sessions. Twenty-eight patients are still alive and 10 are dead, with a mean survival time of 6.5 years from the initial diagnosis.

Among the 28 patients who survived, 22 (one with grade III meningioma) are in good health, while six have a moderate disability. Among the dead patients, in three cases death was due to extracranial causes. In our experience, atypical meningiomas (grades 1 and 2 on the Simpson scale) are treated with surgical excision alone and undergo controlled follow-up. When there is recurrence, the patients undergo surgical excision and radiotherapy or, in few cases, radiotherapy only, depending on the patient’s performance status and the age, site, and the dimensions of the lesion. All of the patients affected by grade III meningiomas are undergoing radiotherapy shortly after the surgical excision.

P121. COAGULATION-FIBRINOLYSIS PECULIARITIES IN INTRACRANIAL MENINGIOMA (IM) PATIENTS IN PRE-, INTRA-, AND POSTOPERATIVE PERIODS

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The aim of the study was to evaluate the functional state of the hemostasis system in IM patients during preoperative, intraoperative, and early postoperative periods, considering a histological structure. A total of 32 IM patients (32 women and 20 men; age, 36–58 years) have been observed. All patients underwent computed tomography and histological investigation. Coagulation-fibrinolysis study was performed in preoperative, intraoperative, and early 1- to 3-day and 7- to 10-day postoperative periods. The following tests were carried out: Willebrand factor (WF) and antithrombin III (AT III) activity, fibrinogen (P), D-dimer level, and plasminogen (P) activity. The levels of hemostatic markers (grades 1 and 2 on the Simpson scale) t-test. Most patients (67.3%) had increased F and D-dimer levels pointing to a prethrombotic state. In atypical and anaplastic IM, significantly low levels of F (P < 0.001) and AT III (68.2 ± 1.55 vs. 87.2 ± 2.04; P < 0.001) were found during operation, with elevated levels of WF (278 ± 18.1 vs. 141 ± 11.2; P < 0.05), D-dimer (2038 ± 78.2 vs. 1047 ± 64.4; P < 0.001), and P (107 ± 2.6 vs. 87 ± 3.7; P < 0.05) in comparison to preoperative values in benign IM. Early postoperative period at 1–3 and 7–10 days is characterized by increased F (P < 0.001) and D-dimer (P < 0.05) levels compared to the controls. WF and D-dimer levels were significantly enhanced during observation and early postoperative periods. The increased D-dimer circulating level is correlated with IM histological structure. During operation, t-PA entering from brain meninges and capillary endothelium creates a danger of intracranial hemorrhage. Local DIC syndrome developed intraoperatively in 12% of patients.

The results obtained demonstrate activation of coagulation-fibrinolysis processes in IM patients. Neurosurgical operations are followed by local, generalized, and chronic DIC syndrome, which may be corrected during the initial cellular tissue thrombomembranous discharges that should be taken into consideration in IM patients.

Background: Meningiomas are usually benign tumors and cyogenetically well characterized. Most tumors show either monosomy 22 or a diploid karyotype. Progression of meningiomas is correlated with increasing hypoploidy and the loss of the short arm of chromosome 1. The aim of this study was to assess intratumoral patterns of clonal chromosomal evolution in order to identify tumor progression pathways and to analyze their correlation with time to recurrence.

Methods: From 1973 to 2004, a total of 661 patients with complete tumor resections and cyogenetic characterization were followed up. We have developed oncogenic tree models for estimating the most likely order of cytogenetic aberrations.

Results: Overall, in 8.0% of the tumors (53 of 661), at least one recurrence was documented during the study. Our results showed a significant correlation between cytogenetic data and recurrence (P < 0.001), location (P < 0.05) and WHO grade (P < 0.15). The estimated model was used to assign a genetic progression score (GPS). The GPS of a tumor is a quantitative measure and allows precise assessment of genetic progression. We classified tumors in three groups: low genetic progression (GPS < 2), intermediate genetic progression (2 ≤ GPS < 6), and advanced genetic progression (GPS ≥ 6). The recurrence rate was 11.2; 3.7; and 11.2, respectively. The increased D-dimer circulating level is correlated with IM histological structure. During operation, t-PA entering from brain meninges and capillary endothelium creates a danger of intracranial hemorrhage. Local DIC syndrome developed intraoperatively in 12% of patients.

The results obtained demonstrate activation of coagulation-fibrinolysis processes in IM patients. Neurosurgical operations are followed by local, generalized, and chronic DIC syndrome, which may be corrected during the initial cellular tissue thrombomembranous discharges that should be taken into consideration in IM patients.
Conclusion: Cytogenetic classification of meningiomas is a powerful tool for predicting tumor recurrence and a valuable parameter for the postoperative management protocol.

P123. FALX MENINGIOMAS: CLINICAL AND RADIOLOGICAL ANALYSIS AND SURGICAL APPROACHES
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Objective: In general, meningiomas are slowly growing intracranial benign tumors. However, falx meningiomas arise adjacent to venous sinuses, and most patients have symptoms of increased intracranial pressure (ICP) earlier than the patients with convexity meningiomas. Therefore, their surgical management is important.

Methods: The authors retrospectively investigated 39 cases of falx meningiomas treated at Goztepe Education and Research Hospital in Istanbul between 1997 and 2005. The patients were evaluated by computed tomography (CT) and magnetic resonance imaging (MRI). A digital subtraction angiography (DSA) and/or a MR venography were performed in 12 cases. The location of the tumors was anterior in 12 cases, middle in nine cases, posterior in eight cases, and junctional areas in eight cases. In 12 of 39 cases, CT scans and MR images showed peritumoral edema. The tumor size was less than 8 cm in six cases, between 4 and 8 cm in 10 cases, and larger than 8 cm in 23 cases. In 39 patients, 44 surgical interventions were performed to remove the tumors. Tumors were removed totally in 32 patients. There was no mortality. Morbidity was 30.7%.

Conclusions: Falx meningiomas can be safely managed by thoroughly evaluating neuroimaging studies and using microsurgical techniques. Additionally, preoperative DSA and MR venography studies are very helpful in achieving complete resection of tumor tissues.

P124. OLFACTORY GROOVE MENINGIOMAS: CLINICAL AND RADIOLOGICAL ANALYSIS AND SURGICAL APPROACHES
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Objective: Olfactory groove meningiomas (OGMs) arise over the cribiform plate and frontonasal suture and comprise approximately 10% of intracranial meningiomas. OGMs may become very large prior to producing symptoms. Many OGMs are quite large on initial presentation and, in these cases, surgery may be the only option. The large size of these tumors makes resection challenging.

Methods: The authors retrospectively investigated 14 cases of OGMs treated at Goztepe Education and Research Hospital in Istanbul between 1997 and 2005 (female–male ratio = 0.4; ages, 44 – 72 years; mean age, 58 years). The patients were evaluated by computed tomography (CT), magnetic resonance imaging (MRI), digital subtraction angiography (DSA), and/or MR venography. In 9 of 14 cases, CT scans and MR images showed peritumoral edema. The size of the tumor was between 4 and 7 cm. The surgical approach was bifrontal craniotomy in nine cases, unilateral frontal craniotomy in four cases, and personal cranial in one case. There were 11 complete resections and three subtotal resections. One patient was operated on twice. There was no mortality. Morbidity was 21.4% (three cases).

Conclusions: To decrease the mortality and morbidity in olfactory groove meningiomas, it is important to evaluate the radiological findings carefully and choose the most appropriate surgical approach combined with microsurgical techniques.

P125. A CASE OF MENINGOTHelial MENINGIOMA WITH MULTIPLE INTRACRANIAL AND CERVELICAL RECURRENCES
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Objectives: Most meningiomas are benign central nervous system tumors. Multiple recurrences of meningothelial meningiomas are rare. Case description: The case of a 51-year-old male patient with multiple meningiomas is presented. A patient was diagnosed as having an intracranial mass lesion in the posterior parietal parafalcian region. The tumor was removed subtotally. Histopathological examination proved it to be a meningothelial meningioma. The patient received radiotherapy after the surgery. Twenty-four months later, the tumor recurred in the same place and was removed totally. Twenty months after that, a mass lesion appeared in his neck. This lesion was removed totally, and the histopathological diagnosis was meningothelial meningioma. Six months after that, the patient was operated on again because of three separate intracranial parafalcian meningiomas. One of them was in the same place as the first intracranial meningioma. These tumors were removed totally by microsurgery. The patient was neurologically intact after the surgery.

Conclusions: This case report shows that meningiomas may recur in a short time even if the histopathological diagnosis is a benign tumor. The relation of the recurrences and radiotherapy in this case may be discussed. However, other predisposing factors, such as genetic, molecular, and hormonal factors, are under investigation in this case.

P126. EPITHELIAL DIFFERENTIATION AND VASCULARITY IN SECRETORY MENINGIOMAS
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Introduction: The unique features of secretory meningioma—namely, pseudopsammoma bodies, vessel proliferation, and edema formation—and the relationship of these features to a presumed secretory activity have not been completely understood so far. We present new data concerning both the epithelial differentiation of “secretory” cells and the topographical relation of newly formed vessel to such cells.

Materials and Methods: Among 1400 meningiomas diagnosed in our institutions over the last 25 years, 13 cases of secretory meningiomas were identified. The pathological features and clinical behavior of the tumors were analyzed retrospectively.

Results: Many secretory meningiomas exhibit pericytic perivascular proliferation in connection with an increased number of mast cells that express CD117 and partly serotonin and coarsely aggregated vascular endotelial growth factor (VEGF). Light and electron microscopy revealed increasing vacuolization within and between tumor cells around proliferated vessels, a feature considered as the origin of edema formation. Furthermore, the pseudopsammoma bodies that characterize those tumors are surrounded by cell complexes that react vividly with cytokeratin antibodies. Cytokeratin subtypes CK 7 and CK 8 were weakly expressed while the strafittication-related CK 3 and CK 6 were strongly expressed in these cells. A more thorough investigation with the PAS reaction in combination with immunohistochemistry revealed that pseudopsammoma body–forming complexes were preferentially arranged in the immediate neighborhood of newly formed vessels, and that cells of these complexes express not only cytokeratins but also desmosomal proteins, such as certain desmocollines and desmogleins, that all belong to the strafitticitation group.

Conclusions: Mast cells shedding VEGF and edema-producing factors in these tumors may explain pericytic vessel proliferation and edema formation of secretory meningiomas. Obviously, vessels are intimately connected to secretory complexes; the latter by analysis of their cytokeratin and desmosomal composition display features typical of stratified epithelial cells.

P127. TOLERABILITY OF INTRATHECAL LIPOSOMAL CYTARABINE AT A DOSE OF 25–50 MG IN 10 CHILDREN 11 MONTHS TO 15 YEARS OLD
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Neoplastic meningitis remains one of the greatest treatment challenges in cancer medicine. Because of the limited penetration of systemically administered anticancer drugs across the blood-brain/CSF barrier, there is a compelling need for new drugs that can be administered intrathecally. Liposomal cytarabine (DepoCyt) is a novel slow-release formulation of cytarabine that is hoped to result in cell kill in patients with leptomeningeal disease from solid tumors. The recommended dose for patients between 3 and 21 years of age is 35 mg versus 50 mg in adults.

We report on the tolerability of intrathecal DepoCyt in 10 children who were either younger than 3 years or received higher doses. From October 2004 to January 2006, 10 children aged 11 months to 15 years (median, 12 years) with various highly malignant solid tumors located in the CNS were treated with intrathecal DepoCyt. The dose was 25 mg in children 11 months to 3 years of age, 35 mg in a 5-year-old, and 50 mg in older patients. DepoCyt was administered either intraventricularily (n = 31) or intralumbarly (n = 25) every 2 weeks with a median of 6 applications/patient and a maximal cumulative dose of 450 mg (median 225 mg). All patients received concomitant dexamethasone. Except for headache grade 2 in two patients and transiently decreased vision, unsteadiness, gait, decrease in bladder control, and fatigue in one patient, no adverse effects were noted.
Repetitive intrathecal DepoCyt therapy at a dose of 25 to 50 mg with concomitant oral dexamethasone appears to be feasible and is well tolerated. Further research is warranted to determine the threshold dose required for solid tumors in children.

P128. SURFACE RECEPTOR GENE REARRANGEMENT ANALYSIS IN CEREBROSPINAL AND VITREOUS FLUID OF PATIENTS WITH BENIGN AND MALIGNANT LYMPHOPROLIFERATIVE DISEASES
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Objective: To determine the sensitivity and specificity of immunoglobulin heavy-chain (AIGHR) and T-cell receptor gene rearrangement analysis (ATCRR) of cerebrospinal fluid (CSF) and vitreous fluid in the diagnosis of benign and malignant lymphoproliferative disorders.

Backgrounds: The diagnosis of nervous system lymphoma generally relies on morphological analysis of biopsy tissue or CSF. Although surface receptor gene rearrangement studies have been accepted as a valuable adjunct to classic methods of diagnosis in lymphoma outside the nervous system, this modality has not commonly been applied to paucicellular specimens, and thus its sensitivity and specificity in this setting are unknown.

Methods: Patients have been recruited to this prospective study since January 2005. To qualify for study enrollment, patients had to have a clinical history or radiographic findings suspicious for a lymphoproliferative disorder involving the leptomeninges or the eye. For AIGHR/ATCRR, cells from CSF or vitreous samples were separated by centrifugation. The cell pellet was resuspended in 100 μL of the supernatant and boiled for 10 min; 10 μL of the lysate served as the template for polymerase chain reaction (PCR) using HGK or TCR consensus primers. PCR products were separated by capillary electrophoresis (AIGHR) or polyacrylamide gel electrophoresis (ATCRR), which was then stained in ethidium bromide and photographed under ultraviolet light. Clonal rearrangement was defined as the occurrence of one or two identical prominent bands from parallel PCR assays of two aliquots of the same specimen.

Results: As of today, 51 patients yielding 57 specimens have been enrolled in the study. Twelve analyzed specimens were obtained from patients in whom leptomeningeal or vitreous lymphoma was confirmed. Of those, six tested positive for clonal rearrangement. There were 42 cases (45 specimens) with benign lymphoid processes.

Conclusions: Specimen acquisition is ongoing. AIGHR/ATCRR appears to be a useful addition to cytopathology and flow cytometry in lymphoproliferative diseases of the nervous system. As clonal rearrangement is not a marker of malignancy per se, clonal lymphoproliferative diseases may be identified at a stage when transformation into a malignant process may still be reversible.

P129. SEQUENTIAL INTRA-OMMAYA CHEMOTHERAPY AND SYSTEMIC TARCEVA PROLONG SURVIVAL IN A PATIENT WITH BRAIN AND LEPTOMENINGEAL METASTASES FROM LUNG ADENOCARCINOMA
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It is estimated that 5% of patients with non-small-cell lung cancer (NSCLC) develop leptomeningeal metastases (LM). The prognosis for these patients is very poor, with a median survival of four months, even with treatment. We present the case of a patient with NCLC with brain metastasis and LM treated with multimodality therapy, who achieved a longer survival with good quality of life.

Our patient is a 60-year-old man who presented in May 2005 with seizures and was diagnosed with lung adenocarcinoma with a solitary left frontotemporal brain metastasis. He had resection of the brain metastasis and started RT to the brain. Three days later, he was admitted with headache, blurred vision, back and neck pain, and left arm weakness. KPS was 70. CSF was positive for malignant cells. The patient started erlotinib (Tarceva) chemotherapy and sequential intra-Ommaya chemotherapy with thiopeta two weeks weekly, followed by DepoCyt (cytarabine liposome–injectable) every two weeks and then alternating thiopeta and DepoCyt. Although the CSF remains positive for malignant cells, the symptoms resolved, the left arm weakness improved, and the patient is now nine months after diagnosis of LM and maintains a KPS of 80 and good quality of life. His systemic disease is stable on erlotinib and he continues intra-Ommaya chemotherapy.

We conclude that sequential long-term intra-Ommaya chemotherapy and systemic treatment including erlotinib may help prolong survival in patients with LM from NSCLC even when the CSF remains positive for malignant cells. A clinical trial using this approach would be worthwhile.

P130. BREAST CANCER CARCINOMATOUS MENINGITIS: THE ROLE OF MULTIMODALITY TREATMENT
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Purpose: The aim of the study was to assess the efficacy of multimodality treatment of patients with carcinomatous meningitis and to establish whether systemic chemotherapy can improve the survival of those patients.

Materials and methods: We reviewed 67 breast cancer patients treated in the Cancer Center, Warsaw, between 2000 and 2005. Karnofsky performance status (KPS) > 60 was established in 63% of patients and KPS < 60 in 37% of cases. The treatment was undertaken in 62 patients (93%). Intrathecal methotrexate was administered to 57 patients (85%). In 33 patients (49%), the whole brain was irradiated. Forty-one patients (61%) received systemic chemotherapy after intrathecal treatment. In 27 cases (40%), three methods of treatment were undertaken.

Results: Clinical response was achieved in 49 patients (76%). The median survival calculated from diagnosis of carcinomatous meningitis was 16 weeks (range, 1–408 weeks). Patients in whom three methods of treatment were used had better survival (22 weeks) than did those with suboptimal treatment (14 weeks) (P = 0.003). Survival was 20 weeks for patients with KPS > 60 who were treated with systemic chemotherapy, 13 weeks in patients with KPS > 60 who were without chemotherapy, 9 weeks for patients with KPS < 60 who were treated with systemic chemotherapy, and 4 weeks for patients with KPS < 60 who were without systemic chemotherapy. The log-rank test stratified for KPS was highly statistically significant (P = 0.00009).

Conclusions: Our observations suggest that breast cancer patients with carcinomatous meningitis treated with systemic chemotherapy, intrathecal therapy, and radiotherapy achieved better survival. Intravenous chemotherapy is an important part of multimodality treatment.

P131. INTRACELLULAR TARGETING OF SODIUM MERCAPTOUCDEHYDRODODECARBOATE (BSH) TO MALIGNANT GLIOMA BY TRANSFERRIN-PEG LIPOSOMES, FOR BORON NEUTRON-CAPTURE THERAPY (BNCT)
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Introduction: Malignant glioma is one of the most difficult tumors to control with the usual therapies. In our institute, we chose boron neutron-capture therapy (BNCT) as an adjuvant radiation therapy after surgery. This therapy requires the selective delivery of a high concentration of 10B compounds to malignant tumor tissue. In this study, we focused on a tumor-targeting drug delivery system for BNCT that uses sodium borocaptate (BSH)–encapsulating, transferrin (TF)-conjugated polyethyleneglycol liposome (TF-PEG liposome), and compared 10B uptake of the tumor among BSH alone, PEG liposome containing BSH, and TF-PEG liposome.

Methods: In vitro, we analyze the 10B concentration of the cultured human U87 glioma cells incubated in medium containing 20 μg 10B/ml derived from each BSH delivery system by inductively coupled plasma atomic emission spectrometry (ICP-AES). In vivo, human U87 glioma–bearing nude mice were administered each BSH delivery system (35 mg 10B/kg) intravenously. We analyzed 10B concentration in tumor, normal brain, and blood by ICP-AES.

Results: The TF-PEG liposome showed higher absolute concentration more than did the other BSH delivery system. Moreover, TF-PEG liposome decreased 10B concentration in blood and normal tissue while it maintained high 10B concentration in tumor tissue for a couple of days. This showed the TF-PEG liposome caused the selective delivery of a high concentration of 10B compounds to malignant tumor tissue.

Conclusions: The TF-PEG liposome is a more potent BSH delivery system for BNCT to obtain an absolute high 10B concentration and good contrast between tumor and normal tissue than are BSH alone and PEG liposome.

P132. NEOADJUVANT THERAPY WITH TEMOZOLOMIDE AND 13-CIS-RETINOIC ACID IN MALIGNANT GLIOMA (RNO-05): A PHASE II STUDY
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Introduction: A number of studies have evaluated the benefit of neoadjuvant chemotherapy after resection in anaplastic glioma and glioblastoma (WHO grade III/IV). For example, Friedman et al. (1998) demonstrated a 54% progression-free survival at six months with neoadjuvant temozolomide. Nevertheless, a clear benefit concerning median time to progression

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Patients: We enrolled 23 patients (16 men; median age, 56.4; age range, 34.3–74.7) with these tumors: 19 glioblastomas (GBs), 2 grade III anaplastic astrocytomas (AAs), 1 anaplastic meningioma (AM), and 1 anaplastic ependymoblastoma (OL). The ECOG PS number of patients was 0/5, 1/8, 2/8, and 3/2. Of these patients, 13 with GB had previous surgery, 18 had radiation therapy, and patients with AM and AA had both surgery and radiation therapy. In the last 9 GB patients, MGMT promoter methylation was evaluated: 2 patients were methylated, 5 were unmethylated, and 2 had inadequate samples.

Methods: All pts received C 75 mg/m² i.v. on day 1 and oral T at 100–150 mg/m² on days 1–5, every 21 days. Seventeen received oral 100–150 mg TH per day continuously. Hematological toxicity was assessed the day before each subsequent cycle delivery.

Results: A total of 24 cycles were administered; 18 cycles had to be delayed because of recovery from toxicity. In the 22 evaluable patients, toxicity WHO grade 1–2 was (type of toxicity and number of patients) as follows: anemia, 9; nausea, 8; vomiting, 6; allergic reaction, 5* (cutaneous, 4; and dyspnea, 1); fatigue, 4; urticaria, 2; epigastralgia, 2; thrombocytopenia, 2; granulocytopenia, 2 (G1 not assessed); arthralgia, 1; myalgia, 1; somnolence, 2; depression, 1; and oral dryness, 1. WHO grade 3–4 was as follows: granulocytopenia, 8 (febrile G4, 1); vascular venous, 6* (DVT, 3; and PE, 3); vascular arterial, 1; thrombocytopenia, 1; anorexia, 2; weight loss, 1; and conclusion, 1. (Only patients receiving TH.) The 18 evaluable patients with GB had 1 PR, 12 SDs, and 5 PDS. Median TTP was 3.7+ months (range, 0.7–13.2 months) and median OS was 7.9+ months (range, 0.7–15.6+ months). The 2 patients with AA III had been treated in adjuvant setting and had, respectively, a TTP of 5.2 (OS = 37.0+) and a PFS of 18.4+ months. The patient with AM had SD, TTP = 8.3 months, and OS = 16.3 months. The patient with OL had SD, TTP = 5.1 months, and OS = 8.2+ months.

Conclusions: Administration of C and T every 21 days is feasible and safe. The addition of TH enhances the risk of thrombosis, and a low-dose heparin prophylaxis is recommended. The effectiveness of present therapy seems to be promising, and study is ongoing.

Diffuse, intrinsic brainstem gliomas (DBSGs) are diagnosed primarily in children, and only less than 20% of these tumors occur in adults. DBSGs are inoperable and incurable by radiation therapy and chemotherapy. Prior reports from our group indicated that patients with GB and INMB had significant responses to combined treatment with radiation and chemotherapy. ANP was given intravenously daily by a subclavian venous catheter and a double-channel infusion pump. The median duration of ANP administration was 6.5 months, and the average dosage of ANP was 350 g/m²/day of AS2–1 was 0.28 g/kg/day. Responses were assessed by gadolinium-enhanced MRIs and confirmed by PET scans in the majority of patients. Eleven patients had recurrence and were treated with ANP. ANP was well tolerated and provides encouraging results in the treatment of malignant gliomas. Nevertheless, patients with grade III tumors may benefit significantly from this combined approach with very limited toxicity.

Adjuvant chemotherapy has a significant but limited impact on survival of malignant gliomas. However, the role of salvage chemotherapy (CT) at recurrence after first-line treatment is still debated, and there are few data about the real benefit and toxicity of second- and third-line CT. We analyzed PFS, response to CT, and toxicity in a series of 127 patients affected by malignant gliomas treated in our institution with second- and third-line CT after recurrence. Patients were affected by glioblastoma in 44 cases, anaplastic astrocytoma in 24, progressive low-grade astrocytoma in 17, and anaplastic oligodendroglioma or mixed in 42. Patients received as first-line treatment temozolomide in 61 cases and PCV in 66. Second-line treatment was temozolomide in 58, PCV or CCNU in 38, fotemustine in 21, and others in 10. Thirty patients received a third-line CT consisting of fotemustine in 11 cases, temozolomide in 7, PCV or CCNU in 5, and others in 7. PFS after second-line CT was 6 months in GBMs, 9 months in anaplastic astrocytomas, 20 months in progressive low-grade astrocytoma, and 11 months in oligodendroglioma or mixed. Third-line treatment consisted of fotemustine, 7 months in progressive low-grade astrocytoma, and 4 months in anaplastic oligodendroglioma or mixed. Overall response rate to second-line chemotherapy (CR and PR) was 26.1% (GBM 4.5% and no-GBM 32.8%). Overall response rate to third-line chemotherapy was 20% (no GBM 27% and no response in GBM). Hematologic toxicity higher than grade 2 was observed in 23% of patients. Our data show that salvage chemotherapy may be considered an effective treatment option only in a subset of patients with chemosensitive disease.
From July 2004 to February 2006, we have treated 25 patients, who participated in different clinical trials involving CED for recurrent GBM. A total of 57 catheters were inserted: 12 intratumoral catheters in 9 procedures and 45 intraparenchymal catheters in 16 procedures after resection with GBM, 13 (52%) were in or near eloquent brain tissue. Complications included increased edema (36%), infection (8%), bleeding (8%), and seizures (16%). Difficulties in adhering to the CP guidelines that were encountered included location (superficial frontal/parietal lesions, mesial temporal lobe, vicinity to CSF spaces and resection cavity, and shifting proximity to eloquent cortex), tissue-density changes that interfered with the trajectory, and technical limitations with stereotactic instruments.

Conclusions: (1) Mortality included increased edema, infection, bleeding, and seizure, which were associated with neurological deterioration. Neurological deterioration was also associated with distance to eloquent cortex. (2) Guideline adherence was limited to superficial or eloquent location. Tissue density (both of tumor and brain tissue), artificial dura, and eloquent location did effect the accuracy of catheter placement both for intratumoral and intraparenchymal placement. Technical limitations with normal/stereotactic equipment (NAVIGUS vs. BrainLab) were encountered.

P137. FOTEMUSTINE-DACarbacINE IN NEWLY DIAGNOSED HIGH-GRaDE GLIOMAS
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Background: The combination of fotemustine-dacarbazine (F-D) given concomitantly in radiotherapy and as adjuvant treatment was proven to be a feasible drug combination in patients newly diagnosed with GBM, yielding a median survival duration of 14.5 months, with an acceptable toxicity profile. At our institution since 1998, 143 patients with newly diagnosed malignant glioma have received F-D as first-line therapy.

Methods: Histologic diagnoses were glioblastoma multiforme in 122 patients, anaplastic astrocytoma in 18, and PNET in 1. All patients were treated with the combination of dacarbazine (D) (200 mg/m²) and fotemustine (F) (100 mg/m²) q 21 days, and concomitant radiotherapy (2 Gy/day, 5 days per week using limited fields up to 60 Gy).

Results: Median survival reached 16.3 months for GBM patients (CI, 13.42–19.26), patients with anaplastic astrocytoma lived for a median period of 37.7 months, and the patient with PNET lived 22.2 months. The 12-, 24-, and 36-, and 60-month survival rates for GBM patients were 57.4%, 24.6%, 12.3%, and 4.9%, respectively. The survival rates for patients with anaplastic astrocytoma were 94.4%, 61.1%, 38.9%, and 5.6%, respectively. Median time to progression was 7.8 months in GBM and 37.8 months in patients with anaplastic astrocytoma. Patients were given 709 cycles of therapy, and the median number of cycles given to patients was 6 (range 1–20). Forty-two patients did not complete therapy (36%). Toxicity was mainly grade 3 and grade 4 thrombopenia after the first cycle in eight patients, and after further cycles in five more patients, whereas febrile leukopenia occurred in four patients (one died). Other severe toxicities were rash in one patient after the first cycle, leading to cessation of therapy.

Conclusion: In patients where oral therapy with temozolomide appears difficult, F-D provides an arguable alternative.

P138. EFFICACY OF FOTEMUSTINE IN RECURRENT/PROGRESSIVE GLIOMAS: A PHASE II STUDY
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Objective: The chloroethyl-nitrosourea fotemustine has been shown to be effective as single agent against malignant melanoma, but few data are available regarding malignant glioma. The objective of this phase II study was to evaluate the efficacy and toxicity of fotemustine in glial tumors progressing or recurrent after surgery and/or radiotherapy and one or more lines of chemotherapy.

Materials and methods: From July 2004 to February 2006, we have treated 40 patients (14 women and 26 men; median age, 51 years; age range, 19–78 years). Histological diagnosis was as follows: GBM, 17 of 40; A, 7 of 40; O II, 6 of 40; O III, 4 of 40; O I, 4 of 40; O II, 4 of 40; O I, 4 of 40, and ganglioglioma III, 1 of 40. Fotemustine was administered weekly for three consecutive weeks (days 1, 8, and 15) at 100 mg/m² (induction treatment), fol- lowed by a five-week rest period. A maintenance therapy started in patients with nonprogressive disease consisted of fotemustine 100 mg/m² every three weeks until progression or unacceptable toxicity. MRI was performed at baseline, at the end of the induction treatment, and at the end of every cycle thereafter. Tumor response was evaluated according to Macdonald criteria. Fotemustine was administered as second-line chemotherapy (after concomitant or adjuvant temozolomide) in 14 patients, as third-line therapy (after temozolomide and PCV) in 21 patients, and as fourth-line (after PCV, temozolomide, and tamoxifen) in 5 patients. Response was evaluable for 39 patients and assessable in two patients treated as second line, in four patients treated as third line, and 1 point treated as fourth line. Myelosuppression was the most common adverse event and occurred mainly during induction treatment in 5 patients. To date, 39 of 40 patients are evaluable for response. An objective response was observed in 7 (18%) of 39 patients (4 PR and 3 MR), with 1 SD and 19 PD. Among responders (7), the original histological diagnosis was O III in 2, O II in 2, A III in 2, and GBM in 1. Response was observed in two patients treated as second line, in four patients treated as third line, and one patient treated as fourth line. Myelosuppression was the most common adverse event and occurred mainly during induction treatment in 5 patients. Grade III–IV thrombopenia was observed in 18 (46%) and grade IV–V thrombopenia in 17 (43%) of 39 patients. A severe hepatotoxicity (grade IV) was observed in one patient.

Conclusions: Fotemustine has showed some activity, especially in oligodendroglial tumors. Further studies are needed to fully evaluate the efficacy of fotemustine as second-line treatment.

P139. DELTA-LIKE LIGAND 4 (DLL4) OF THE NOTCH-SIGNALING PATHWAY IS EXPRESSED IN ENDOTHELIAL CELLS OF GLOBLASTOMA: POSSIBLE TARGET FOR ANTIANGIOGENESIS
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Introduction: Glioblastoma is characterized by prominent vascular proliferation, and antiangiogenic therapy may be efficient in glioblastoma patients. Notch signaling is considered a new important mechanism of tumor angiogenesis. Delta-like ligand 4 (DLL4) is an essential factor in the notch-signaling pathway and is upregulated in endothelial cells of the vasculature of some human tumor types. Therefore, DLL4 has been suggested as a potential novel target for antiangiogenic therapy. In our study, we investigated expression of DLL4 in glioblastoma.

Materials and methods: Using in situ hybridization, we studied DLL4 mRNA expression in 20 surgical glioblastoma specimens and 10 nonneoplastic control specimens from the temporal lobes of patients surgically treated for intractable temporal lobe epilepsy.

Results: We found expression of DLL4 in glioblastoma in endothelial cells of capillaries (16 of 20 cases [80%]) and glomeruloid (11 of 20 [55%]) vascular proliferates. Additionally, tumor cells also expressed DLL4 (4 of 20 [20%]). We did not find DLL4 expression in parenchymal or vascular cells in any temporal lobe specimen (0 of 10 cases).

Summary and conclusions: DLL4 mRNA is expressed in glioblastoma but does not seem to be expressed in nonneoplastic CNS tissue. DLL4 is expressed in endothelial cells both of capillaries and bizarre vascular proliferates of glioblastoma. Therefore, DLL4 is not to be considered as a target for antiangiogenic therapy in glioblastoma.

P140. COMBINED REGIMEN OF TEMOZOLOMIDE AND LIPOSONAL PEGYLATED DOXORUBICIN IN GLOBLASTOMA: TOXICITY AND EFFICACY— AN UPDATE
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Introduction: Temozolomide (TMZ, Temodar™) recently showed promising efficacy in an EORTC trial of first-line therapy of glioblastoma (R. Stupp, 2005). PEGylated liposomal doxorubicin (PEG-DOX, Caelyx™) was evaluated in patients with recurrent high-grade glioma and showed an overall response rate of 40% (P. Hau, 2002). Therefore, a combination of both agents is promising.

Methods: TMZ was given orally at 75 mg/m² daily during standard radiotherapy (initiation) and at 150 to 200 mg/m² on days 1 to 5 over 28 days, starting four weeks after radiotherapy (maintenance). PEG-DOX was given as a short-term infusion in a dose-escalation regimen once prior to radiotherapy and on days 1 and 15, starting four weeks after radiotherapy. The PEG-DOX dose was raised in steps of 5 mg/m² in groups of three patients, starting with 5 mg/m² (group 1) up to a highest dose of 20 mg/m² (group 4)
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Results: In the dose-escalation part of this study, the regimen was feasible, tolerable, and able to induce objective responses and stabilization in patients with glioblastoma. In the first treatment group (5 mg/m² of PEG-DOX), one of seven evaluable patients had a dose-limiting toxicity (DLT). In the second, third, and fourth treatment groups, the regimen was tolerated without DLT. With regard to efficacy in the “treated patients” analysis of 41 patients from both the toxicity and efficacy phase, one had a partial response in MRI, and 20 patients had tumor stabilization four weeks after conclusion of radiotherapy in MRI. Only five patients did glioblastoma progress early. Thirty-one patients responded with progression-free survival time of 12 to 149 weeks.

Conclusion: After conclusion of the toxicity phase, the efficacy phase was started. Together, 41 patients have been included thus far. In addition, three additional study centers (Ludwigsgaarden and Dülmen, Germany; and Innsbruck, Austria) were initiated recently. Results will be compared to the published study EORTC 26981/22981 (R. Stupp et al., 2005), which did set a new standard in the first-line treatment of glioblastoma as survival times of more than 14 months and a two-year overall survival of 26% were reached. Regarding the promising preliminary survival data of study RNome-09, we expect even better results with the regimen used in this protocol, which would mandate a phase II trial comparing both studies.

PI42. ENZASTAURIN: AN INTRODUCTION TO A NEW, TARGETED AGENT FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME
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Enzastaurin, an orally administered serine/threonine kinase inhibitor, targets PKC and PI3K/AKT pathways and suppresses tumor growth through multiple mechanisms: inhibition of cell proliferation, induction of cell death, and inhibition of tumor-induced angiogenesis. The PKC and PI3-Kinase/AKT signaling pathways are activated by growth factors such as VEGF or by mutations such as PTEN loss in many human cancers, including gliomas, non-Hodgkin’s lymphomas, and cancers of the lung, breast, colon, and prostate. Preclinically, enzastaurin suppressed the proliferation and increased apoptosis of various cell lines, including U87MG glioblastoma cells. Enzastaurin also inhibited the growth of U87MG glioblastoma xenografts in mice. Antiangiogenic effects of enzastaurin were demonstrated in rat corneal micropocket assays, in which VEGF-stimulated growth of new vasculature was suppressed. Enzastaurin also decreased VEGF expression and microvessel density in human tumor xenografts. In animal models, enzastaurin showed antitumor and antiangiogenic activity in several malignancies, including non-small-cell lung, colon, renal cell, and hepatocellular carcinomas. Enzastaurin also showed anticancer activity and an exceptional safety profile in patients with relapsed/advanced solid and hematological tumors.

Given that VEGF is the most potent angiogenic factor in the highly vascular and angiogenic gliomas, inhibition of angiogenesis may be an effective approach for reducing the growth of gliomas. Enzastaurin, as a potent inhibitor of PKCβ, an important mediator in VEGF signaling, is being investigated in clinical trials for the treatment of glioblastoma multiforme. Enzastaurin, administered as a daily oral dose of 525 mg, showed promising activity and was well tolerated in a phase II trial of patients with recurrent high-grade glioma. Overall, 23% of the heavily pretreated patients had radiological responses, while 6% had stable disease for longer than 3 months. Based on the promising results observed in the phase II study, a phase III study has been designed to compare the safety and efficacy of enzastaurin to lomustine, using progression-free survival as the primary endpoint.

Enzastaurin is also being investigated in combination with other drugs, including Alimta®, Xeloda®, Gemzar®, and cisplatin in various tumors.

PI44. PHASE I/II TRIAL (UKT-03) OF CCNU/ TEMOZOLOMIDE CHEMOTHERAPY IN ADDITION TO RADIOTHERAPY AS FIRST-LINE THERAPY FOR GLIOBLASTOMA: FINAL REPORT
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The phase III UKT-03 trial evaluates toxicity and efficacy of a combination therapy with CCNU, temozolomide (TMZ), and involved-field radiotherapy in patients newly diagnosed with glioblastoma (GBM). Thirty-one adult patients (median Karnofsky score, 90%; median age, 51 years) in two centers were enrolled for this study. The patients received involved-field radiotherapy (60 Gy in 2-Gy fractions) and chemotherapy with CCNU 100 mg/m² (day 1) and TMZ 100 mg/m²/day (days 2–6) with individual dose adjustments according to hematotoxicity. A median of five courses (range, 1–6) were applied. WHO grade 4 hematotoxicity was observed in five patients (16%), one of whom died of myelosuppression-associated sepsis. Nonhematological toxicity included one patient with WHO grade 4 drug-induced hepatitis leading to discontinuation of CCNU/TMZ and one patient with WHO grade 2 lung fibrosis leading to discontinuation of CCNU. The median progression-free survival (PFS) was nine months (95% CI, 5.3–11.7 months), and the median overall survival (OAS) was 22.6 months (95% CI, 12.5–NA), with a two-year OAS rate of 44.7%. Patients with a methylated O6-methylguanine-DNA methyltransferase (MGMT) promoter in tumor tissue showed a longer PFS (P = 0.014, log-rank test) and OAS (P = 0.037) than did patients with a nonmethylated MGMT promoter. In conclusion, CCNU/TMZ therapy is feasible with acceptable acute toxicity. CCNU/TMZ therapy was highly effective in GBM with substantially prolonged survival times, particularly in patients with a methylated MGMT promoter. The survival data appear to be superior to previously published trials on chemotherapy in GBM and to the Radiation Therapy Oncology Group database.

From January 2000 to December 2004, 410 patients with supratentorial glioblastoma multiforme were treated in our institutions. Removal of the tumor was distinctively at least subtotal in 350 patients. Routinely, a bio- molecular study was performed in all histopathological specimens (EGFR, PTEN, MGMT, VEGF, and other indexes of proliferation). The entity of removal was estimated by an MRI study done within 24 h after surgery.
P146. FIRST-LINE CHEMOTHERAPY VS SALVAGE CHEMOTHERAPY: TEMOZOLOMIDE AND GBM

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The treatment of glioblastoma multiforme (GBM) is still the object of much discussion. The present guidelines provide for a multimodal approach consisting of surgical treatment, radiotherapy, and chemotherapy. In the last years, chemotherapy has assumed an essential role. One of the most important drugs is temozolomide, used at the beginning for the treatment of relapsed GBM and, subsequently, as first-line chemotherapy. In this retrospective, multicentric study, we analyze the efficacy of temozolomide as first-line drug compared with its use in salvage chemotherapy of patients with GBM.

Between January 2000 and March 2005, at the Division of Neurosurgery of the Department of Neurological Sciences of Ospedale Maggiore Policlinico (Milan) and at the Division of Neurosurgery of Istituto Galleazzi (Milan), we have operated on 68 patients who had a histological diagnosis of GBM. All of these patients, after surgical treatment, underwent conventional radiotherapy. We divided the patient population into two groups according to the timing of temozolomide administration: Group 1 underwent first-line chemotherapy, while group 2 received a second-line treatment with temozolomide.

We have analyzed progression-free survival at 6 months and at 12 months (PFS-6 and PFS-12), time to progression (TTP), and overall survival (OS) in the two groups. We have made a clinical and radiologic follow-up: PFS-6 was 85% for group 1 and 65% for group 2. PFS-12 was 21% for the first group and 15% for the second. The median TTP amounted to 10 months for group 1 and 7 months for group 2. The median OS was 16.3 months for first-line treatment and 12.4 months for salvage chemotherapy. The median follow-up was 14 months.

P147. A MULTICENTRIC PHASE II STUDY OF TEMOZOLOMIDE NEOADJUVANT, CONCOMITANT AND SEQUENTIAL TO RADIOThERAPY IN Glioblastoma MULTIFORME: IS THE IMPROVEMENT OF THERAPEUTIC RESPONSE A FEASIBLE TARGET?

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Introduction: The new protocols combining temozolomide (TMZ) and radiotherapy (RT) have improved clinical results in glioblastoma multiforme (GBM), without significant increase in either acute or late toxicity. Given the high cellular kinetics and regrowth of GBM, it can be hypothesized that the neoplastic progression frequently occurring between surgery and radiotherapy would be limited by early administration of TMZ. Furthermore, in responding patients, extending TMZ therapy beyond the standard of six cycles should positively affect time to progression and overall survival.

Materials and methods: The study was a prospective, multicentric study, we analyze the efficacy of temozolomide as first-line chemother AP y (CT) with temozolomide.

Methods: All 89 patients with newly diagnosed GBM treated with this schedule from March 2002 to December 2005 were recruited retrospectively from 8 centers in Catalonia and Mallorca. Nine patients were excluded by reason of follow-up of less than 30 days or age less than 18 years. A Kaplan-Meier method and a multivariate analysis using the Cox model were performed to study time to progression (TTP) and overall survival (OS).

Results: For the 52 men and 28 women (median age, 56.5 years; age range, 18–80 years), median follow-up was 7.3 months (range, 1.3–39.1 months). Median time of surgery to complete resection (PR) in 47.5%, and biopsy (B) in 20%. Median Karnofsky (KI) after surgery was 90 (range, 40–100), and median time from surgery to start of RT (SRI) was 41 days (range, 13–79 days). During the RT, 35% of patients received dexamethasone (DVMX), and only 17.5% prophylactically against Pneumocystis. Three patients received a boost of stereotactic RT and one whole brain irradiation. Seventy-four patients (92.5%) completed the concomitant TMZ, and tumor assessment at that time was as follows: 19 patients free of disease, 7 patients with partial responses, 26 patients with stable disease, 14 patients with progression disease, and 8 with status unknown. Sixty-four patients (80%) started adjuvant TMZ (five patients assessed as having disease progression). The median number of cycles administered was 4: 2 patients completed 6 cycles, disease progressed in 11, 2 dropped out due to toxicity, and 11 are still under treatment. Median TTP was 6.6 months (range, 5.2–8.0 months). After disease progression, 29 patients (36.7%) received adjuvant chemotherapy (CT), and 5 surgery. In multivariate analysis, age and extent of surgery were significant for recurrence, with HR = 2.7 (range, 1.56–4.66) in patients older than 60 years; HR = 2.042 (range, 1.09–3.84) between CR and PR; and HR = 2.99 (range, 1.42–6.27) between CR and B. No statistical difference was seen for gender, KI, DVMX, and SRI.

Conclusions: GBM patients treated with this schedule out of trial’s selection criteria (>70 years, KI < 70, and SRI > 6 weeks) achieve a TTP similar to that in the EORTC and NCIC trials. Extent of surgery and age were significant for the disease recurrence, but not KI. Currently, follow-up is too short to evaluate OS, but an update of results and survival analysis will be presented.
Abstracts for the Seventh Congress of the European Association for Neuro-Oncology (EANO)

each inhibitor its therapeutic window toward vessel normalization when administered at a certain stage of tumor progression. Furthermore, we designed tailored schemes of treatment in which the inhibitors were sequentially administered alone or in combination according to their therapeutic windows established at a certain stage of tumor progression. Our findings indicate that by the strategy of carefully tailoring the administration of antiangiogenic agents, it is possible to maintain a long-term status of vessel architecture normalization, and that this results in long-term tumor growth control.

P149. POSTOPERATIVE CONCOMITANT RADIOCHEMOTHERAPY IN THE TREATMENT OF PRIMITIVE CNS HIGH-GRADE GLIOMAS: A RETROSPECTIVE ANALYSIS

Background: The authors analyze retrospectively the impact on tumor control and toxicity of concomitant radiotherapy (RT) and temozolomide (TMZ) in high-grade gliomas (HGGs) of the CNS, in patients treated in the Clinical Oncology Institute, Oporto, Portugal.

Methods: This cohort represents all patients with HGGs who were treated between January 2002 and January 2006, with concomitant RT a median total dose of 60 Gy, 2 Gy per treatment given once daily, five days/week and TMZ 75 mg/m² for 42 days, followed by adjuvant treatment with TMZ five-day schedule every 28 days (150 mg/m² for the first cycle increased to 200 mg/m²). The cohort was retrospectively analyzed for general information, age, extent of surgical resection, initial KPS, median overall survival (OS), and hematochemical toxicity.

Results: Twenty-three patients (6 female and 17 male; median age, 58 years; age range, 17–72 years) with HGGs were treated with concomitant RT (44–72 Gy) and TMZ, followed by adjuvant TMZ. Median KPS was 80 (range, 40–90). Three patients had complete resection, 17 had partial resection, and three biopsy. All patients except one, who had treatment interruption for thrombocytopenia, continued the concomitant phase of treatment; 19 patients continued to received adjuvant treatment with TMZ (median number of cycles, 5; range, 1–20). Median OS (measured from the date of diagnosis to the date of death) was 20.4 months, and 1-year OS was 54%. During the concomitant phase, one patient had grade 3–4 thrombocytopenia. During adjuvant TMZ therapy, four patients had grade 3–4 hematochemical toxicity (anemia, 1; thrombocytopenia, 1; and leukopenia and thrombocytopenia, 2).

Conclusions: The results of concomitant RT + TMZ followed by TMZ in these patients with HGGs showed values in accordance with the latest data published on literature for this association. Differences observed might be due to the small sample size. RT+TMZ followed by adjuvant TMZ is a well-tolerated treatment with better results in median OS comparatively with previous results of RT-only treatment in HGGs. Treatment-related toxicity was within acceptable levels, and this approach became routine practice in this set of patients.

P150. TEMOZOLOMIDE IN GLIOMATOSIS CEREBRI: RESULTS OF A MULTICENTER RETROSPECTIVE STUDY OF THE AINO (ITALIAN ASSOCIATION OF NEURO-ONCOLOGY)
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Objectives: To assess the efficacy and toxicity of temozolomide (TMZ) in patients with gliomatosis cerebri, a diffusely growing neuroepithelial tumor, whose optimal treatment is unclear.

Methods: Since 1999, 41 patients with histologically confirmed gliomatosis cerebri were treated with TMZ either at progression after radiotherapy/chemotherapy or up front. Horizontal diagnosis was gliomatosis in 3 cases, malignant glioma in 6, anaplastic astrocytoma in 7, gemstocytocytic astrocytoma in 2, astrocytoma in 12, anaplastic oligoastrocytoma in 1, oligoastrocytoma in 1, oligodendrogloma in 4, and glioblastoma proliferation typical gliomatosis cerebri in 5. Patients characteristics were as follows: 21 males and 20 females; median age, 49 years (range, 14–70 years); and median KPS at diagnosis, 80 (range, 50–90). Presenting symptoms included seizures (20 patients), intracranial hypertension (8), motor deficits (7), cognitive deficits (2), drowsiness and diplopia (3), and dizziness and vomiting (1). Of 41 pre-treatment MRIs, 19 demonstrated some contrast enhancement. Twenty-two patients were treated up front, while 19 received either radiation therapy or nitrosourea-based chemotherapy prior to TMZ. All patients were treated with TMZ 200 mg/m² per day for 5 days every 4 weeks until progression or unacceptable toxicity. Response was evaluated, according to Macdonald criteria, on T1 weighted with gadolinium and FLAIR MRI.

Results: The median number of cycles was 7 (range, 1–20). Two patients (5%) showed a CR of the contrast-enhancing area, 2 patients (5%) a PR of the FLAIR hyperintense area, 5 (12%) a minor response, 16 (39%) an SD, and 16 (39%) a PD. Overall response rate (CR + PR + "minor response”) was 22%, 14% in patients treated up front, and 4% at disease progression. Median time to tumor progression (TTP) was 9 months (range, 1–27 months). PFS was 66% at 6 months and 45% at 12 months, and median survival was 13 months (range, 3–123 months). Oligodendrogliomas showed a 43% response rate and a 11-month TTP. A clinical benefit was observed in 12 (29%) of 41 patients, consisting of a reduction in seizures (6 patients) and improvement in motor (1 patient) or cognitive (1 patient) deficits. Four patients showed grade III–IV hematological toxicity (10%).

Conclusion: TMZ in conventional regimens seems to be moderately effective and safe in gliomatosis cerebri. To improve efficacy further, a phase II study with an extended schedule as first-line treatment has been launched.

P151. THERAPY AND SURVIVAL TIME OF 18 PATIENTS WITH GLIOSARCOMA
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Background: Gliosarcoma (GS), a subtype of glioblastoma with sarcomatoid and/or epithelioid phenotype, is an uncommon, highly malignant brain tumor with poor prognosis. It represents 2%–3% of all glioblastomas. Because of the varied morphology of this tumor, adequate sampling of the tissue is crucial for the accurate diagnosis.

Results: Materials and methods: We retrospectively analyzed 18 patients with GS diagnosed from 1994 to 2005 and treated at the Institute of Oncology in Ljubljana. Ten patients were men and eight were women. Age ranged from 18 to 72 years. They had signs and symptoms of a rapidly growing brain tumor. The diagnosis was confirmed by microscopic examination using H&E, silver stain for reticulin, and immunohistochemistry for GFAP, GMA, EMA, and CK. Ten patients had gross total excision of the tumor, five had partial debulking surgery and, for the rest, there was no clear data on the extent of operation. Postoperatively, 13 patients received radiotherapy, with a median TD of 50 Gy (range, 20–60 Gy). Chemotherapy (temozolomide) was delivered to three patients.

Conclusions: The median survival was 9 weeks (95% CI, 17–55 weeks). Of the 18 patients, 16 patients died, and two are still alive 5 and 10 months after surgery.

Conclusion: The survival of our patients is poor and does not much differ from the results of other published studies.

P152. CHEMOTHERAPY COMBINED WITH RADIOTHERAPY IN TREATMENT OF HIGH-GRADE GLIOMAS
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Background: In our previous study of patients with high-grade glioma treated with postoperative radiotherapy between 1995 and 2004, 137 patients with glioblastoma multiforme (GBM) and 95 with anaplastic astrocytoma (AA) showed an overall survival of 32% for GBM and 49% for AA. The role of chemotherapy in the treatment of high-grade gliomas is still discussed. We retrospectively analyzed our experience in temozolomide (TMZ) chemotherapy plus radiotherapy for high-grade glioma with the aim of evaluating time to tumor progression, toxicity, and overall survival.

Methods: We treated 47 high-grade gliomas (between 1998 and 2004) with postoperative radiotherapy (RT) and chemotherapy with TMZ: 31 patients (65.96%) with GBM and 16 (34.04%) with AA. The median age was 46 years (range, 18–64 years). There was a preponderance of male patients (28 vs. 19 female). Postoperative RT was administered to all patients with 1.8 to 2.0 Gy/day to a total dose of 50 to 60 Gy. Chemotherapy was delivered according to the TMZ treatment schedule was TMZ concomitant with RT (75 mg/m² daily for 5–6 weeks, and then 200 mg/m² p.o. on days 1–5 every 28 days for 6 cycles) for 20 patients (42.5%) and TMZ adjuvant to RT (27 patients, 57.5%) started 3 weeks after the end of RT (200 mg/m², days 1–5, every 28 days, for 6 cycles).

Results: The median follow-up was 18.1 month (between 5 and 76 months). Fifteen patients (31.9%) lived and 32 died (68.09%) by the end of the study. The overall RR (CR + PR) was 46.81%, stable disease was achieved in 40.43% of patients and progressive disease in 12.77%. At last follow-up, 10 patients (32.6%) with GBM were free of disease, and 21
patients (67.74%) had progressive disease. The overall survival at 18 months was 49% for patients with GBM who had TMZ concomitant with RT and 35% for patients who had TMZ started after the completion of RT (P = 0.92 NS). Among patients with AA, nine (56.25%) and 40 million cells were treated with adjuvant TMZ after RT, and seven (43.75%) with TMZ concomitant with RT. The overall survival was 69% at 18 months. The disease-free survival for all patients (GBM and AA) with objective response was 58% (CI, 37%–76%). Toxicity was acceptable for all patients: without toxicity, 61.67%; hematologic toxicity, 6.67%; nonhematologic toxicity, 28.33%; and hematologic + nonhematologic toxicity, 3.33% of patients.

Conclusions: Concomitant radiotherapy plus TMZ is well tolerated and seems to increase progression-free survival and overall survival. Considering this, our next study will combine TMZ with hyperfractionated accelerated radiation therapy and a higher total dose.

P153. LOCOREGIONAL HYPERTHERMIA IN PATIENTS WITH PROGRESSIVE ASTROCYTOMA WHO III OR GLOBLASTOMA WHO IV (RNO-10): A PROSPECTIVE SINGLE-ARM PHASE I/II STUDY

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Introduction: Despite promising data from phase II studies in solid tumors, locoregional hyperthermia in the treatment of malignant brain tumors has not been investigated thus far in a controlled clinical study. In the animal model, combined treatment with hyperthermia and chemother-apy showed an additive inhibition of brain tumor proliferation (Fugl et al., 2004). Existing data on recurrent malignant brain tumor patients treated with the combination of chemotherapy or radiotherapy with hyperthermia showed a median survival of 44.2 months in patients with WHO grade III astrocytoma and 23.2 months in patients with grade IV glioblastoma (Sahinbas et al., 2005).

Methods: This protocol is designed as a prospective single-arm phase I/II study for patients with recurrent malignant glioma after first-line treat-ment. In the first part of the study (toxicity and dose finding), in addition to ACNU 90 mg/m² given as a short-term infusion (day 7 of a six-week cycle), locoregional hyperthermia is applied in a dose-escalation regimen starting with two sessions per week over a four-week interval (days 1–28 of a six-week cycle) in a group of three patients (group 1) for up to five sessions a week in the highest dosing group (group 4). Maximum tolerated dose and dose-limiting toxicity will be evaluated. In the second part of the study (exploratory efficacy), another group of patients will be treated with the evaluated maximal tolerated dose (MTD), and median time to progression will be observed. The cases are followed closely, including MRI imaging (every six weeks), and H-proton spectroscopy and diffusion and perfusion MR-imaging (first and fourth cycles).

Results: Until now, three patients have been included in the first dose-escalation group, and one patient was excluded because of dose limiting toxicity (local paresthesia during hyperthermia).

Conclusion: The updated data will be available at the EANO. Observing some toxicity already at this low dose level, it may be concluded that such a protocol is urgently needed, because many patients currently receive hyperthermia on a commercial basis. This study will be the first to evaluate objective feasibility/toxicity of regional hyperthermia as a noninvasive, potentially additional, therapeutic approach in malignant gliomas.

P154. IMMUNE THERAPY SIGNIFICANTLY PROLONGS SURVIVAL OF GBM PATIENTS

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During the last few years, remarkably promising results have been reported from at least four clinical trials based on immune therapy against glioblastoma multiforme (GBM): the Victor trial using the EGF/FGF/III peptide conjugated with keyhole limpet hemocyanin (KLH) combined with autologous dendritic cells for immunization (Darel Bigner’s group, Duke, NC, reported at the sixth EANO meeting, Neuro-Oncology, July 2005); the use of protein extracts from GBMs in combination with autologous dendritic cells (J. Wheeler, LA, CA, Cancer Res. [2004] 64:4973–4979); and the use of cultured autologous GBM cells irradiated and infected with Newcastle disease virus before immunization (Herold-Mende, Heidelberg, J. Clin. Oncol. [2004] 22:4272–4281).

Our group has completed the first part of a human immunogene therapy study in 10 patients, 50 to 69 years old, operated on for GBM and immunized with their individual cells. When these cells are karyotyped as malignant, 36% of the patients are alive, and 40 million cells are collected, the immunizations are given intradermally 3-weekly. Seven days after immunization, a skin biopsy sample is taken from one of the injection sites. Peripheral blood is sampled before and after the operation and after each immunization. Coculture of this blood with the tumor cells allows for a selection of T cells that recognize tumor-specific antigens.

The method is safe for the patients and gives positive DTH reactions and an increase in infiltrative CD8+ and CD4+ T cells at the immunization site. Cocultures show that the IFN-γ production is higher in lymphocytes from patients with a prolonged survival. Of nine immunized patients, four survived for 19.5, 21, 26.5, and 24.5 months. The mean survival time of these nine patients (mean age, 61.4 years) is 16.4 months, as compared with the 9.7-month survival (P < 0.03, Mann-Whitney) of the 11 patients (mean age, 60.3 years) included in the study, but where the cells did not grow sufficiently well in the cultures to make immunization possible. A tenth patient (age 57) who is under immunization has a remarkable regression of her tumor and has also shown stronger DTH reactivity than any of the nine patients treated earlier.

The aforementioned other studies report significantly prolonged sur- vival. They include, however, also patients younger than 50 years of age, with their presumably stronger immunoreactivity. Our conclusion is that the results described strongly support the possibilities in immune therapy against GBM.

P155. IMAGING FINDINGS RELATED TO ADJUVANT DENDRITIC CELL (DC) VACCINATION IN PATIENTS WITH RELAPSED HIGH-GRADE GLIOMA

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Introduction: Malignant gliomas still have a dismal prognosis regard-less of the most advanced combinations of microsurgical resection, radio-therapy, and chemotherapy. For four years, we have been exploring the feasibility, working mechanisms, and potential clinical benefits of adjuvant dendritic cell–based vaccination for patients with relapsed high-grade gli-oma. Having treated 61 patients until now in an ongoing cohort comparison trial, our group gained wide experience in this active immunotherapeutic approach.

Aim: In this subanalysis, we define typical imaging responses and pit-falls related to treatment, where the classic response criteria do not always apply.

Patients and methods: DC vaccination against relapsed high-grade gli-o ma can be offered as a treatment option if the recurrence can be surgically removed. After intentional macroscopic complete resection, a tumor cell lysate is prepared to serve as source of tumor-associated antigens. Autolo-gous, monocyte-derived DCs are generated and loaded with tumor lysate. The loaded DCs are reinfused intradermally. Cases are followed clinically, biochemically, and radiologically. The apparent tumor response on standard gadolinium-enhanced MRI is compared to clinical evolution and to perfu-sion MRI, MR spectroscopy (MRS), and 11C-methionine positron emission tomography (PET) scan findings.

Results: Contrary to other oncological therapies, objective tumor responses according to the McDonald criteria are not applicable, not only because of the incomplete resection, but also because the immune response may create confusing areas of contrast enhancement, presum-ably due to inflammatory reaction. A wide range of MR evolutions can be classified as follows: type 1, tumor progression; type 2, stable disease; type 3, (partial) remission; and type 4, transient inflammatory reaction in the margins of the resection cavity. In five patients (8%), this last evolution falls related to treatment, where the classic response criteria do not always apply.
P156. COMBINATION OF META-
TETRAHYDROXYPHENYLCHLORIN (FOSCAN®) – MEDIATED PHOTODYNAMIC DIAGNOSIS AND PHOTODYNAMIC THERAPY FOR RECURRENT GliOBlastOMAS
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Background: The median survival of patients suffering from malignant brain tumors is 12 months despite all available therapy. Photodynamic therapy (PDT) and photodynamic diagnosis (PDD) are currently undergoing intense clinical investigations. We recently developed intraoperative PDD for fluorescence-guided tumor resection and combined PDD with PDT by using the second-generation photosensitizer meta-tetrahydroxyphenylchlorin (mTHPC).

Methods: In 24 patients suffering from recurrent glioblastomas, photosensitization was performed by 0.35 mg mTHPC/kg.BW four days prior to standard craniotomy. Intraoperative fluorescence was induced by a UV light source at 370 ± 440 nm. A standard neurosurgical microscope was optimized for fluorescence detection. Fluorescence was detected by the naked eye through the operating microscope, CCD camera, and spectrosco- py. Intraoperative PDT was carried out by a diode laser at 652 nm and 20 J/cm². Within 48 h, the extent of the surgical resection was investigated by MRI. The results of this group were compared to a matched group of 23 patients who did not receive photosensitization.

Results: The fluorescence sensitivity and specificity in 172 tissue samples were 87.9% and 95.7%, respectively. Residual tumor could be predicted at an accuracy of 91.7%. The median survival time for the PDD+PDT group was nine months, compared to 3.5 months for the matched pair group. Macroscopic and MRI-documented radical resection was achieved in 15 patients, compared to 10 in the matched-pair group. PDT was generally well tolerated, and side effects consisted of slight intracranial pressure and prolonged skin sensitivity against direct sunlight.

Conclusion: We demonstrated that intraoperative mTHPC-mediated PDT and fluorescence-guided resection combined with intraoperative photodynamic treatment is well tolerated by the patients and is a promising concept for brain tumor surgery.

P158. MGMT expression correlates with response rate and survival in patients with inoperable Glioblastoma (GBM) treated with NeoAdjuvant Temozolomide (TMZ)
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Background: Methylator of the promoter of O6-alkylguanine alkyl-transferase (MGMT), a DNA repair gene, may enhance chemosensitivity to alkylating agents. In GBM, this methylation has been correlated to survival, as well as to the benefit of adding TMZ concomitant and adjuvant to radiotherapy (RT). We examine the relationship between MGMT expression and objective response rate to dose-intensive TMZ schedule administered neo-adjuvant treatment before RT in inoperable GBM, as previously presented.

Patients and methods: Thirty patients were included in this phase II trial that tested TMZ (150 mg/m²/day) on days 1 to 7 and 15 to 21 of each 28-day cycle for up to four cycles prior to RT. We retrospectively analyzed MGMT expression by immunochemistry (streptavidin-peroxidas) after antigen retrieval using anti-MGMT antibody (Abycys, 1:100) in 25 formalin-fixed paraffin-embedded samples from the study population.

Results: In the eligible population (n=38), response rates (RRs) were 25% (95% CI, 6.3%–41.3%); stable disease (SD), 32%; progressive disease (PD), 43%; Median progression-free survival (PFS) and overall survival (OS) were 3.8 and 5.8 months, respectively. MGMT expression was analyzed in 25 patients while material was considered as inadequate in three patients because of insufficient tumor material. The median percentage of cells that expressed MGMT in the tumor nucleus was 35%, and the number of copies of the MGMT gene was chosen as the cutoff. Low MGMT expression was significantly associated with a high RR (55%), whereas tumor that exhibits high MGMT expression was associated with an RR of 7%, with progression disease to 9% and 71% (p=0.004). MGMT was also strongly correlated with PFS at 3.5 and 1.9 months (log-rank P=0.009) and OS at 16 and 5 months (log-rank P=0.003).

Conclusion: Despite the limited number of patients, our study strongly supports the predictive value of MGMT expression for objective response to TMZ in addition to its prognostic value for PFS and OS in GBM. If confirmed in a prospective study, MGMT expression may help to guide therapeutic decisions, as well as more targeted trial design.

P159. PRERADIOThERAPY CHEMOTHERAPY (cHT) IN HIGH-Grade gliOMAS 1 – HIGH GRADe BRAIN tumOrs (HG-BG) with TEMOzolomIDE (TMZ) and cisplAtin (cDDP): RESULTS with lower-dose cDDP in AND oNLY TWO cYcles C. Balañà,1 R. Ballester,1 J. Capellades,2 O. Etxanitz,3 M. Alamar,3 R. García,1 C. Carrasco,1 A. Arellano,1 R. Rosell,1 and V. Vallés1; 1Institut Català d’Oncołgia, Badalona/Barcelona, Spain, 2Institut Diagnòstic per l’Imatge, Badalona/Barcelona, Spain, and 3Hospital Universitari Germans Trias i Pujol, Badalona/Barcelona, Spain

Preradiotherapy (P-RxT) chemotherapy seems a reasonable option for high-grade glioma (HGG) non-oligodendroglioma that has had only a biopsy and not debunking surgery, because standard treatment has not demonstrated a survival advantage in this setting. A previous report of treatment with TMZ and CDDP has shown an increase in median survival in these patients but at the expense of too much toxicity. Another ChT combination schedule must be explored.

Objective: To evaluate the clinical activity and overall survival of biopsy-alone patients with newly diagnosed GBM of anaplastic astrocytoma (AA) over 50 treated with reduced doses of CDDP and TMZ for only two cycles before radiotherapy.

Patients and methods: Two cycles of TMZ (200 mg/m⁲/day [days 1–5]) plus CDDP (70 to 75 mg/m² on day 1 every 28 days) were administered before RxT of 60 Gy over primary tumor. MRT was repeated after ChT and one month after RxT if no signs of neurological worsening were suspected. Concomitant RxT to TMZ (75 mg/m²/day for 42 days) was added after completion of P-RxT. Twenty-five patients were analyzed.

Results: Twenty-four patients have been included: male–female ratio, 10:14; average age, 61 years (range, 19–72 years) (83% were over age 50); GBM–AA ratio, 19:5; KPS >70, 89%; Barthel index >70, 33%; multi-centricity, 33%; neurological deficit, 75%; neurological scale, 0 to 1, 54%; multi-vascular, 35%; complex surgery, 75%; median survival, 11 months. MGMT was not demonstrated a survival advantage in this setting. A previous report of treatment with TMZ and CDDP has shown an increase in median survival in these patients but at the expense of too much toxicity. Another ChT combination schedule must be explored.

Conclusion: Despite the limited number of patients, our study strongly supports the predictive value of MGMT expression for objective response to TMZ in addition to its prognostic value for PFS and OS in GBM. If confirmed in a prospective study, MGMT expression may help to guide therapeutic decisions, as well as more targeted trial design.
P160. EFFICACY OF RADICAL SURGERY UNDER NEURONAVIGATION AND POSTOPERATIVE TEMODAL PLUS RADIATION FOR SUPATENTORIAL MALIGNANT GLIOMAS
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Aim: The aim of the present study is to analyze the efficacy and safety of neuronavigation radical surgery and postoperative Temodal (temozolomide) plus radiotherapy for supratentorial glioblastomas (GBMs) and anaplastic astrocytomas (AAs).

Materials and methods: A total of 86 patients with cerebral malignant gliomas underwent microsurgical tumor removal by the same surgical team and in identical surgical conditions. In 43 patients (neuronavigation group), gliomas were resected completely under neuronavigation (Voyager SX). Spiral computed tomography (IQ 5000, Picker) contrast-enhanced data or magnetic resonance imaging (Open View, Picker) were employed for the image guidance. In the control group (43 patients), glioma resection was performed using no neuronavigation. There was no significant difference in age, gender, GBM/AA ratio, and Karnofsky scale before surgery between groups. In neuronavigation group, 36 patients were given chemotherapy with Temodal plus radiation postoperatively. In subgroup I, 24 patients with AA had external beam radiation (6000 cGy) and Temodal (200 mg/m², six cycles). In subgroup II, 12 GBM patients received radiotherapy plus continuous daily Temodal (75 mg/m²) followed by six cycles of adjuvant Temodal (200 mg/m²).

Results: Microsurgical malignant glioma removal in patients with a preparooperative high Karnofsky score (above 60) has a low risk of postoperative severe complications. No patients died after surgery in either group. In the neuronavigation group, 15 patients (35%), in comparison with seven patients (16%) of control group, demonstrated functional improvement after glioma surgery. There was functional deterioration in three (7%) and 12 (29%) patients, respectively.

In subgroup I, the median survival (MS) was 25.9 ± 3.8 months. The median progression-free survival (MDFS) was 23.1 ± 3.7 months. The two-year survival rate was 54% (13 patients). There were adverse effects in six patients (25%). Two patients (8%) had only grade 3 toxic effects. In subgroup II, the MS was 17 ± 7.1 months. The MDFS turned out to be 14.8 ± 6.2 months. The two-year survival rate was 17% (two patients). There were adverse effects in four patients (33%). Two patients (17%) had grade 3 and 4 toxic effects.

Conclusions: Computer-assisted technologies improve the postoperative outcomes for high-grade glioma patients. Temodal is a rather safe chemotherapy agent. Postoperative chemotherapy therapy with Temodal plus radiotherapy extends high-quality survival in patients with supratentorial malignant glioma.

P161. A PHASE II STUDY WITH TEMOZOLOMIDE AS FIRST-LINE CHEMOTHERAPY IN RECURRENT/PROGRESSIVE OLGODENDROGLIAL TUMOR
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Aim: There are few data regarding the efficacy of first-line chemotherapy in patients with oligodendrogliomas responding, including the seven patients with CR (six grade III tumors and one grade II), compared to 11 (67.6%) of 16 oligoastrocytomas, including two CR (OAI/II). Twenty-three (44%) of enhancing tumors responded, compared to 8 (53.3%) of 15 nonenhancing tumors and 63.3% of grade III responded, compared to 55% of grade II. Median TTP was 16 months (range, 2–44), with a PFS at six months of 82% and at 12 months of 50%. Grade III–IV myelotoxicity was observed in 22% of patients. Of the 50 patients, 24 had a disease progression and thus received radiotherapy or PCV. Preliminary data suggest a positive correlation between response to TMZ and combined 1p/19q loss.

Conclusions: Temozolomide shows activity as first-line treatment in oligodendrogial tumors at first relapse, its activity similar being to that previously observed with PCV. Long-term treatment with temozolomide is safe.

P162. DNA TOPOLOGY-MODULATING AGENT CHLOROQUINE ACTIVATES P53-DEPENDENT TRANSCRIPTIONAL RESPONSE AND INDUCES APOPTOSIS IN GLIOMA CELLS
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The major biological function of the tumor-suppressor p53 is that of a sequence-specific transcriptional activator that regulates expression of a large number of functionally diverse genes involved in the regulation of cell cycle, DNA repair, or apoptosis. The potential to activate genes with distinct or even antagonist functions comprises the molecular basis underlying the remarkable functional versatility of p53, whose activities are essential for the maintenance of a balance between survival-promoting and apoptosis-inducing signals in a cell. Not surprisingly, the search for agents that would be effective in (re)activating the p53 transcriptional response in tumor cells has been an actively developing area of cancer research. Recent advances revealed that the potential of p53 to activate transcription is dependent on the local structure of DNA that can either facilitate or inhibit interaction of p53 with its cognate sites, a prerequisite for transcriptional activation by p53. In agreement with the general idea that p53 transcriptional activity is sensitive to DNA topology, we found that DNA intercalating agent chloroquine activates sequence-specific DNA binding and transcriptional activity of p53 in gloma-derived cell lines. Importantly, chloroquine-activated p53 transcriptional response is functional as it leads to the activation of proapoptotic p53 target genes and induction of apoptosis in glioma cells. Furthermore, chloroquine treatment sensitizes glioma cells to gamma-irradiation and to drugs used for glioma treatment, such as BCNU. The mechanism underlying the p53 activation induced by chloroquine involves interaction with Temodal and extends high-quality survival in patients with supratentorial malignant glioma.
To investigate efficacy and toxicity of second-line PCV in glioblastoma.

Results: A total of 336 courses of Caelyx™ were administered. In 49 patients, 5 PRs and 21 SDs were observed. The response rate for patients with WHO grade III was 61% and with WHO grade IV was 48%. The median TTP for patients who were treated with definitive RT between 1997 and 2004. We examined the effect of age (<60 vs. ≥60 years, as the most characteristic age borderlne), performance status (PS < 70 vs. PS ≥ 70), duration of PCV (radical vs. partial), T stage (T1 vs. T2–4), and adjuvant chemotherapy (CHT) with Gemcitabine and/or BCNU (yes vs. no). One patient died 14 months after having received three cycles of Caelyx™. The cases were followed by MRI every eight weeks. The mean injected volume into the cavity was 4 to 8 ml. The patients were followed by MRI every 4 weeks up to three times. The mean injected volume into the cavity was 4 to 8 ml. The patients were followed by MRI every 4 weeks up to three times.

Conclusion: The regimen is well tolerated and has activity in patients with recurrent glioblastoma.

P165. TARGETED RADIONUCLEOTIDE THERAPY WITH 90Y-DOTA-TOC FOR RECURRENT HIGH-GRADE GLIOMA: FIRST RESULTS


Objective: To evaluate the efficacy and tolerability of ifosfamide, carboplatin, and etoposide (ICE) in patients with recurrent glioblastoma.

Methods: Patients with recurrent malignant brain tumors who failed standard treatment were investigated for the expression of somatostatin receptors by 111In-DOTA-TOC scintigraphy or 68Ga-DOTA-DOC positron emission tomography. The primary end point was progression-free survival at six months (PFS-6), and secondary end points were response rate, toxicity, and survival. Chemotherapy consisted of ifosfamide (700 mg/m² on days 1, 2, and 3), carboplatin (100 mg/m² on day 1), and etoposide (70 mg/m² on days 1, 2, and 3), every six weeks.

Results: PFS-6 was 37%. The median PFS was 17 weeks. Response rate was 27%. Adverse events were generally mild (grade 1 or 2) and consisted mainly of alopecia.

Conclusion: This regimen is well tolerated and has activity in patients with recurrent glioblastoma.

P166. RADIOTHERAPY DOSE ESCALATION AS THE PART OF A MORE AGGRESSIVE THERAPEUTIC APPROACH IN GLIOBLASTOMA

Introduction: The aim of our retrospective study was to investigate the value of dose-escalated radiotherapy (RT) in glioblastoma patients.

Materials and methods: We analyzed the data of 126 glioblastoma patients who were treated with definitive RT between 1997 and 2004. We examined the effect of age (<60 vs. ≥60 years, as the most characteristic age borderlne), performance status (PS < 70 vs. PS ≥ 70), duration of PCV (radical vs. partial), T stage (T1 vs. T2–4), and adjuvant chemotherapy (CHT) with Gemcitabine and/or BCNU (yes vs. no). One patient died 14 months after having received three cycles of Caelyx™. The cases were followed by MRI every eight weeks. The mean injected volume into the cavity was 4 to 8 ml. The patients were followed by MRI every 4 weeks up to three times. The mean injected volume into the cavity was 4 to 8 ml. The patients were followed by MRI every 4 weeks up to three times.

Results: Younger age, good performance status, and T1 stage had a positive effect on survival: the median survival times (MSTs) were 12 vs. 10.5 months (P = 0.0284), 14 vs. 9 months (P = 0.0001), and 13 vs. 11 months (P = 0.0317), respectively. Regarding the aggressiveness of surgery, a nonsignificant tendency was observed: the MSTs were 12.0 vs. 10.5 months (P = 0.1227). CHT and RT dose escalation significantly prolonged the survival: the MSTs were 13.5 vs. 8.5 months (P = 0.0001) and 13.5 vs. 9 months (P = 0.0001), respectively. Eight patients were alive two years after initial diagnosis, seven of whom had been treated with CHT, and all of the two-year survivors received higher RT doses. Multivariate analysis revealed that good KPS (P = 0.0011; RR = 0.31, 95% CI, 0.14–0.76), RT dose escalation (P = 0.0002; RR, 0.46; 95% CI, 0.31–0.69), and the addition of CHT (P = 0.0001; RR, 0.45; 95% CI, 0.30–0.67) had an independent positive effect on survival. The IOFH-RT showed a significant positive effect on survival compared with conventional RT. As well, the MSTs were 13 vs. 9 months (P = 0.0048). We found that the choice of IOFH-RT did not improve significantly the effect of CHT; nevertheless, it is more advantages in the groups of patients having radical surgery, or of younger age, or with higher T stage or lower KPS.

Conclusion: This study indicates that, besides CHT the RT dose escalation, moreover IOFH-RT 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.

P167. SECOND-LINE PCV IN RECURRENT OR PROGRESSIVE GLIOBLASTOMAS: A PHASE II STUDY


Objective: To investigate the efficacy and toxicity of second-line PCV in patients with recurrent glioblastoma after surgery, external radiotherapy, and first-time chemotherapy with temozolomide. End points of this study were response rate (according to Macdonald’s criteria), time to tumor progression (TTP), survival (S), and toxicity.

Methods: Inclusion criteria were age ≥ 18 years, histologically confirmed diagnosis of glioblastoma (WHO grade IV); measurable enhancing tumor on MRI; Karnofsky performance status ≥ 60, and adequate laboratory test results. PCV was administered at a standard schedule (CCNU, day 1; procarbazine, days 8–21; and vincristine, days 8 and 29) every six weeks, with 75% standard dose of CCNU on the first cycle.

Results: Since 2001–2003, 54 patients (male–female, 40:14) were evaluable: median age, 57 years (range, 27–70 years); and median Karnofsky performance status (KPS) of 80 (60–100). Overall 133 cycles were administered (median, 2; and range, 1–10). Responses to PCV in the 54 patients for which data were available were CR, 0 of 54; PR, 2 of 54; MD, 6 (11%) of 54; and PD, 45 (83%) of 54. Median TTP after PCV was 2.4 months, and PFS at 6 and 12 months was 13% and 6%, respectively. Median survival was 5.1 months. Three patients with PR (KPS ≥ 90) had a TTP of 10.2, 10.3, and 37.8 months, and survival of 12.1, 14.6, and 43.9 months, respectively. Grade 3–4 toxicity included thrombocytopenia (15%) and neutropenia (17%). In one patient, procarbazine was stopped (skin rash). Five patients (9%) showed peripheral
neuropathy and, in 2004, vincristine was withdrawn from the schedule (six patients). In two responding patients, PCV was stopped because of overwhelming fatigue.

Conclusion: Second-line PCV may be useful in some patients with good performance status. Toxicity is the limiting factor.

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P170. DOSE INTENSITY OF TEMOZOLOMIDE DETERMINES THE BONE MARROW TOXICITY PROFILE
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Current schedules of temozolomide (TMZ) for glioma treatment include a five-day regimen of 200 mg/m² and dose-intense 21-day regimens of 75 or 150 mg/m². Preliminary observations on side effects suggest that the latter regimen would mainly cause thrombocytopenia, and extended dose-intense schedules mainly lead to neutropenia and lymphopenia.

Methods: Prospectively, we examined three series of chemonaive patients: Group A consisted of patients with low-grade or high-grade gliomas receiving TMZ 200 mg/m² on days 1 to 5 every four weeks during six or more cycles. Group B had recurrent low-grade or high-grade gliomas receiving TMZ 75 mg/m² on days 1 to 21, every four weeks during six or more cycles. Group C had de novo glioblastoma multiforme receiving concomitant radiation and oral TMZ 75 mg/m² during six weeks, followed by adjuvant TMZ 200 mg/m² on days 1 to 5, every four weeks during six cycles. Side effects were recorded at days 21 and 28 according to CTC criteria, with standardized rules for dose modification or discontinuation of TMZ.

Results: Regimen A was applied in 62 patients receiving a total of 348 cycles (median, 4.5 cycles per patient) with 39 (11%) grade 1 – 2 and 2 (1%) grade 3 – 4 neutropenia, and 94 (27%) grade 1 – 2 and 13 (10%) thrombocytopenia. Complete and partial response rates were evaluated for six months both with MRI and with neurological evaluation. Time to tumor progression was 6 months. Toxicity is the limiting factor.

Conclusion: Second-line PCV may be useful in some patients with good performance status. Toxicity is the limiting factor.

P169. CHEMORADIATION AND PROLONGED ADJUVANT TEMOZOLOMIDE IN HIGH-GRADE GBM PATIENTS
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Background: Evaluated were the efficacy and safety of concomitant radiotherapy plus temozolomide (TMZ) therapy followed by prolonged adjuvant TMZ therapy in patients with newly diagnosed high-grade gliomas.

Materials and methods: Thirty-three patients with glioblastoma multiforme (GBM) (79%) or mixed anaplastic astrocytoma–GBM (21%) at a mean age of 52.9 years (range, 23–73 years) received concomitant radiotherapy followed by 6 cycles of TMZ 200 mg/m² every four weeks. TMZ was administered on days 1 to 5, in 28-day cycles up to tumor progression.

Results: Chemoradiation was completed without interruption in 82% of the cases. Causes for early discontinuation in the other patients were toxicity (9%), tumor progression (3%) or other reasons (not drug related) (6%). Grade 3 or 4 anemia or neutropenia were observed in 3% and thrombocytopenia in 9%. Seventeen patients received more than 6 cycles (up to 12 cycles) of adjuvant TMZ. During adjuvant TMZ, no grade 3 hematological toxicity occurred; however, thrombocytosis was seen in 15% of the cases. Grade 1 or 2 nausea or vomiting occurred in 24%, grade 3 or 4 in 6.2%.

Conclusion: Preliminary data presented in this study suggest that TMZ is well tolerated in 80% of the patients, without any significant side effects. Significant, although statistically not proven, better prognostic results, even a complete response, were achieved in the sequential administration of TMZ and temozolomide in glioblastoma patients.

Other ways of administration discussed, such as i.v. TMZ or locoregional chemotherapy, have yet to be established.

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resistance in glioblastoma cells. This gene may be an attractive target for therapeutic modulation of glioblastomas.

P172. PARANEOPlastic BRAINSTEM ENCEPHALITIS AND LAMBERT-EATON MYASTHENIC SYNDROME

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Paraneoplastic neurological syndromes (PNS) are a heterogeneous group of uncommon neurological disorders that can occur in patients with tumors without being directly caused by the tumor itself, metastases, bolic or nutritional deficits, or side effects of cancer treatment. The most common are paraneoplastic cerebellar degeneration, sensory neuromopathy, Lambert-Eaton myasthenic syndrome, and encephalomyelitis.

Frequently they are associated with the presence of antineuronal antibodies in serum and CSF, the most common being anti-Hu, anti-Yo, and anti-Ri antibodies. Concomitance of distinct PNS in single patients has been described. We report on a 63-year-old male patient who initially presented in the ENT department with nyctagmus and vertigo. Persistence of these symptoms without response to conservative treatment and additional development of progressive brainstem symptoms—in particular diplopia, dysarthria, dysphagia, and severe ataxia—led to the suspicion of a paraneoplastic brainstem encephalitis. Repeated MRI of the brain did not show any changes, and CSF was unremarkable; in particular, no oligoclonal bands could be detected. Antineuronal antibodies were negative in serum and CSF. Despite immunoglobulin and high-dose steroid therapy, the patient clinically deteriorated. In addition to the previously described symptoms, the patient developed a progressive proximal tetraparesis and areflexia with facilitation of deep tendon reflexes. A repetitive stimulation in the repetitive in the repetitive stimulation in EMG proved the diagnosis of LEMS. Voltage-gated calcium channel (VGCC) antibodies were positive in the serum. A tumor screening was performed and a SCLC detected. The patient underwent chemotherapy with etoposide and cisplatin in which he has received six cycles up to now and was symptomatically treated with 3,4-diaminopyridine for LEMS. All his neurological symptoms improved significantly, and tumor size decreased markedly.

This case demonstrates the unusual concomitance of an antineuronal antibody-negative paraneoplastic brainstem encephalitis and LEMS that were both reversible under therapy. Whereas the improvement of the myasthenic syndrome can be explained by the symptomatic treatment with 3,4-diaminopyridine, the nearly complete remission of the brainstem symptoms could be secondary to immunosuppression due to chemotherapy, which makes an inflammatory pathogenesis in the form of encephalitis likely.

P173. THE EFFECT OF EXPERIMENTAL NEOPLASTIC DISEASE ON ACTIVITIES OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE IN DIFFERENT REGIONS OF THE RAT BRAIN

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Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are membrane-bound enzymes. The first is localized mainly in neurons and the second in glia, endothelial cells, and neurons. It is not clearly known if cognitive impairment observed in breast cancer patients results from neuroplastic disease as such or if it is an effect of adjuvant chemotherapy. This study evaluated the activity of AChE and BChE in brain regions in experimental breast cancer. We used transplantable hepatoma for comparison, because there is no evidence for cognitive impairment associated with this neoplasm.

Materials and methods: Male Buffalo rats, 3½ months of age, inoculated with Morris hepatoma, and female Wistar rats, 3½ months of age, with breast cancer, were used for the experiments. Morris hepatoma-bearing rats were sacrificed under halothane anesthesia after one, two, and three weeks, and breast cancer rats after 1 and 2 weeks. The frontal, temporal, and occipital lobes, cerebellum, and brainstem were used for analysis. The activity of AChE and BChE were estimated in homogenates spectrophotometrically. The activities were expressed as units per milligram of protein estimated by means of the Lowry method. Results: Rats bearing Morris hepatoma for one week showed increased AChE activity in cerebellum (P < 0.01). However, after five weeks, it was increased in the occipital lobe (P < 0.05) and cerebellum (P < 0.05). In breast cancer rats, AChE activity was increased in cerebellum during weeks 1 and 2 of the tumor growth (P < 0.05). That was also the case after two weeks in brainstem (P < 0.05). On the other hand, we observed increased BChE activity following weeks 1 and 2 after hepatoma inoculation in brainstem (P < 0.01 and P < 0.001, respectively). In rats bearing hepatoma for two and three weeks, BChE activity was increased in the frontal lobes (P < 0.001 and P < 0.01, respectively), temporal lobes (P < 0.001 and P < 0.01, respectively), and occipital lobes (P < 0.01 and P < 0.01, respectively). In cerebellum, it was higher when estimated one, two, and three weeks after breast cancer transplantation (P < 0.01, P < 0.0001, P < 0.01), compared to the control.

Conclusion: The experimental neoplastic disease leads to co-stimulation of both esterases involved in hydrolysis of acetylcholine. Cerebellum is the structure affected commonly at all stages of tumor growth in which increased esterases activities may accompany paraneoplastic cerebellar degeneration.

P174. THE SHORT-TERM EFFECT OF CYTOKINES ON THE EXPRESSION OF HEAT SHOCK PROTEIN 70 KDA IN THE RAT BRAIN

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Introduction: Heat shock protein 70 kDa (Hsp 70) as a chaperone is expressed in central nervous system as a result of ischemia and seizures and accompanies neurodegeneration and autoimmune disorders. Our previous studies have shown increased Hsp 70 expression in the central nervous system in experimental neoplastic disease. Cytokines involved in reactions between the host and the tumor may lead to stimulation of Hsp 70 expression in paraneoplastic syndromes. This study examines the short-term effect of selected cytokines on the expression of Hsp 70 in the central nervous system.

Materials and methods: Cytokines were injected intraperitoneally into male Buffalo rats, 3½ months of age, in the following doses: 4 μg/kg for tumor necrosis factor (TNF), 2 μg/kg for interleukin 1 (IL-1), and 4 μg/kg for interleukin 6 (IL-6). The same volume of physiological salt was injected intraperitoneally into the control animals. After 24 h, the animals were sacrificed under halothane anesthesia. The brain was dissected macroscopically into the white and gray matter, brain stem, cerebellum, and basal ganglia. The expression of heat shock protein 70 kDa was analyzed after polyclonal antibody gel electrophoresis and subsequent Western blotting. Hsp 70 was identified with monoclonal mouse anti-rat antibodies, as the second antibody goat anti-mouse conjugated with horseradish peroxidase was used. The semiquantitative analysis was performed using a BioRad densitometer. The densities of bands corresponding to Hsp 70 were normalized to the actin band.

Results: IL-1 and IL-6 caused a significant (P < 0.05) increase in expression of Hsp 70 in gray matter. No statistically significant expression of Hsp 70 was found in white matter, cerebellum, brainstem, and basal ganglia as a result of IL-1, IL-6, and TNF administration.

Conclusion: We have demonstrated that the short-term effect of the IL-1 and IL-6 on the expression of heat shock protein 70 appears in the gray matter. The interplay of cytokines and Hsp 70 seems to be involved in pathomechanisms associated with paraneoplastic syndromes.

P175. PROTEIN KINASE C: AUTOMMUNITY IN PARANEOPlastic CEREBELLAR DEGENERATION AND NON-SMALL-CELL LUNG CANCER

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Background: The clinical and immunological profile of patients with paraneoplastic cerebellar degeneration (PCCD) and non-small-cell lung cancer (NSCLC) is not well known. The authors reviewed the clinical and immunological features of patients with PCD and NSCLC, and without well-characterized onconeuronal antibodies.

Methods: The clinical features of nine patients included in our archives with the diagnosis of classic PCD and NSCLC were retrospectively reviewed. The presence of antibodies against cerebellar components was determined by immunohistochemistry and immunoblot of human cerebellum. A cDNA library of human cerebellum was screened with the positive sera from NSCLC patients. The presence of antibodies against cerebellar components was determined by immunohistochemistry and immunoblot of rat cerebellum. A cDNA library of rat cerebellum was screened with the positive sera from NSCLC patients.

Results: We identified nine patients with PCD and NSCLC. Six patients were men, and the median age at PCD diagnosis was 63 years (range, 47–73 years). PCD completely recovered in two patients, and partially in one, after tumor treatment. The serum of one of the PCD patients showed a unique autoantibody directed against the antigen named PCD. The autoantibody directed against the antigen named PCD was strongly present in the sera of eight of the nine patients and showed a different intensity of staining of the human cerebellum and immunoprecipitation of a polypeptide of 40–50 kDa from the human cerebellum.

Conclusion: The clinical and immunological profile of patients with paraneoplastic cerebellar degeneration (PCCD) and non-small-cell lung cancer (NSCLC) is not well known. The authors reviewed the clinical and immunological features of patients with PCD and NSCLC, and without well-characterized onconeuronal antibodies.
reactivity with Purkinje cells. The screening of a cerebellar-expression library resulted in the isolation of protein kinase C gamma (PKCγ). PKCγ immunoactivity was not observed in the serum of 170 patients with non-neoplastic neurological syndromes; 27 with PCD, no oncounal antibodies, and small cell lung cancer; and 52 with NSCLC without paraneoplastic neurological syndromes. The NSCLC from 11 patients without PCD did not express PKCγ either at the RNA or protein level. However, many tumor cells of the NSCLC of the patient with PKCγ antibodies exhibited PKCγ.

Conclusion: Our data indicate that PCD occurs in NSCLC patients without typical onconeural antibodies and associates with immune reactions against key proteins of the Purkinje cells.

P176. PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: RESULTS OF A PILOT AND PHASE II STUDY OF SYSTEMIC AND INTRAVENTRICULAR CHEMOTHERAPY WITH DEFERRED RADIOTHERAPY: FINAL REPORT

Introduction: Dexamethasone is highly efficient in PCNSL. A substantial fraction of patients under 60 years of age can obviously be cured with this regimen. 6) – based systemic therapy (including dexamethasone, vinca alkaloids, ifosfamide, and lactate metabolites) was quantified. Furthermore, ratios of Cho/Cr and NAA/Cr were calculated. PCNSL diagnosis was confirmed by biopsy.

Results: All 30 lesions investigated were located in close proximity to the subarachnoid space. The tumors were most frequently situated in the cerebral hemispheres (10), the corpus callosum (five) and the basal ganglia (three). In seven patients, multiple lesions were present. On CTC scans PCNSL appeared hypodense to isodense. In contrast, T2 weighted MRI showed hypointense lesions. PCNSL on T2 weighted MRI appeared hyperintense, whereas in diffusion weighted images the lesions were hyperintense in seven patients and hypointense in two. None of the tumors was located in all patients. MRS revealed a strong decrease in the NAA/Cr ratio in all patients investigated and showed an increase in Cho/Cr ratio in 10 patients. Previous reports suggested that the presence of a lipid or lactate peak is a specific sign for PCNSL. However, lipid or lactate peaks could be detected in only eight patients and were absent in five. At the time of MRS investigation, 10 patients had already received steroid treatment of different durations, and all patients with lipid or lactate peak negative PCNSL were also treated with steroids.

Conclusions: Specific MRS patterns have been suggested for PCNSL; however, in the clinical situation, MRS patterns of PCNSL often appear less distinct. Unspecific alterations may be caused by unspecific changes in the treatment. Although MRS in combination conventional imaging techniques is helpful in predicting the lesion entity, biopsy remains a necessary means for diagnosis.

P177. MAGNETIC RESONANCE SPECTROSCOPY IN PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL)

Introduction: The incidence of primary central nervous system lymphoma (PCNSL) has markedly increased during the past 20 years. Its diagnosis often poses problems because the entity appears unspecific on conventional imaging techniques. Up to today, diagnosis of PCNSL was confirmed by biopsy.

Methods: Twenty-eight MRS studies in 13 patients and 10 age-matched healthy controls were conducted in addition to CCT and MRI. Spectra were collected by single voxel MRS (1.5T, TE: 31 and 136), and a number of different peaks corresponding to the RNA or protein level. However, many tumor cells of the NSCLC of the patient with PKCγ antibodies expressed PKCγ.

Conclusion: Our data indicate that PCD occurs in NSCLC patients without typical onconeural antibodies and associates with immune reactions against key proteins of the Purkinje cells.

P178. AN UNUSUAL CASE OF A PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

T-cell-type lymphomas are rare, representing less than 10 percent of central nervous system malignant lymphomas. We describe the unusual MR imaging findings, including spectroscopy, and perfusion- and diffusion-weighted imaging (DWI), in a 53-year-old woman.

The patient was admitted with a history of subacute aphasia to our neurological department. Previous CT and MR examinations had shown two frontal expanding lesions with cortico-subcortical location. Laboratory examinations, including cerebrospinal fluid, were unremarkable. At a first biopsy, a histologic diagnosis of inflammatory changes was made.

A second MR study, performed two weeks later, demonstrated an increase of the lesions and new foci in the left thalamus, corpus callosum – cingulate gyrus, despite steroid therapy. The cortical lesions in the right frontal lobe showed areas of subacute hemorrhage. Pain and irregular enhancement on MR perfusion did not show any increase in perfusion parameters at the level of the evolving lesions. Neither evident increase in choline peak nor reduction of N-acetyl-aspartate were seen on MR spectroscopy.

Because of subsequent rapidly progressive neurological worsening, the patient underwent a second operation during which the lesion was subtotally removed. The histologic diagnosis was of peripheral small T-cell lymphoma with perivascular cuffing involving both leptomeninges and brain parenchyma. The patient received postoperative radiation and chemotherapy and had an otherwise uneventful clinical course in the following four months. Lesions have been shown to be dramatically reduced in a recent MR study.

P179. PRIMARY PITUITARY LYMPHOMA: CASE REPORT

Primary CNS lymphoma (PCNSL) is now thought to constitute 3% of all intracranial neoplasms. PCNSL in sella turcica region is extremely rare. Primary pituitary lymphomas as infrequent tumors have only hypothetical risk factors, such as immunodeficiency states, pituitary adenomas, and lymphocytic hypophysitis. We present a case report on a 37-year-old male patient with primary pituitary lymphoma treated in our hospital. The patient, who had no previous illnesses, was admitted to the hospital because of bilateral blurred vision. Findings on physical examination were normal except for a visual-field defect on the temporal side of his eyes. No abnormalities were found in his bilateral ocular movement, facial nerve function, or motor function. His blood count and biochemical profile were normal. Basal hormonal studies revealed no symptoms of panhypopituita-
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P180. ENHANCED DELIVERY TO THE CENTRAL NERVOUS SYSTEM OF MONOCLONAL ANTIBODY-BASED THERAPY IN RELAPSED PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL)

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Background: Treatment for PCNSL has centered around high-dose methotrexate and radiotherapy. At PCNSL recurrence, therapeutic options are limited. Rituximab has shown success in certain B-cell non-Hodgkin’s lymphomas and may synergize with platinum-induced apoptosis. However, it has been reported that rituximab may diffuse poorly across the blood-brain barrier (BBB).

Methods: We reviewed and report our clinical experience with five cases of relapsed PCNSL treated in an innovative care context with rituximab (375 mg/m²), i.v. on day 1, followed by carboplatin (200 mg/m²/day) and methotrexate (2500 mg/m²) opening, on days 2 and 3, every four weeks. All cases had radiographically evaluable disease prior to treatment and had failed prior methotrexate-based therapy.

Results: Four patients achieved radiographic CR and survived 171+, 82, 42, and 38 weeks; one had SD and survived 62+ weeks after the aforementioned treatment. The most common side effects were hematologic. At relapse after the treatment, one patient received indium 111-ibritumomab tiuxetan i.v. for imaging, and yttrium 90-ibritumomab tiuxetan i.v. for therapy, both without osmotic BBB disruption. The patient achieved CR in enhancing tumor (where the BBB was leaky) one month after treatment. However, CR in enhancing tumor was accompanied by multiple new enhancing lesions in other brain regions, where the BBB was intact at the time of yttrium-90 infusion. Estimates of the fractional uptake of yttrium 90, and radiation doses to tumor and other brain regions, will be presented.

Conclusions: These five cases suggest that enhanced delivery of rituximab, carboplatin, and methotrexate has the potential to improve outcomes in relapsed PCNSL, with acceptable toxicity. However CRs are often not durable. Imaging with indium 111 and efficacy with yttrium 90 suggests the importance of the BBB for antitumor response when using monoclonal antibody-based radiolabeled antibody-based therapy for relapsed PCNSL.

P181. EXPRESSION OF SURVIVIN, PLATELET-DERIVED GROWTH FACTOR A (PDGF-A), AND PDGF RECEPTOR ALPHA IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Primary central nervous system lymphomas (PCNSLs) are rare tumors occurring in the brain. Their biology and the factors predicting survival are not well known. This study investigated expression of the antiapoptotic protein survivin and platelet-derived growth factor A (PDGF-A) and receptor (PDGFRα) in PCNSLs.

Fourty-four patients with histologically confirmed PCNSL treated between 1992 and 2004 were included in this study, and tumor specimens were investigated immunohistochemically for expression of survivin, PDGF-A, and PDGFRα. Protein expression and clinical variables were analyzed statistically.

Forty-three (98%) of the 44 tumors were diffuse large B-cell non-Hodgkin’s lymphomas (NHL) and one was a T-cell NHL. Thirty-seven (84%) of the examined PCNSL specimens showed expression of survivin, 16 (37%) of PDGF-A, and 34 (77%) of PDGFRα. Tumors expressing coexpressed PDGFRα and PDGF-A occasionally. Expression of the aforementioned proteins was not predictive for survival in this patient group except for age and therapy, no other clinical variables correlated significantly with overall survival.

In conclusion, PCNSLs express survivin and PDGFRα in the majority of cases investigated. PDGF-A is less expressed frequently. Immunohistochemical detection of these proteins does not correlate with overall survival and cannot be used as a prognostic factor.

P182. LEFTLEPTOMENINGEAL SPREAD AND LYMPHOMATOSIS AS PRESENTING SYMPTOM OF PRIMARY T-CELL CNS LYMPHOMA

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Background: Primary CNS lymphomas (PCNSLs) are usually of B-cell origin. T-cell phenotype incidence, according to various series from Western countries, is only 1% to 6.6%. Higher incidence was reported in Far East countries (8.5%–16.7%). Primary leptomeningeal lymphoma, of either T- or B-cell origin, is a rare condition. We have followed the cases of 100 PCNSL patients in our center over the previous 10 years; only five (5%) had T-cell tumors and all presented with leptomeningeal involvement as a sole manifestation.

Objectives: To describe five patients with T-cell PCNSL who presented with a disease confined to the subarachnoid space. All demonstrated involvement of cranial and peripheral nerves, with neurolymphomatosis forming in three of them.

Case reports: The mean age of the three females and two male patients was 31 years (range, 19–62 years). Presenting symptoms included elevated intracranial pressure signs and sixth cranial nerve palsy; headache and bilateral third nerve palsy; systemic signs, uveitis, mononeuritis multiplex, and unilateral hearing loss; and bilateral seventh nerve paralysy and bilateral uveitis. Even though CSF was abnormal in all of them, repeat CSF cytology and CSF studies were occasionally suspicious, but not conclusive, and results of recurrent examinations for PCR gene rearrangement were normal in four of them. MRI revealed leptomeningeal spread, which appeared relatively late in the course of the disease (two patients), cranial nerve imaging involvement (two patients), and a normal MRI (one patient). Diagnosis was finally revealed by meningeal biopsy (three patients), by CSF analysis (one patient), and from orbital tissue (one patient). Three patients died 10, 19, and 19 months from disease onset, and two patients are still alive at 2.3 months and 10 months following diagnosis.

Discussion: Leptomeningeal spread or neuronal lymphomatosis is a common manifestation of T-cell lymphoma. Such an appearance can imitate other neurological conditions, as pseudo tumor cerebi or vasculitis, and therefore should be considered in their differential diagnosis. However, diagnosis can be difficult because neither CSF nor the MRI is informative, requiring meningeal biopsy.

P183. INTRACRANIAL TUMORS IN THE INFANCY: EXPERIENCE OF TWENTY YEARS

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Background: The incidence of brain tumors within the first year of life is estimated at 1.1 per 100,000 live births.

Objectives: The clinical features, presenting symptoms, histological types, localization, and treatment results of central nervous system tumors in children under one year of age in our institute are reviewed.

Results: From January 1985 to December 2004, 69 infants with newly diagnosed central nervous system tumors from 11 institutions were registered in Hungary. Of these 69 infants, 11 were treated at the Oncological Unit of the Second Department of Pediatrics of the Semmelweis University in Budapest. The male–female ratio was 6:6, and mean age at the time of diagnosis was 5.5 months. The neoplasms were localized in the posterior fossa in seven patients and in the supratentorial compartment in four. The histological types of the tumors were medulloblastoma/primitive neuroectodermal tumor (five), astrocytoma (two), choroid plexus center (two), ependymoma (one), and one case was not specified. In five infants, the first presenting sign was vomiting, in four cases hydrocephalus, and in one case the intracranial tumor was detected already in the intratrauterine period. Ten patients received chemotherapy. In three cases, we achieved complete remission, in two cases partial remission, and in four cases the tumor progressed.
in spite of the chemotherapy. The disease relapsed in three patients, two of whom died within one year. One patient who relapsed four years later, but after chemotherapy he is in complete remission. Three patients (28%) are still alive. The other eight patients died because of the progress of their brain tumor or the complications of the treatment. The cause of death in the remaining patients was the disease itself. The follow-up period ranged from 10 to 68 months. Partial or complete rehabilitation was observed in 18 patients, and 2 patients had no improvement. Conclusion: Patients with suspected brain tumors, mainly low-grade tumors, were included in this study. The results of MET PET scans were normal. No correlation of MET uptake and outcome existed in the patient cohort investigated. Therefore, MET PET seems to be helpful in differentiating low-grade brain tumors from nontumorous lesions, but clinical impact has not yet been observed during median 32-month follow-up period (range, 10–68 months). Partial or complete rehabilitation of visual and/or endocrine and/or midbrain disturbances was attained in most cases. Conclusions: Our data suggest that the combined chemoradiotherapeutic regimen probably has an advantage over standard radiotherapy in primary CNS pure germinoma patients because of its absolute effectiveness in the combination with a more intelligible influence on normal and compromised brain tissue.

P178. GIANT CELL GYLOBLASTOMA WITH A NEAR HAPLOID KARYOTYPE AND MICROSATellite INSTABILITY IN A YOUNG MAN: TWO RARE GENETIC ANOMALIES, POSSIBLY INVOLVED IN THE ONCOGENESIS OF THE TUMOR IN YOUNG PEOPLE

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General cell giant cell glioblastoma (GGG), a rare variant of glioblastoma, usually occurs de novo in adults. This variant is very uncommon in children and young people, and the oncogenesis of the tumor in this population remains unknown. We studied the karyotype and the microsatellite stability of a GGG occurring in a 19-year-old man.

Methods: The karyotype was determined according to standard procedures. By analyzing loss of heterozygosity, five microsatellites (D1S508, D1S2734, D19S219, D19S412, and D19S596) were compared between the fresh tumor and the blood. Expression of MLH1 and MSH2 proteins in the tumor was studied using immunohistochemistry.

Results: Tumor karyotype was near haploid (26–29c), and MSH2 and MSH1 proteins in gliomas and have never been described together. Each anomaly has been independently described in gliomas, particularly in young people, and some cases were GGG. MSH1 is a mechanism involved in the genesis of some cancers and is often caused by a deficiency of proteins involved in the DNA mismatch repair system. In our case, MSH1 was detected by sequencing of the MSH1 gene and MSH2, a protein of this system. Near haploid karyotype is extremely rare and has been described in two cases, one of which was a GGG in a child.

Conclusion: Near haploid karyotype and MSI might be involved in the oncogenesis of GGG in young people. A systematic analysis of karyotype and microsatellite stability in gliomas of young children and young people could resolve this issue.
P189. MULTIMODAL MANAGEMENT OF MEDULLOBLASTOMA
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The purpose of the current study is to investigate a hospital series of 15 patients with medulloblastoma who were admitted to and treated at the First Neurosurgical Department, Cluj-Napoca County Hospital, Iuliu Hatieganu–Cluj-Napoca University of Medicine and Pharmacology, in January 2001–February 2006. Medulloblastoma arises from the primitive neuroepithelial cells located in the roof of the fourth ventricle. The medulloblastoma occurs most commonly in infancy and childhood. It is characterized by its tendency to expand along the neuraxis, following CSF pathways, and represents one of the few brain tumors that metastasize to extraneural tissue.

Taking into consideration the cases studied, the gender ratio shows a predominance of medulloblastoma in male patients (male–female ratio, 9:6). Twelve patients were children. Among the total of 77 cases of pediatric brain tumor, medulloblastoma is second in frequency (18.18%), following astrocytoma. The distribution in age demonstrates a predominance of children aged 4 to 6 years (five cases), compared with children aged 0 to 3 (three cases), children aged 7 to 9 (three cases), and children over age 9 (one case).

From a clinical aspect, the duration of symptoms usually is less than one month at diagnosis (12 cases). On physical examination, cerebellar signs dominate, e.g., nystagmus, nausea, and vomiting occurring in 80% to 90% of patients, secondary to increased intracranial pressure. With patients in a sitting position, surgery was performed through midline posterior fossa craniectomy. Total removal was accomplished in 80% of the cases, and subtotal removal in 20% of the cases, with persistence of the tumoral sequence sticking to the cerebral peduncles. Subtotal removal is predictive for posterior fossa recurrence. This occurred in five cases in our study.

The treatment of these tumors includes surgical resection, craniospinal radiation, and chemotherapy, but only 60% of affected children are cured, and most of them suffer long-term side effects in response to the aggressive treatment (neuropsychiatric and neuroendocrine sequelae and cognitive dysfunctions). Overall, the one-year survival rate was 100%, and the five-year survival rate was 60%.

P190. PEDIATRIC BRAIN TUMORS
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Background: The purpose of the current study is to investigate a hospital series of 77 cases of pediatric brain tumors.

Methods: The authors reviewed the database of primary pediatric brain tumors, including less than 18 years of age who were operated on between January 2001 and February 2006 in Cluj-Napoca County Hospital, First Neurosurgical Department, and Iuliu Hatieganu University of Medicine. Age and gender distribution, location, symptoms, types of operations, and classification of brain tumors were analyzed. Intracranial tumors with the potential therapeutic effects of somatostatin.

Results: The pediatric brain tumors represent around 9% of all brain tumors operated on in this period. The mean age of these 77 patients was nine years, and the male–female ratio was 1.21:1. Infratentorial located tumors (51.9%) were predominant to supratentorial tumors (48.1%). Histological dispersion showed that 38.9% were represented by astrocytoma (pilocytic astrocytoma, 18.18%), followed by medulloblastoma (18.18%), craniopharyngiomas and ependymomas (both with 6.5%), cosinophil granulomomas and gliomas (both with 5.19%), and choroids plexus carcinomas, retinoblastomas, and oligodendrogliomas (2.59%). There was also one case each of teratoma, schwannoma, testicular carcinoma, retinoblastoma, and meningioma, bipolar adenoma, primitive neuroectodermal tumor, and hemangioendothelioma.

Conclusions: Due to continuous improvement of neuroimaging procedures, this five-year review showed a gradual increase in number of cases operated on in our department. The most common tumor was astrocytoma. All 77 tumors were operated on, and 16 developed hydrocephaly that demanded drainage. Histological dispersion was similar to that in the latest data in the literature.

P191. COMPARISON OF IRRADIATION TECHNIQUES USED TO BOOST POSTERIOR FOSSA TUMOR BED IN CHILDREN
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The pediatric brain tumors represent around 9% of all brain tumors. The purpose of the current study is to investigate a hospital series of 15 patients with medulloblastoma who were admitted to and treated at the First Neurosurgical Department, Cluj-Napoca County Hospital, Iuliu Hatieganu–Cluj-Napoca University of Medicine and Pharmacology, in January 2001–February 2006. Medulloblastoma arises from the primitive neuroepithelial cells located in the roof of the fourth ventricle. The medulloblastoma occurs most commonly in infancy and childhood. It is characterized by its tendency to expand along the neuraxis, following CSF pathways, and represents one of the few brain tumors that metastasize to extraneural tissue.

Taking into consideration the cases studied, the gender ratio shows a predominance of medulloblastoma in male patients (male–female ratio, 9:6). Twelve patients were children. Among the total of 77 cases of pediatric brain tumor, medulloblastoma is second in frequency (18.18%), following astrocytoma. The distribution in age demonstrates a predominance of children aged 4 to 6 years (five cases), compared with children aged 0 to 3 (three cases), children aged 7 to 9 (three cases), and children over age 9 (one case).

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The treatment of these tumors includes surgical resection, craniospinal radiation, and chemotherapy, but only 60% of affected children are cured, and most of them suffer long-term side effects in response to the aggressive treatment (neuropsychiatric and neuroendocrine sequelae and cognitive dysfunctions). Overall, the one-year survival rate was 100%, and the five-year survival rate was 60%.

P192. PRELIMINARY RESULTS OF DIAGNOSTIC AND THERAPEUTIC USE OF SOMATOSTATIN IN CHILDREN WITH BRAIN TUMORS
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Introduction: Malignant solid tumors and leukemias are the second most common causes of death in childhood. The most frequent pediatric solid tumors are the brain tumors. Brain tumors, especially medulloblastoma, should be treated by surgery, irradiation, and chemotherapy. However, chemotherapy has only a moderate effect. Pediatric brain tumors (especially medulloblastomas) express somatostatin receptors. This study investigated the expression of somatostatin receptors in pediatric brain tumors and the potential therapeutic effects of somatostatin.

Patient and methods: Scintigraphic imaging [(111)In-diethylenetriamine-pentaacetic acid-octreotide] was performed in 20 children treated for brain tumors at the Second Department of Pediatrics, Semmelweis University. Somatostatin receptors were identified in 12 cases: seven patients had medulloblastomas, three had astrocytomas, one had ependymomas, and one had optic glioma. Octreotide (Sandostatin, Novartis) was administered subcutaneously in two patients—with relapsed chemotherapy-resistant and somatostatin receptor–positive tumors—at a dose of 5 to 10 µg/kg daily.

Results: The disease in one of the patients (diagnosed with ependymoma) was stable after 1.5 years; the other patient is in partial regression (optic glioma) after one year of octreotide treatment.

Summary: Octreotide treatment may have therapeutic value in somatostatin receptor–positive pediatric brain tumors, but further investigation is warranted. This work was supported by OTKA T-46938 and NKFP 1A/002/2004 grants.

P193. LEVEL OF ANXIETY IN BREAST CANCER WOMEN
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Anxiety in cancer patients can be acute (associated with symptoms and treatment), can be chronic, or can be a consequence of previous pathology exacerbated by impact of a new disease (J. Holland, 1999). Anxiety preceding the medical appointment should be taken into account. Pain can provoke symptoms of acute anxiety. Previous states of chronic anxiety that can exacerbate anxiety are anxiety disorders, phobias, and panic disorders. Of special relevance is anxiety in women with breast cancer who are con-
ditated by a cosmetic defect and loss of sexuality after the mastectomy. In most women, this is the leading anxiety.

To study the peculiarities of women's mental health in a cancer clinic, to assess the psychiatric and psychotherapeutic assistance needed, and to develop jointly with cancer specialists a complex program of rehabilitation in which, along with special methods of therapy, psychotherapeutic assistance was complemented with psychopharmacologic therapy to improve the psychoemotional state and quality of life of cancer patients.

We examined 22 women with breast cancer under treatment in the Oncology Research Institute (Department of General Oncology). In addition to traditional clinical-dynamic examination, the clinical Hamilton Anxiety Scale was used in the investigation. This personality questionnaire is directed at revealing constitutional and situational anxiety. It consists of 14 groups of symptoms regarding mental, somatic, and neurovegetative aspects of anxiety. The score for the level of anxiety according to the Hamilton Scale was 13.2. The score for the level of somatic anxiety was 4.6, that for neurovegetative anxiety was 1.8, and that for mental anxiety was 6.8. A significant percentage of midlevel anxiety was revealed (31.5%), which was greater than the levels of somatic anxiety (34.8%) and neurovegetative anxiety (13.7%). Thus, in women with breast cancer, a sufficiently high level of personal anxiety is detected that has preferentially a mental and, to a lesser extent, somatic and neurovegetative character.

P195. SCREENING FOR DEPRESSION AND ANXIETY IN NEO-URO-ONCOLOGY PATIENTS BEFORE, DURING, AND AFTER RADIOTHERAPY: A PILOT STUDY USING THE HOSPITAL ANXIETY AND DEPRESSION SCALE

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Previous investigations of depression and anxiety in cancer patients demonstrate significantly raised rates of affective disorder, which has been associated with reduced quality of life. Published studies in neuro-oncology are sparse, although the course, treatment, and neurological/cognitive symptoms of brain tumors present substantially different risks to mental health. Patients in busy oncology clinics do not always raise emotional issues, and screening for affective disorders is an often-recommended strategy to address this. Data were collected by patient-completed Hospital Anxiety and Depression Scale (HADS) questionnaire and by clinician-completed checklist from consecutive CNS radiotherapy clinic attendees before radiotherapy, halfway through, two months after, and at follow-up. Preliminary results are reported and discussed in relation to the clinical utility of the HADS as a screening measure in this group, and the relationships between affective disorder, tumor grade/prognosis, tumor location, radiotherapy mode, and clinician-rated cognitive impairment. Issues of the relationship between biological symptoms of depression and steroid dose are also discussed.

P196. BRAIN TUMOR AND EPILEPSY: TREATMENT WITH LEVETIRACETAM

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Purpose: Tumor-related seizures are often difficult to control, and the clinical picture is complicated by frequent interactions between antiepileptic drugs (AEDs) and antineoplastic agents. Levetiracetam (LEV) is a new AED, with a mode of action that differs from other AEDs, and almost ideal pharmacokinetic characteristics. We study the safety and efficacy of LEV in patients with brain tumors associated with epilepsy.

Methods: We studied 19 patients (6 women; mean age, 48 years) affected by supratentorial gliomas (7 patients by glioblastoma multiforme, 2 by anaplastic astrocytoma, 9 by low-grade astrocytoma, and 1 by meningioma). Seizures types were simple partial in 6 patients, complex partial in 4, complex partial with secondary generalization in 7, and generalized tonic-clonic from the onset in 4. LEV was added to the existing AED treatment because of persisting seizures at dosages of 1500 to 3000 mg/day. Follow-up ranged between 5 and 47 months (mean, 22.1 months; median, 17 months).

Results: At the end of the observation period, 8 patients were seizure-free (seizure-free period ranging from 5 to 33 months [mean, 14.9]; and median, 10.0]), and 5 patients showed a seizure frequency reduction greater than 50%. Seizure frequency was unchanged in 5 patients and increased (less than 50%) in 1 patient. No LEV-related adverse effects were observed, and no significant changes in blood chemistry were found. LEV plasma concentrations monitored in 13 subjects ranged from 11.9 to 93.7 µg/ml.

Conclusions: Our preliminary open data indicate that add-on treatment with LEV in patients with brain tumors is safe and efficacious. Controlled studies on larger populations are warranted to confirm these open observations.
Background: NeuroCogFX is a standardized computer-based test battery aimed at identifying treatment-related cognitive dysfunctions in patients with brain tumors. This test battery evaluates memory span, working memory, information-processing speed, and simple and complex choice-reaction tasks, as well as verbal and figural memory and lexical fluency. In the present study, NeuroCogFX was validated against a neuropsychometric standard test battery (STB) representing the same cognitive domains to establish external validity as well as practicability.

Patients and methods: 24 patients (13 men and 11 women; age, 34 years; SD = 14 years) were included in the validation study. Patients included were those with (a) potential neurotoxic brain tumor therapy, lasting at least one year back; (b) no tumor activity at present; and (c) Mini Mental State Examination (MMSE) score > 19. All patients underwent both cognitive test procedures (NeuroCogFX and STB). Single-subtest scores and total score, as well as administration time, were noted for both procedures. Convergent validity was evaluated on the basis of Pearson correlations indicating associative strengths between equivalent cognitive dimensions of the respective test procedures.

Results: The mean testing time amounted to 23.5 min (SD = 3 min) for NeuroCogFX and 80 min (SD = 15 min) for STB. The range of correlations between the subtest scores of the different test procedures (NeuroCogFX and STB) varied between r = 0.32 and r = 0.81. The lowest correlation coefficient was found in the working-memory domain, while the simple-choice reaction task had the highest correlational strength. Convergent validity was evaluated on the basis of Pearson correlations indicating associative strengths between equivalent cognitive dimensions of the respective test procedures.

Conclusions: On the basis of our preliminary results, it can be concluded that NeuroCogFX is a valid and time-economic neuropsychological test battery for assessing treatment-related cognitive dysfunction in patients after successful brain tumor therapy. To further improve the external validity of this computerized battery, the validation sample is being currently extended.
P202. OUTCOME AND TOLERABILITY OF TOPIRAMATE IN BRAIN TUMOR–ASSOCIATED EPILEPSY

Purpose: In patients with brain tumors, conventional antiepileptic drugs (AEDs) may interact with chemotherapeutics and corticosteroids, leading to reduced efficacy and/or more frequent side effects. Topiramate is a newer, well-tolerated broad-spectrum AED with low potential for interactions. We selected topiramate for its favorable pharmacokinetics in order to investigate its efficacy and tolerability in brain tumor–associated epilepsy.

Methods: In this single-center, open-label, observational study, 46 patients (20 men and 26 women; mean age, 48 years) with epilepsy related to brain tumors received topiramate according to normal prescription practices. Twelve patients were treatment naïve, and 34 were converted from other AEDs to topiramate add-on or monotherapy because of poor seizure control or intolerable side effects. Seizure frequency was captured retrospectively (baseline) for up to 12 months prior to study entry and at the final follow-up visit (mean, 13.1 months; range, 5–43 months).

Results: During topiramate administration (67.4% monotherapy and 32.6% add-on), 54.4% of patients were seizure-free; at least 50% seizure reduction was observed in another 23.9%. Seizures remained unchanged in 17.4% and worsened in 4.3% (increasing less than 50%). Seizure control remained stable during tumor progression. The overall responder rate was 78%: 75% with versus 83% without tumor progression. Side effects during topiramate administration (one case of paresthesia, one case of weight loss, and one case of confusion) were mild and never led to changes in therapy.

Conclusions: Topiramate appears to be a good choice for patients with brain tumor–associated epilepsy. The data support its adequate seizure control (also stable during tumor progression) and paucity of adverse events.

P203. TOXICITY OF TEMOZOOLMIDE AND IMPLICATIONS FOR INTERVENTIONS BY THE NURSE PRACTITIONER IN NEURO-ONCOLOGY


Purpose: To compare toxicities of standard schedules of temozolomide (TMZ) with a dose-intensive schedule, and to determine whether observation of toxicities would imply a change in nursing strategies in the management of brain tumor patients.

Methods: In the setting of a nurse-led TMZ clinic, three groups of patients with high-grade and low-grade gliomas treated with TMZ were evaluated: group A (n = 62), TMZ daily for 5 days, 200 mg/m²/day, every 4 weeks; group B (n = 18), TMZ daily for 21 days, 75 mg/m²/day, every 4 weeks; and group C (n = 10), treated with TMZ during radiotherapy (6-week schedule B), 4-week interval followed by schedule A. Toxicity recordings included blood counts and nausea and vomiting according to CTC criteria. Critical toxicities were defined as neutropenia (<1000 µl), grade 3; lymphopenia (defined as an absolute lymphocyte count [ALC] < 500 µl), grade 3 CTC criteria, version 3; and thrombocytopenaia (<50,000 µl), grade 3. Interventions by the nurse practitioner consisted of either dose reductions or delay of administration of TMZ, growth-factor support (GFS), or administration of thrombocyte suspensions.

Results: Thrombocytopenia was the most frequent toxicity seen in all three groups, causing either a delay of the next cycle or a dose reduction of TMZ. Neutropenia and subsequent interventions occurred more often in groups B and C. Group C differed from groups A and B in lower overall blood counts and a higher total number of interventions. If treated for a longer duration with TMZ, lymphopenia (grade 4) occurred significantly more often in group B, necessitating interventions like Pneumocystis carinii pneumonia prophylaxis. Neourea occurred more frequently in group B compared to groups A and C.

Conclusions: Responsibility of the nurse practitioner in neuro-oncology is the evaluation of lab results and of potential side effects in patients treated with TMZ, and the consideration of interventions in relation to observed toxicities. The chance of and occurrence of toxicities may lead to decision making by the nurse practitioner together with the responsible physician on necessary interventions. Future research should focus on the effect of these interventions on the quality of life of the patient and on adaptation of guidelines for oral TMZ in brain tumor patients.

P204. AN APPROPRIATE TIMING OF POSTSURGICAL EVALUATION FOR PATIENTS WITH ACROMEGALY
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Introduction: Judgment of cure for acromegaly after surgery is somewhat uneasy because the GH-HIR system may take a long time to normalize. This study investigated the appropriate timing for postsurgical endocrinological examination of acromegaly.

Methods: Forty patients with acromegaly were included in this study. Seventeen patients were operated during 1998 to 2004, and 23 patients were operated during the period between January 2005 and June 2006. The median follow-up period was 17 months. After surgery, blood samples were taken at 0, 1, 2, 3, 6, 9, and 12 months postoperatively. Serum IGF-1 and GH were measured. The IGF-1 levels were normalized if below 1.95 mg/l. The GH levels were normalized if below 0.4 µg/l.

Results: Eight patients with acromegaly were included in this study. Seventy-seven of the patients were men and 33 were women, mean age 48 years. Between January 2000 and January 2006, 100 patients with brain tumor were treated. The follow-up visit (mean, 13.1 months; range, 5–43 months).

Discussion: This study investigated the appropriate timing for postsurgical endocrinological examination of acromegaly.

Conclusions: Postsurgical endocrinological evaluation of acromegalic patients should be performed within two to three months after surgery. Repeated examination in late term would be meaningful when the earlier one did not fulfill care criteria even though immediate GH and IGF-1 satisfied its criteria.

P205. MINIMALLY INVASIVE AWAKE CRANIOTOMY USING STEINER-LINDQUIST STEROTACTIC LASER GUIDANCE
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Objective: Awake craniotomy permits continuous assessment of intraoperative neurological functions, and the use of Steiner-Lindquist stereotactic laser guidance aids in performing a minimally invasive procedure related to the resection of lesions located in cortical, subcortical, and deep brain regions.

Materials and Methods: Between January 2000 and January 2006, 100 consecutive patients with various intracranial tumoral lesions underwent 115 resection procedures.

Results: Seventy-seven of the patients were men and 33 were women, and their mean age was 52.1 ± 8.0 years (range, 22–78 years). Majority of the lesions were located within the parietal lobe. Of the lesions, 27 (23.5%) were located within the cortex, while 85 (74.8%) were subcortical. The most common pathologies were metastasis (55 cases) and glioblastoma multiforme (18 cases). In 13 of the cases, lesions were multiple, and 13 and two of these patients underwent two and three craniotomies, respectively. The mean operative time was 72 ± 0.3 min, and the mean time of hospital stay was 3.4 ± 2.1 days (range, 1–11 days). The average lesion size was 9.06 ± 5.02 cm² (range, 0.25–56.0 cm²). Seven (6%) of the patients experienced transient new postoperative neurological deficits, and one patient (0.8%) died of postoperative intracerebral hemorrhage.

Conclusions: Awake craniotomy with the aid of Steiner-Lindquist stereotactic laser-guidance is a safe procedure that helps in performing a minimally invasive resection of lesions located in eloquent brain regions. This method decreases the operative time, hospital stay, and postoperative new neurological deficits.

P206. SEIZURES AND AWAKE SURGERY FOR LOW- AND HIGH-GRADE GLIOMAS: A RETROSPECTIVE STUDY OF 42 PATIENTS
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Objective: To describe the incidence of seizures in patients undergoing awake surgery for low-grade glioma (LGG) and high-grade glioma (HGG).

Methods: Asleep–awake anesthesia with propofol and remifentanil infusion was used to enable the evaluation of language and motor functions. Patients underwent functional cortical and subcortical brain mapping

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during tumor resection. The Ojemann cortical stimulator, electrocorticography (EcoG), EEG, and EMG monitoring were used. The occurrence of intraoperative and early postoperative seizures was carefully monitored and reported.

Results: We retrospectively studied 42 patients (21 men; age, 42 years; age range, 22–76 years) admitted between January 2004 and February 2006 for tumor resection (20 LGGs and 22 HGGs). All patients underwent EcoG for registration of cortical activity and for determination of stimulation current, which was adjusted to immediately below that inducing afterdischarge potential. The intensity of currents used was 2 to 8 mA, with a duration between 2 and 4 s. Intraoperatively, eight patients developed four seizures, which occurred immediately after the application of the current. In three patients, seizures appeared after cortical stimulation and, in five patients, after subcortical stimulation. In five patients, seizures resolved within 1 min after cold saline irrigation; three patients required drug administration. In these patients, the procedure was discontinued, they were allowed to rest, and the procedure was restarted after the EcoG registration was normalized and the patients became fully awake. The replacement of the laryngeal mask was not necessitated for any patients. Postoperatively, three patients had seizures that were treated pharmacologically.

Conclusions: Intraoperative seizures should be taken into account when performing cortical and subcortical stimulation during awake surgery. Nevertheless, in most cases, seizures did not necessitate pharmacological treatment and did not affect surgical and anesthetic conduct.

P207. PERSONAL EXPERIENCE IN BRAIN TUMORS
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We present an epidemiological report presenting our experience in brain tumor surgery, based on a group of 846 patients admitted to the First Neurosurgical Department, Cluj-Napoca County Hospital, between January 1, 2001, and December 31, 2005, and treated by surgery, and compared our results with the latest data in the literature. We have thoroughly evaluated all aspects implicated in this study. Overall, we emphasize that cerebral tumors represent 20% of the cases treated by surgery during this period.

This five-year review shows an increasing number of cases being admitted to our department: 129 cases in 2001, 147 in 2002, 161 in 2003, 169 in 2004, and 240 in 2005. This may obviously be due to the continuous improvement in neuroimaging procedures, but other factors cannot be excluded. Histological features show a broad dispersion corresponding with previously published data. The majority of cases were represented by gliomas (simple or mixed) in 39%, followed by meningiomas in 22%, metastases in 17%, vestibular schwannomas in 5%, tumors of the sellar region in 5%, ependymomas in 2%, medulloblastomas in 2%, and other types in 8%.

Of the patients, 58% were male. This analysis arrived at the conclusion that the peak tumor incidence (27%) was in the sixth decade of life, while noting that the two youngest patients (both of them 14 months old) harbored, respectively, a hamartoma and a choroid plexus papilloma. Of the tumors, 81% were situated supratentorially and 19% were localized infratentorially.

P208. SURGICAL STRATEGIES IN THE TREATMENT OF GLIOMAS INVOLVING CRITICAL BRAIN AREAS
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We report a series of 43 patients surgically treated between November 2001 and March 2006 for gliomas involving critical brain areas. We regarded as critical the Rolandic and insular-rolandic areas (primary motor area, primary sensory area, and supplementary motor area), the speech areas, and the thalamus. Presenting symptoms were focal epileptic seizures in 20 patients, focal neurological deficits in 17, and nonspecific symptoms (headache and dizziness) in six. In all patients, surgical planning was performed through a three-dimensional rendering of volumetric MRI scans. In all the lesions involving the Rolandic/Insula-rolandic areas or the insular lobe, cortical and subcortical intraoperative brain mapping, including direct cortical stimulation and MEP recording, enabled the identification of the primary motor area and monitoring of the pyramidal tract under general anesthesia. The eight patients with lesions involving the speech areas underwent extraoperative brain mapping after implantation of subdural electrodes. The procedure included a thorough neuropsychological evaluation. In the patients with minimal neurological deficit, we performed through a three-dimensional rendering of volumetric MRI scans. In all patients, the procedure was discontinued, they were allowed to rest, and the procedure was restarted after the EcoG registration was normalized and the patients became fully awake. The replacement of the laryngeal mask was not necessitated for any patients. Postoperatively, three patients had seizures that were treated pharmacologically.

Conclusions: Intraoperative seizures should be taken into account when performing cortical and subcortical stimulation during awake surgery. Nevertheless, in most cases, seizures did not necessitate pharmacological treatment and did not affect surgical and anesthetic conduct.

P209. SURGICAL MANAGEMENT OF BRAIN-STEM PARAGANGLIONA: PRESENTATION OF AN UNUSUAL CASE
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Summary: The paragangliomas (chemodectomas or glomus tumors) are unusual neuroendocrine tumors. They include neoplasms of the adrenal medulla (pheochromocytomas) and paraganglia and are located in diverse anatomic sites of the body. They are slow-growing tumors (< 2 cm in five years) and histologically benign (<10% associated with lymph node involvement or distant spread). The most usual localizations of these tumors in the central nervous system (CNS) is considered to be the region around the jugular bulb. Paragangliomas originating from the brain stem are exceptionally infrequently reported in the international literature.

Case presentation: A 72-year-old female patient with a disease-free individual history presented with disorders of stance and gait, and diplopia gradually worsened during the last month. MRI demonstrated a sizable compact lesion, primarily considered to be meningioma, in the region of the medulla oblongata. Intraoperatively, we observed that the tumor originated exclusively from the brain stem and was very vascular. The morphological features, based on histological and immunocytochemical analyses, established the diagnosis of primary paraganglioma. In the present project, apart from the presentation of the case, we analyze the radiological and morphological characteristics of this unusual tumor.

Conclusion: Extra-axial paragangliomas are assumed to originate from elements of the dispersed neuroendocrine system (paraganglia), which are derived from neural crest progenitor cells. Brain stem paragangliomas are rare, and their diagnosis and surgical treatment represent a real challenge for neurosurgeons.

P210. COLLAGEN BIOMATRIX IN DURA REPAIR: A NEW TECHNIQUE IN BRAIN TUMOR SURGERY
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Introduction: Graft to replace dura after resection of infiltrating tumors can be necessary. Recently, in cranial and spinal surgery, minimally invasive approaches are becoming much more common, but a “watertight” dura closure can not always be achieved. In these cases, complications associated with the failure of adequate dural healing have been reported: for example, CSF fistulas, meningitis, and arachnoiditis. Different materials (human or animal patches, fat grafts, synthetic grafts, and fascial grafts) have been evaluated over the past decades in the quest for the ideal dura replacement or repair, but no product fully meets all the applicable criteria.

Method: We tested a natural dural graft that is an acellular equine collagen biomatrix (Tissudura, Baxter), looks like a foil, consists of reconstructed naturally cross-linked collagen fibrils, and provides a nonporous primary liquid-tight structure. The foils have several sizes and can be adapted to the defect. Tissudura is also adhesive and sutureless or can be attached by fibrin glue.

Results: Tissudura as dural graft was used in 12 patients who underwent tumor resection of meningiomas, acoustic neuromas, and spinal tumors. The dural defect size ranged from 2.5 to 2.5 cm to 10 × 10 cm. In six infiltrating meningiomas, dura was totally replaced by Tissudura. In three acoustic neuromas and three spinal tumors operated on with a minimally invasive approach, a “watertight” dura closure by Tissudura was achieved. In all cases, Tissudura was less technically demanding and less time consuming, compared to traditional durel or patch repair. Two days after surgery, no meningiomas, and no patients required CSF drainage or reoperation. In the follow-up (median, 10 months), no wound infection, fistula, or other complications were seen.

Conclusion: These are preliminary data, but in our hands Tissudura was extremely successful. It provides for biological repair of dura. Water-tight dura closure can be achieved at inaccessible areas, too. By prevent-
ing CSF leakage, Tissudura reduces the morbidity and the hospital stay of patients affected by brain or spinal tumors.

P211. MULTIPLE RESECTIONS FOR GLOBLASTOMA MULTIFORME
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The role of multiple resections in the management and prognosis of glioblastoma multiforme is unclear. Data on reoperation for resection of recurrent glioblastoma are sparse. We retrospectively searched our prospectively collected database from two major Tel-Aviv medical centers for primary (de novo) glioblastoma patients who underwent three or more re-resections from 1995 until the present. From a total of 633 patients with a diagnosis of glioblastoma, we excluded patients with a prior diagnosis of lower-grade tumor that had transformed to glioblastoma. We considered only tumor resection as one of three or more reoperations and did not consider diagnostic biopsy, shunting for hydrocephalus, or wound revision as one of the three or more surgeries. We identified 11 patients from our database with three or more reoperations for primary glioblastoma. Of the 11 patients, two were diagnosed as having gliosarcoma. Six of these patients underwent three resection/resective operations, four underwent five resections, and one underwent five operations for tumor re-resection. Five patients were men, and six were women. Ages ranged from 32 to 65 years, with median age 54 years. All tumor resections were right-sided and three were left-sided. There was fairly even location representation of all lobes, with several tumors spanning several adjacent lobes. Range of survival from date of diagnosis to date of death was nine months (case with four resections) to 45 months (also four resections). We examined the survival of this cohort, in relation to number of operations, and will compare this with our internal glioblastoma control group undergoing less than three operations. We also will present data of time spans between first and second, second and third, third and fourth, fourth and fifth surgeries, and last surgery and date of death, to look for increasing or decreasing trends between the increasing number of surgeries and related complications.

P212. INFLUENCE OF SURGERY ON SURVIVAL OF ELDERLY PATIENTS WITH GLOBLASTOMAS
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Background: Glioblastomas (GBLs) have a very poor prognosis, with 28% patient survival at one year. Independently from other factors (extent of resection, Karnofsky score, presenting symptoms, and adjuvant oncological therapies), age is considered in the literature as a poor prognosis factor. Overall survival decreases with age. The age-specific incidence rates (<45: 40 – 70, >70 years). Considering the oldest patients, the survival can be influenced by many different factors, such as comorbidity, infections, decision regarding extensive resection, and revealing symptoms.

Patients and methods: Among the data on 1069 patients with primary brain tumors and the 376 GBLs collected in the Nice Neuro-Oncological Database, patients over 70 years of age with GBL were identified. Overall survival decreases with tumor age-specific incidence rates (<45: 40 – 70, >70 years). Considering the oldest patients, the survival can be influenced by many different factors, such as comorbidity, infections, decision regarding extensive resection, and revealing symptoms.

Results: Eighty-nine patients were identified: 46 men and 43 women; median age at diagnosis, 74.5 years; and median age at first symptom, 74 years. The first symptom was mostly neurological deficit (70% of cases). For the whole database group with GBLs (median patient age, 60 years), median survival time was 10 months (±0.6), compared to 6 (±1) months for elderly patients. Median survival decreased according to neurosurgical procedure, regardless of whatever oncological adjuvant treatment was received (i.e., chemotherapy, radiotherapy, or both); P < 0.00001: 3 months (presumed diagnosis on brain MRI, no histological confirmation), 5 (±0.7) months (biopsy), 7 (±3) months (partial resection), and 9 (±2) months (complete resection). In comparison, median overall survival was 15 months for GBL patients less than 40 years of age (P < 0.0001) and 11 months for GBL patients between 40 and 70 years of age (P < 0.0001).

Conclusions: These data suggest the following: (1) GBL patients over age 70, who had partial resection or biopsy only, had a poor survival time regardless of whatever adjuvant treatment they received. (2) Neurosurgical complete resection influences survival, even after age 70.

P213. RECURRENT GLOBLASTOMA: THE ROLE OF SECOND SURGERY AND SECOND-LINE CHEMOTHERAPY IN PATIENTS WHO RECEIVED TEMOZOLOMIDE AS FIRST-LINE REGIMEN
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Several reports have demonstrated the efficacy of temozolomide (TMZ) when administered in newly diagnosed glioblastoma either in the concomitant radiotherapeutic regimen (Stupp protocol) either in alternative scheduled (neoadjuvant and adjuvant). According to different published series, the median time to tumor progression (TTP) reached 9 to 10 months. The decision making of what to offer to patients at the time of recurrence is not currently standardized and is influenced by the general condition of the patient (Karnofsky performance score [KPS] and Mini Mental State Examination [MMSE]). We present a series of 38 patients with supratentorial GBM submitted to open surgery and standard radiotherapy (60 Gy) at the time of diagnosis. Furthermore, they all received a first cycle of TMZ in a neoadjuvant setting and several adjuvant cycles after completing radiotherapy. The median TTP was 10 months. Only one patient is actually free of recurrence. At the time of recurrence, 33 of the patients were considered for additional therapies, while the remaining four (with TTP less than four months) were not. The median KPS at the time of recurrence was 70 (quite similar to the median KPS at the time of diagnosis), and the median MMSE was 24 (also similar to the score at the time of diagnosis). Thirteen patients (with a median TTP of at least eight months and after MR1) had a second surgery and received adjuvant therapies (additional cycles of TMZ or locoregional chemotherapy and resection). We considered only tumor with a high dose of temozolomine. Five patients received additional cycles of TMZ. Most patients received combined treatment. Our aim was to prolong the survival of the patients while maintaining a good quality of life and minimizing hospitalization. The median survival after the treatment for recurrence was 7.3 months (range, 1 – 27 months). The administration of additional cycles of TMZ has been effective only in patients who underwent reoperation of a recurrent glioblastoma with a high dose of temozolomine. Temoxustine has been well tolerated, and its administration in the day-hospital regimen enabled patients to have good quality of life and short hospitalization. Locoregional therapy was effective only in the patients who underwent reoperation (median overall survival, 16 months), while patients who had only locoregional therapy had a median overall survival of 12 months.

P214. MAGNETIC RESONANCE DIFFUSION TENSOR IMAGING AND INTRAOPERATIVE MOTOR EVOKED POTENTIAL FOR BRAIN TUMOR SURGERY NEAR THE CORTICOSPINAL TRACT
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Objective: Advances in preoperative neuroimaging (i.e., functional mapping) and neuromonitoring (i.e., neuronavigation systems, fluorescein tumor staining, and neurophysiological techniques) have improved recognition of the motor eloquent area on the brain surface at the time of brain tumor surgery. However, the corticospinal tract (CST) is still impossible to distinguish anatomically from the intrinsic brain tumor in the white matter during microsurgery. We report five cases of deep-seated intrinsic brain tumors near the CST, in which the tumors were evaluated topographically in the context of the CST by using preoperative magnetic resonance (MR) diffusion tensor imaging (DTI) and removed successfully without any additional neurological deterioration by using intraoperative motor evoked potential (MEP).

Materials and methods: Two patients with glioblastoma, one with anaplastic astrocytoma, one with ependymoma, and one with cavernous angioma underwent surgery using MR DTI and intraoperative MEP. MR DTI was performed using a 1.5T MRI system (Sigma; GE Medical System, Milwaukee, WI, USA) and was analyzed to visualize the CST with diffusion tensor visualization software developed by Martuzzi et al. Intraoperative MEP was derived by transcranial electric stimulation (TCS) and monitored with Neuropack MEB2200 (Nihon-Koden, Tokyo, Japan). The change in MEP amplitude during surgery was evaluated to monitor damage to the CST.

Results: The topographical relationship between tumors and the CST was easily visualized by MR DTI and was extremely informative for the care of the CST during surgery in all cases. The lack of significant deterioration in MEP amplitude by repeated TCS during removal of the tumors led surgeons to confirm that the operation was safely performed to preserve the motor function of the CST in all cases. No additional neurological deterioration was observed postoperatively in any of the patients.
P216. IMAGE-GUIDED CRANIAL SURGERY: EXPENSIVE TOY OR COST-EFFECTIVE TOOL? A.D. Kane and P.J. Kane; James Cook University Hospital, Middlesbrough, United Kingdom

Background: The use of image guidance is well established in neurosurgical practice, but its use is not universal. For many smaller neurosurgical units, the capital cost of investing in image-guidance systems can be prohibitive. Previous studies on the use of image-guidance systems in neurosurgery have tended to focus on the benefits relating to tumor localization and the extent of resection rather than on issues that might have a bearing on cost-effectiveness.

Aim: To audit our experience of the use of the Stealth neuronavigation system in a view to assessing potential cost benefits.

Methods: These included a retrospective case note audit of the use of the Stealth neuronavigation system in patients undergoing craniotomy for primary treatment of intracranial tumor and comparison with a similar audit in patients treated prior to the introduction of neuronavigation. Specific factors audited included duration of operation (time of induction of anesthesia to reversal), postoperative complications, and postoperative stay (date of operation to date of discharge).

Results: (1) The records of 21 consecutive patients treated prior to the introduction of Stealth and 99 patients treated using Stealth were audited (no Stealth: age range, 28–77 years [mean, 55.9 years], vs. Stealth: age range, 21–78 years [mean, 52.3 years]). (2) The setup time for Stealth did not increase the length of operation (no Stealth mean duration, 3.42 h ± 0.87 SD; vs. Stealth mean duration, 3.64 h ± 0.95 SD, Students t-test, t = 0.98, NS); (3) The number of complications in both groups of patients was small, and no conclusions can be drawn. (4) The mean duration of postoperative stay was reduced in the group whose surgery involved the use of image guidance (no Stealth: 7.6 days ± 4.5 SD; vs. Stealth, 5.4 days ± 2.8 SD, one-tailed Student’s t-test, t = 2.895, P = 0.003).

Conclusions: This study is limited by the factors that can be levied at all retrospective case note audits. However, the use of image-guided surgery appears to have a significant cost benefit in terms of the reduction of inpatient stay. Further work is needed to unbundle the effects in different tumor types and evaluate the cost savings associated with use of neuro-navigation.

P217. TRANSCRANIAL EPIDURAL APPROACH FOR GIANT PITUITARY ADENOMAS INVADING THE Cavernous Sinus and Parasellar Regions T. Tanimoto, M. Kawashima, K. Miyake, M. Kagawa, Y. Matsumoto, N. Kawai, and S. Nagao; Kagawa University School of Medicine, Kagawa, Japan

Objective: Dolenc’s transcranial epidural approach has recently been used in the treatment of the parassellar, infrachiasmatic, or intracavernous regions. In this approach, the temporal (superficial) dural layer is separated from the deep layer (inner cavernous membrane) to expose the cavernous sinus extradurally. We report our experience with nine patients in which a giant pituitary adenoma invading the cavernous sinus and parassellar region was resected via the transcranial epidural approach.

Patients and methods: Between January 1995 and December 2005, 169 patients with a pituitary adenoma, one had a growth hormone–secreting pituitary adenoma, and other had a PRL-secreting pituitary adenoma. Results: The operations resulted in one total, six subtotal, and two partial excisions. There was no operative mortality or major morbidity. Transient oculomotor palsy occurred in four cases postoperatively. This approach provided excellent exposure of the tumor, relevant cranial nerves, and arteries in and around the cavernous sinus through extradural retraction of the temporal lobe, allowing for sufficient resection of the cavernous and parasellar portions of the tumor. Tumors invading the inferior portion of the clivus or the contralateral cavernous sinus could not be removed through this approach.

Conclusion: Our findings suggest that the transcranial epidural approach is useful for resection of giant pituitary adenomas invading the cavernous sinus and parassellar regions.
operative MRI obtained either within 48 h or 30 to 45 days after surgery. Postoperatively, no neurological deficits were observed, and only one patient experienced transient memory impairment. Overall, serial follow-up neuropsychological evaluation at one and two years showed a trend toward improvement in cognitive performance.

P223. CLINICAL RESULTS OF BORON NEUTRON CAPTURE THERAPY (BNCT) FOR 40 CONSECUTIVE MALIGNANT GLIOMAS IN THREE DIFFERENT PROTOCOLS USING BSH AND BPA

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In 2002–2006, we applied boron neutron capture therapy (BNCT) in 40 cases of malignant gliomas with 46 intracranial neutron irradiations. We used three different protocols. In each protocol, we used sodium borocaptate (BSH) and boronophenylalanine (BPA) simultaneously. In protocol 1, BSH 5 mg/kg and BPA 250 mg/kg were used for 15 consecutive cases of gliomas. BSH was administered 12 h prior to neutron irradiation with 1-h intravenous drip infusion, and BPA was administered just prior to neutron irradiation with 1-h intravenous drip infusion. The median survival time (MST) of five patients with newly diagnosed glioblastoma (GBM) was over 23 months after diagnosis. Two patients were still alive. All cases, including recurrent ones, showed radiographic improvement on MRI. Ten of 14 cases showed more than 50% mass reduction on images. Major cause of death was CSF dissemination. In protocol 2, BNCT was applied in four patients twice with a one- to two-week interval. All four patients were lost, and MST after BNCT by this protocol was 13.3 months. In protocol 3, BSH 5 mg/kg and BPA 700 mg/kg were used with 20 to 50 Gy fractionalized external x-ray (XRT) boost after BNCT. XRT boost was applied especially for the deeper part of the tumor, where insufficient neutron flux was speculated by dose simulation. In protocol 3, BPA was administered for 6 h, just prior to neutron irradiation to obtain a more uniform distribution of the compound. By this protocol 3, six patients newly diagnosed with GBM were observed for more than 16 months. Three had died and three were still alive at the time this abstract was prepared. The MST of these six patients was 16.8 months after diagnosis. Air instillation in the tumor-removed cavity increased the neutron flux and absorbed dose by BNCT, especially for the deeper part of the tumor. Air instillation was performed via Ommaya’s reservoir, whose catheter had been inserted into the tumor-removed cavity at craniotomy. In each protocol, radiation necrosis was the problem for patients with recurrent tumor who had already been treated with full-dose XRT, and removal of the necrosis prolonged the patients’ survival and recovery from the neurological deficits. Surgical specimens obtained at the removal of necrotic tissue proved the existence of neurons by immunohistochemistry using neuronfilament antibody, while no surviving neurons were observed in the necrotic tissue of patients treated by greater than 80 Gy XRT at one protocol. MR spectroscopy data also suggested tumor-selective damage by BNCT.

P224. CYBERKNIFE RADIOSURGERY IN THE TREATMENT OF SPINAL LESIONS: PRELIMINARY RESULTS

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Aim: Conventional EBRT lacks the precision necessary to allow for the delivery of a large dose of radiation near radiosensitive structures such as the spinal cord. If the radiation dose could be confined more precisely to the treatment volume, the likelihood of success increases and the risk of spinal cord injury is minimized. The aim of this study was to evaluate the feasibility and the effectiveness of the treatment of spinal lesions with a limited number of fractions in the radiosurgical technique using the CyberKnife.

Methods: Between August 2004 and January 2006, 21 patients (10 men and 11 women; median age, 53.4 years; age range, 25–76 years) who had spinal lesions (five cervical, eight thoracic, three lumbar, four sacrum, and one cauda equina) were referred to our CyberKnife Center with the intent of controlling pain and improving neurological symptoms. Histological types included 13 metastases, one recurrence of hemangiopericytoma, three meningiomas, one ependymoma, one chordoma, one myeloma, and one fibrosarcoma. In eight cases, the lesions had already been treated with EBRT (range, 36–60 Gy). The mean follow-up was 7.5 months. In all patients, the lesions were tracked to place gold fiducial bone markers. Radiation dose plans were calculated based on TC scans acquired using 1.25-mm slices. In only one case was Mield T2c TC performed. PTV was calculated as the radiographic tumor volume with no margin. The tumor dose was 10 to 35 Gy (mean, 22.9 Gy) to a 65%–80% isodose in 4 to 5 fractions. Only two patients received radiotherapy in a single fraction. The mean of maximum tumor volume was 46.4 mL (maximum, 230 mL). The mean homogeneity index was 1.4 (range, 1.3–1.5), and the mean new conformality index was 1.43 (range, 1.3–1.9). The mean of maximum dose to the spinal cord was 17.9 Gy.

Results: All patients tolerated the procedure well. No acute radiation-induced toxicity was observed. No additional neurological deficit during the follow-up period was observed. Pain relief was achieved by analyzing the decrease in drug dospose. Radiologic response (McDonald criteria) was scored as stable in 11 patients, PR in three, and PD in two.

Conclusions: Spinal stereotactic radiosurgery in the spinal lesions was found to be feasible and safe, offering an alternative therapeutic modality in the palliation of pain with a benefit in quality of life. A larger number of patients and a longer follow-up period are needed to determine late complications and tumor control rates.

P225. EFFICACY OF GROSS TOTAL RESSECTION, EXTERNAL BEAM RADIATION THERAPY, AND STEREOTACTIC LINAC RADIOSURGERY IN PATIENTS WITH GLOBLASTOMA MULTIFORME

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Objective: It was reported that stereotactic radiosurgery (SRS) for glioblastoma multiforme (GBM) did not contribute to the prolongation of overall survival time (OST), but it was not clear whether SRS following surgery was effective in local control in the cases in which gross total resection (GTR) had been achieved. The aim of this study was to evaluate the efficacy of the combined treatment with GTR, external beam radiation therapy (EBRT), and SRS for OST in patients with GBM.

Methods: From September 2000 to December 2005, a consecutive series of 38 patients with GBM (group A) (23 men and 15 female; mean age, 61 years) were retrospectively analyzed and compared with 52 patients who had undergone surgery and EBRT (group B) before SRS had been used in our institution. Our therapeutic strategies were first, surgery, second, EBRT with 40 to 50 Gy, and third, SRS with a mean marginal dose of 20.9 Gy. Before SRS, methionine positron emission tomography (met PET) was performed to evaluate the residual tumors. The mean follow-up period was 17.8 months. The survival curve was drawn using the Kaplan-Meier method. The multivariate analyses were performed with the proportional-hazards model.

Results: The median survival time in group A was 19 months, which was significantly longer than 11 months in group B (P < 0.02). The cases confirmed to be GTR by the met PET were as follows. Case 1: A 59-year-old man. The parietal lesion did not relapse until he died of intracranial dissemination (ICD) 19 months after the surgery. Case 2: A 64-year-old man. The parietal lesion did not relapse until he died of pneumonia 24 months after the surgery. Case 3: A 39-year-old woman. She survived for 27 months after the surgery without relapse of her insular lesion. It has since relapsed with ICD. Case 4: A 71-year-old man. His frontal lesion has not relapsed in the 17 months after surgery, although the lesion remotely metastasized in the temporal lobe. Case 5: A 77-year-old woman. The lesion did not relapse until she died of ICD 13 months after surgery. The combination of three factors (GTR, EBRT, and SRS) was a significantly independent prognostic factor in multivariate analysis (P < 0.05).

Conclusions: These results raised the possibility that SRS could prolong OST by controlling local recurrence in cases in which GTR of the tumors had been achieved. However, we should be careful of ICD, which cannot be controlled by local irradiation.

P226. IMAGE-FUSION COMPUTER TOMOGRAPHY–MAGNETIC RESONANCE FOR TREATMENT PLANNING OF HIGH-GRADE GLIOMA WITH THREE-DIMENSIONAL CONFORMAL RADIOThERAPY

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Purpose: To establish the technique of image-fusion computer tomography–magnetic resonance (CT–MR) for planning treatment of high-grade glioma with three-dimensional conformal radiotherapy (3-D CRT). We describe the different stages of the procedure and the impact of fusion on tumor contours and the consequences on planning.

Materials and methods: The protocol was established at Hospital Universitario de la Princesa in 2003. Thirty patients with high-grade glioma were selected and a MR was obtained between September 2003 and February 2006 at our institution. Both 18 patients for histopathological analysis were obtained from five patients. Fifteen patients had partial surgical resection, and 10 had complete surgical resection. All patients received 3-D CRT.
P227. MALIGNANT GLIOMAS IN ELDERLY: SHORT-COURSE RADIOTHERAPY IS FEASIBLE AND EFFECTIVE
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The benefit of standard treatment of malignant gliomas in older patients is still debated. To assess the effect of the short-course schedule of radiotherapy, 33 consecutive patients older than 70 years (range, 70–78 years) with malignant supratentorial gliomas were studied. Patients underwent surgery, followed by a course of radiation therapy: A total dose of 45 Gy was administered in two cycles (split 15 days), 2.5 Gy/fraction, three fractions daily for 3 days/cycle with limited fields (ICRU 50). The mean KPS before starting radiotherapy was 70. The overall median survival was eight months, the median time to progression was six months, and 25% of the patients survived over 18 months. This study confirmed that patients older than 70 years with good KPS may benefit from treatment with surgery followed by a short course of radiotherapy.

P228. CONFORMAL RADIOTHERAPY WITH MICROMULTILEAF IN ATYPICAL MENINGIOMA: EXPERIENCE AT THE C. BESTA NATIONAL NEUROLOGICAL INSTITUTE, MILAN
I. Milanesi, M. De Santis, L. Fumagalli, F. Ghielmetti, and L. Farselli; National Neurological Institute, Milan, Italy

Materials and methods: Between June 1999 and September 2004, 17 patients with atypical meningioma (8 women and 9 men; mean age, 57 years; age range, 31–57 years) were treated with conformal radiotherapy using a micromultileaf collimator (MLC). Mean follow-up was 19 months (range, 6–60 months). Sites of the lesions were 7 falx, 5 convexity, 2 cavernous sinus, 2 cerebellopontine angle, and 1 tentorial leaf. Mean total dose was 54 Gy, with a dose fraction of 1.8–2.0 Gy. All patients are evaluated with clinical and radiological follow-up (MRI) after 2 months and then every 4 months.

Results: Six patients died; 3 died of local disease progression, 1 died of intracranial dissemination, 1 died of both causes, and 1 died of other causes. Ten patients (59%) had stable disease (SD), and 1 had complete response (CR) with intracerebral progression. Among patients with local progression, I was treated with surgery, 5 with chemotherapy (Oncosaricbide [hydroxyurea]), and 3 with CyberKnife radiosurgery. Median time to tumor progression was 29 months (95% CI). One patient had an ischemic lesion inside the irradiated volume and ex vacuum hydrocephalus.

Conclusion: Our data suggest a good local control after conformal radiotherapy, without acute and late toxicity.

P229. TREATMENT OF PRIMARY PRIMITIVE NEUROECTODERMAL TUMOR (PNET) IN FIVE ADULTS: OUTCOME AND FOLLOW-UP
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Purpose: PNET is very rare, especially in adults. We report on the therapy—especially the radiotherapy techniques—and outcomes of five adult PNET patients treated at our radiotherapy department between 2001 and 2003.

Materials and methods: We analyzed data of five adult patients (three women and two men) treated in the same neuro-oncological center. Their median age at diagnosis was 48 years (range, 24 to 52 years). Surgical resection was attempted in three patients (one subtotal resection), whereas two had biopsy only. Three patients received adjuvant vincristine chemotherapy, and one received carboptalin and etoposide. All patients had radiotherapy after patient positioning in a fixation system based on a double-vacuum technology developed in our department. To form the actual vacuum mattress, the patient is pressed into the mattress with a vacuum foil that can also be used for daily repositioning and fixation. Four patients received craniospinal irradiation supplemented by local tumor irradiation (total dose, 30.4–60.4 Gy), and one patient received local irradiation only (60 Gy).

Results: Three patients are alive with no evidence of disease (median follow-up, 27 months; range, 3–59 months), and one patient with initial spinal seeding developed recurrent disease. Radiotherapy was well tolerated by all patients. Two patients had mild gastrointestinal side effects.

Conclusion: The overall progression-free survival observed is comparable to that obtained in pediatric series. However, the optimal treatment regimen for adult PNET patients has not yet been established. Complete tumor resection, whenever feasible, adjuvant craniospinal irradiation plus irradiation of the tumor region and chemotherapy are the standard therapeutic modalities.

P230. EFFICACY AND TOXICITY OF POSTOPERATIVE SIMULTANEOUS TEMOZOLOMIDE RADIOCHEMOTHERAPY IN GRADE III AND IV MALIGNANT GLIOMAS WITHOUT INTENDED ADDITIONAL ADJUVANT CHEMOTHERAPY: A MONOCENTER EXPERIENCE
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Purpose: To evaluate the feasibility, safety, and efficacy of daily temozolomide administration concurrent with postoperative radiotherapy in malignant grade III and IV astrocytoma and oligodendroglioma only without intended additional adjuvant chemotherapy.

Patients and methods: From November 1999 to March 2003, 81 patients, aged 15 to 72 years (median, 52 years; Karnofsky score, 80–100 in 83% of the patients), who had primary glioblastoma (n = 47), anaplastic astrocytoma (n = 5), anaplastic oligodendroglioma (n = 16), and recurrent glioma (n = 12) were treated. Patients with primary gliomas received a combination of postoperative radiotherapy (60 Gy/1.8–2.0 Gy daily fractions) and daily oral temozolomide (75 mg/m²) on all irradiation days (30–33 doses), while recurrent tumors were treated with 45–60 Gy/1.8 Gy daily and simultaneous temozolomide.

Results: In total, 70 (86%) of 81 patients completed both radiotherapy and chemotherapy as planned. Grade I nausea/vomiting was seen in 28%, grade II in 11%, and grade III in 1% of the patients. Antiemetics were administered to 41% patients. The hematological toxicities observed were leukopenia grade III/IV, 1%; lymphopenia grade III/IV, 46%; and thrombocytopenia grade III/IV, 1%. Two patients taking dexamethasone suffered herpes encephalitis after 1 and 16 doses of temozolomide (75 mg/m²). Median survival was 15 months for patients with glioblastoma. Patients with oligodendroglioma had a 4-year survival rate of 78%.

Conclusion: Postoperative radiochemotherapy without intended adjuvant chemotherapy is effective in patients with oligodendroglioma and may prolong the survival of patients with glioblastoma.
P231. RADIOTHERAPY FOR HIGH-GRADE GLIOMAS: FROM PAPER TO PRACTICE

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Purpose: To offer an advantage in minimizing irradiated volume, sparing surrounding healthy tissues, and protecting critical structures. Quality assurance procedures during therapy include dosimetry in vivo and positioning accuracy revealed by simulation films and portal vision registration.

Materials and methods: The portal images were taken at the beginning of treatment and were compared with simulation film. A 3-D deviation vector was calculated. When the action level was unacceptable, the patient was repositioned and the procedure restarted.

Results: The results showed mean deviation of the ratio measured to calculate dose at the reference point of 1.4% (± 1.9%) to 4.2% SD = 1.76%. From an analysis of portal films, a deviation in the position was 2 to 5 mm.

Conclusions: The quality assurance procedures during radiotherapy offer the possibility of precise and reproducible treatment. Our system is suitable for the routine verification of doses delivered to patients and for monitoring a patient’s treatment position.

P233. SOLITARY AND Oligo BRAIN METASTASIS: NONINVASIVE HYPOFRACTIONATED STEREOTACTIC RADIOTHERAPY AS ALTERNATIVE TO WHOLE BRAIN RADIOTHERAPY

L. Farsetti, W. De Santis, A. Merlotti, L. Braiti, L. Bianchi, L. Fumagalli, and L. Fariselli

Purpose: The aim of our study was to compare the variation in characteristics and outcomes of patients typically seen in practice by a clinical oncologist as compared with the highly selected patient groups seen in clinical trials. It emphasizes the need to correlate the impact of any treatment with the characteristics of the group as a whole.

Method: Forty-six patients diagnosed with HGG from 1998 onward were identified. Cancer registry data were used to obtain data on patients not referred to an oncologist (group 1, n = 160). Patients who were referred but deemed unfit for or refused treatment made up group 2 (n = 139). Group 3 (n = 147) consisted of patients who received radiotherapy (RT). Variables studied included age, Karnofsky performance status (KPS), and extent of surgery, treatment, and survival.

Results: The median age of patients in groups 1, 2, and 3 was 77 years (range, 56–91), 63 years (range, 19–91), and 56 years (range, 19–75), respectively, for groups 2 and 3, median KPS was 50 (range, 10–100) and 70 (range, 30–100). Surgery was more extensive in group 3 than in group 2, with complete or partial resection is 83.7% vs. 48.1% of patients, respectively. In group 3, 72 patients (48.9%) received 60 Gy in 30 fractions over six weeks, and 67 (45.6%) patients received 30 Gy in 6 to 10 fractions over two weeks. In eight patients (5.4%), RT was truncated because of clinical deterioration. Surgeries in groups 1, 2, and 3 were 63.4% (95% CI, 50.9–79.9%), 96.2 days (95% CI, 90.7–111.8), and 307.8 days (95% CI, 268.8–346.8). For group 3, median survivals were 395.8 days (95% CI, 327.1–464.6) in the 60 Gy group vs. 247.7 days (95% CI, 198.9–296.5) in the 30 Gy group. Survival was significantly better for those patients with a KPS of >80 and for those 55 years of age or younger. Differences in age, KPS, and survival were significant (P < 0.05).

Conclusions: Among patients with HGG, 35.5% were not referred to an oncologist. These patients had the poorest prognosis. Of those referred, 51.4% received RT, and only 48.9% of those were suitable for high-dose RT. This represents 16% of the total number of patients diagnosed with HGG. These patients were younger, had better KPS scores, and more extensive surgical intervention. This highlights the variation in characteristics and outcomes of patients typically seen in practice by a clinical oncologist as compared with the highly selected patient groups seen in clinical trials. It emphasizes the need to correlate the impact of any treatment with the characteristics of the group as a whole.

P232. EVALUATION OF QUALITY ASSURANCE PROCEDURE IN BRAIN TUMOR RADIOTHERAPY IN CHILDREN

K. Fucek, S. Blamek, L. Miszczyk, and K. S Tschibanda

Purpose: Radiation therapy is clearly effective in brain tumors in children when it is accompanied by surgery and chemotherapy. Precision volume treatment and were compared with simulation film. A 3-D deviation vector was calculated. When the action level was unacceptable, the patient was repositioned and the procedure restarted.

Results: The results showed mean deviation of the ratio measured to calculate dose at the reference point of 1.4% (± 1.9%) to 4.2% SD = 1.76%. From an analysis of portal films, a deviation in the position was 2 to 5 mm.

Conclusions: The quality assurance procedures during radiotherapy offer the possibility of precise and reproducible treatment. Our system is suitable for the routine verification of doses delivered to patients and for monitoring a patient’s treatment position.

P234. EVALUATION OF SPECTROSCOPY AS A NEW POTENTIAL TOOL FOR THE OPTIMIZATION OF THE TARGET VOLUME FOR CONFORMAL RADIOTHERAPY OF OPERATED BRAIN TUMORS

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Introduction: Dose escalation for high-grade gliomas has not increased survival. It is possible that the high dose is not delivered at the right location, inducing either a failure to kill malignant cells or a destruction of normal brain tissue. This might hamper the effectiveness of potential adjuvant treatments. After surgery, radiotherapy is usually performed on both the operative bed and the possible residue appearing on MRI. This area, surrounding the nonhomogeneous 2- to 3-cm margin, is usually targeted. Indeed, the latter zone is at risk for microscopic disease. The aim of our study was to compare the results of standard MRI and those of magnetic resonance spectroscopic imaging (MRSI). This technique provides information about tumor activity based on the levels of various cellular metabolites, such as choline and N-acetylaspartate (NAA).

Method: A postsurgery MRI was performed in order to be fused with CT in the treatment position for RT treatment planning. A 3-D chemical shift imaging a multivoxel MRSI sequence was prescribed at the end of the MRI session. Acquisition time limitations restricted the size of the regions of interest from which data could be obtained. Both the MRI and MRSI images of a priori specified voxels were analyzed. Four pairs of voxels were studied per patient. The pair consisted of two successive voxels (one closer and one more distant from the surgical bed) in four directions in the axial plane. Acquisition time constraints restricted the size of the regions that could be analyzed in MRSI. The criterion to qualify a voxel as suspect in MRSI was the NAA–choline ratio. Visual criteria were used for MRI. Images from 16 patients (nine high-grade gliomas and six low-grade gliomas, and one ependymoma) were analyzed in 2005.

Results: Of the 64 proximal and distal voxels, 7 and 13, respectively, were not interpretable in MRI. There was a discrepancy between MRI and MRSI predictions of invasion in 22 (39%) of 57 proximal voxels and in 29 (57%) of 51 distal voxels (P > 0.05, χ² test). In most cases (18 of 22 and 27 of 29, respectively), the MBI prediction was positive and the MRI prediction negative.

Conclusions: A discrepancy between MRI and MRSI predictions of active disease zones was found in a rather high percentage of voxels. This may mean that edematous zones on the MRI were not often invaded in our patients. A next step will be the follow-up of the relapse sites.
P235. ULTRAFRACTIONATION RADIATION THERAPY: A NEW THERAPY FOR HUMAN GLOBLASTOMAS—PRELIMINARY RESULTS OF AN ONGOING PHASE II STUDY

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Background: Ultrafractionation radiation therapy consists of irradiating cells or tumors several times daily, delivering low doses at which hyporadiosensitivity occurs. We recently reported the high efficiency of ultrafractionation radiotherapy in glioma cell lines and xenografts (Beauchene et al., 2003) and are now conducting a phase II clinical trial to determine the effect of an ultrafractionation regimen for glioblastoma patients.

Methods: A prospective, multicenter, phase II study has been conducted since September 2003. Patients over 18 years of age who can give informed consent and have histologically proven newly unresectable, subtotalional glioblastoma multiforme (World Health Organization IV) are eligible. Three doses of 0.75 Gy spaced by at least 4 h are delivered daily, five days a week, for six consecutive weeks. A total of 67.5 Gy is delivered. Irradiation is conformational; the tumor plus a margin was included in the treatment planning. Tolerance and toxicity are primary end points; survival and progression-free survival are secondary end points.

Results: Twenty-five patients are currently enrolled in this study. Nineteen patients were eligible: 11 men and 8 women; median age, 58 years (range, 37–76 years). Their median Karnofsky status is 80 (range, 70–100). The median time between histological diagnosis and the start of treatment is seven weeks. The ultrafractionation radiation therapy is well tolerated; no grade 3 and/or 4 CNS toxicity has been observed. Few minor responses were seen at the end of irradiation. Thus, the treatment has been shown to be safe. Median survival has been reached: 13.53 months. Currently, nine patients are alive.

Conclusions: Ultrafractionation radiation therapy is feasible, safe, and well tolerated. No acute CNS toxicity has been observed. The preliminary results are encouraging. Ultrafractionation radiation therapy regimen seems to be effective in malignant gliomas, and therefore supports the development of further clinical studies testing a concomitant association of ultrafractionation and temozolomide.

P236. CNS NEO-ONCOLOGY CENTER AUDIT: U.K. AND IRELAND

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Aim: To gather in-depth information about all aspects of the neuro-oncology services offered in the United Kingdom and Ireland, following an initial pilot audit to neuro-oncology nurses.

Methods: Fifty-four clinical nurse specialists (CNSs) who work in centers in the U.K. and Ireland were sent a questionnaire, but only 44 of those responded. The newly diagnosed cases seen by these CNSs included a total of 35 centers in the U.K. and Ireland.

Results: Over the last few years, there has been a dramatic rise in the service needs for neuro-oncology patients. The role of the clinical nurse specialist (CNS) has become much more comprehensive. Of the 35 units that responded, the newly diagnosed cases seen by these CNSs included 46% high-grade, 2% low-grade or metastatic, and 5% pituitary tumors. Ten percent of the CNSs worked 6 to 10 h and 7% worked 21 to 25 h overtime every week. Seventy-four percent of the CNSs worked in both surgery and oncology and, surprisingly, 12.5% of centers had no CNS working in neuro-oncology at all. Thirty-three percent of CNSs ran nurse-led clinics, and 75% ran a CNS-led telephone clinic that proved very popular with the neuro-oncology patients. Sixty percent of units had a protocol for the breaking of bad news, and 97% of patients were given their histology results in the neurosurgical unit by their neurosurgeon; 85% had their CNS present. All of the CNSs had teaching or lecturing responsibilities, and 64% of those responded, the newly diagnosed cases seen by these CNSs included a total of 35 centers in the U.K. and Ireland.

Conclusions: There is a need for more resources and personnel to address the growing workload of neuro-oncology clinical nurse specialists. There are gaps in the provision of service for neuro-oncology patients in the U.K. and Ireland today because of this lack of resource and personnel.

P237. UNUSUAL CLINICAL PRESENTATION OF GLOBLASTOMA: REPORT OF THREE CASES

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We report our experience with three patients operated on hemorhagic supratentorial glioblastoma at our institute for. In all of these patients, the clinical history started with acute hemiparesis and headache. Nonenhanced CT scan at presentation showed focal supratentorial hemorrhage in an atypical site, not suitable for surgery. The results of contrast-enhanced CT scan, cerebral angiography, and MRI with spectroscopic analysis were considered negative for underlying tumors. Complete clinical and neuroradiological recovery was observed at follow-up.

All patients underwent a second hospital admission because of a rebleeding within six months. At that time, MRI examination revealed a high-grade glioma in relation to the hematoma site, and all the patients were then operated on; the histological examination showed a glioblastoma. Relying on our experience, the tumoral origin of an atypical cerebral hematoma must be always considered, even if it could be not found at the first MRI examination. Thus, it is advisable in the presence of cerebral rebleeding in the same atypical location to conduct a closer follow-up with enhanced CT scan and MRI.

P238. CEREBRAL GLIOSARCOMA WITH MENINGEAL METASTASIS: CASE REPORT AND REVIEW OF THE LITERATURE

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Background: Gliosarcoma (GS), a subtype of glioblastoma with sarcomatoid and/or epithelioid phenotype, is an uncommon, highly malignant brain tumor with poor prognosis. We describe a patient with spinal meningean metastasis from a gliosarcoma of the temporal lobe.

Case presentation: A 53-year-old man with temporal GS had total excision of the tumor. Postoperatively, he was irradiated to the tumor bed with a TD of 56 Gy and was receiving chemotherapy with temozolomide. Neurologically, he was without deficits. After a few months, the disease progressed in the meninges of the spine, presenting with signs of “cauda equina” syndrome. The gross metastasis was surgically removed, and he was irradiated to the symptomatic regions of the spine. The disease slowly progressed, and he died 22 months after the diagnosis of GS.

Conclusion: To our knowledge, this is the second reported case of cerebral GS disseminating to the meninges of the spine, with a rather long survival time.

P239. CNS GRANULOCYTIC SARCOMA IN LEUKEMIA: TWO CASES AND A REVIEW OF THE LITERATURE

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Background: CNS granulocytic sarcoma (GJS) is a rare manifestation of myeloblastic leukemia and can affect brain and meninges. In the current literature, 38 patients with CNS GS are described in detail.

Results: Case 1: A 61-year-old woman with acute myeloblastic leukemia (FAB M5) received induction chemotherapy. One year later, a left hemiparesis developed. The MFI showed a right parieto-occipital lesion mimicking meningioma. Histological workup showed GS. Partial resection and local radiotherapy were performed, and the neurological deficit improved. Leukemia recurred, but the patient refused therapy and died six months later. Case 2: A 51-year-old man with acute myeloblastic leukemia (FAB M1) received chemotherapy. One year later, he developed a rapidly extending paraparesis due to an epidural tumor ranging from T6 to L2, identified histologically as GS. Despite neurosurgical intervention and local radiotherapy, he became paraplegic and died of sepsis three months later.

Conclusion: Despite its rarity, GS must be considered in myeloblastic leukemia. Radiologically, GS may mimic other tumor entities. A summary on clinical course and therapeutic regimen in 38 previous case reports is provided: Several therapeutic approaches were employed, including sys-
P240. MULTICENTRIC HIGH-DEGREE GLIOMA, A NEURO-ONCOLOGY CHALLENGE: RETROSPECTIVE STUDY IN 23 PATIENTS

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Introduction: Multicentric glioma presents a new neuro-oncological challenge by its histological variability and by the variability of methods used for its diagnosis, which makes its study difficult.

Objective: (1) To define the radiological multicentricity criteria. (2) To define the natural history of multicentric glioma and evaluate the global survival of patients who have it.

Materials and methods: Between September 1995 to December 2005, clinical cases of patients diagnosed with multicentric glioma in the Hospital Germans Trias i Pujol in Badalona (Spain) were retrospectively studied using anamnestic, anatomopathological criteria to define high-degree gliomas (anaplastic astrocytoma—positive or multifocal glioblastoma—positive histology), and using radiological criteria to describe the multicentricity. Demographic, clinical, and radiological variables and modalities of treatment were analyzed in relation to the results and survival time. A statistical analysis was performed by means of the SPSS 11.0 program.

Results: Twenty-three patients who presented radiological criteria of multicentricity had received an anatomoclinical diagnosis of high-degree glioma (five anaplastic astrocytomas and 18 multiformal glioblastomas). The radiological criteria used were the presence of multiple lesions with characteristics of high-degree glioma in magnetic resonance imaging, the absence of macroscopically visible unions, noninvasion of the cerebral commissure, nonextension to cerebral fluids, and absence of satellitosis. The average time until the diagnosis was 7.61 weeks. The more frequent clinical presentation was motor alteration and seizures. The cerebral lobe most affected by the lesion of greatest size was the frontal. The initial treatment was surgery in all the cases, and the most frequent surgical treatment was biopsy (52.2% of the cases). The average survival was 37.8 weeks for patients with anaplastic astrocytoma and 22.27 weeks for those with multiformal glioblastomas.

Conclusions: (1) The survival time of patients with high-degree multicentric gliomas is less than that of patients with isolated gliomas. (2) The time until diagnosis is superior for multicentric gliomas than for isolated gliomas.

P241. NEUROLOGIC MANIFESTATIONS IN HEMATOLOGIC MALIGNANCIES

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Neurologic dysfunction is a well-documented and common complication of hematologic malignancies and their treatment. Thus far, however, literature on neurologic manifestations has focused mainly on specific entities of malignant hematologic disorders, such as leukemias, rather than reflecting the entire spectrum of patients with various types of hematologic malignancies. We performed a retrospective study to characterize the type and frequency of neurologic complications in a more representative cohort of patients with various malignant hematologic diseases. A total of 737 patients with various hematologic disorders were reviewed for the presence of complications affecting the central or peripheral nervous system. There were 143 cases (19%) of neurologic complications. Of these patients, 36% were diagnosed with NHL, 12% with plasmacytoma, 12% with chronic lymphocytic leukemia, and 29% with acute leukemia as the underlying hematologic disease. The most common complication was direct tumor manifestation affecting the central or peripheral nervous system (28%), followed by neurologic disorders unrelated to the malignant hematologic illness (23%), and the neurotoxic sequelae of systemic chemotherapy (22%). Cerebrovascular complications and/or disturbances of coagulation were seen in 9% of the patients, and 8% of the patients had a complication caused by infection of the nervous system. As treatment of malignant hematologic diseases improves and patients survive longer, the incidence of neurologic disorders increases. The goal is to recognize and treat the neurologic complications as early as possible in order to improve survival and prevent neurologic long-term sequelae.

P242. VITAMIN E IN THE NEUROPROTECTION OF PERIPHERAL NEUROTOXICITY AND OTOTOXICITY OF CISPLATIN

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Peripheral neurotoxicity is a well-recognized effect of cisplatin chemotherapy that can result in severe disability and represents a major dose-limiting factor. Several studies have recently investigated the role of vitamin E as neuroprotectant in the prevention of cisplatin-induced peripheral neurotoxicity and ototoxicity. An Italian randomized, placebo-controlled, double-blind multicentric study is ongoing to confirm the role of vitamin E supplementation in the prevention of neurotoxicity and ototoxicity induced by cisplatin. Candidates for cisplatin chemotherapy were randomized to either vitamin E supplementation (α-tocopherol, 400 mg/day) or to placebo. Patients were evaluated with neurological and neurophysiological examination before and after treatment. Neurotoxicity was measured using the total neuropathy score. Ototoxicity was evaluated with audiometric test and acoustic evoked potential before and after treatment. Fifty-eight patients have been enrolled in four Italian oncological centers; at present, 30 patients have completed the treatment and received a cumulative dose higher than 300 mg/mq. An interim analysis is scheduled for June 2006.

This is the first randomized, placebo-controlled, double-blind study exploring the efficacy of vitamin E in the neuroprotection of cisplatin neurotoxicity. The results of this study may represent an important step in the individuation of neuroprotective strategies.

P243. DEVELOPMENT OF A NORMAL 11C-METHIONINE PET UPTAKE MAP: A NOVEL APPROACH TO THE EVALUATION OF BRAIN TUMORS USING PET

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Background: Conventional interpretation of methionine PET (Met-PET) scans in suspected brain tumors uses the ratio of the tracer uptake within the lesion to the corresponding contralateral area. The precise location at which the region of interest used to calculate the reference value is placed is vital, because local variations in methionine uptake may significantly alter the calculated ratio. Identifying a precise mirror region is complicated by the distorting effect of the tumor and the need for manual realignment of the image.

Method: Patients with low-grade primary brain tumors or benign lesions were identified on the basis of a tissue diagnosis or surveillance neuroimaging that excluded a high-grade tumor. These conditions were selected because they primarily involve a single hemisphere, with methionine uptake in the unaffected hemisphere being essentially normal. A total of 180 Met-PET scans performed during 2003–2005 were identified from the database at the Max Planck Institute for Neurological Research, coded, and anonymized for analysis. Scans demonstrating midline lesions, significant mass effect, or evidence of substantial previous surgery were then excluded. A methionine template was prepared using data from patients who had undergone both FDG and Met-PET scans within eight weeks, with normalization to a previously developed FDG template. Methionine scans were coregistered to the template, after masking any of tumor, and the diseased hemispheres stripped. Mean uptake maps for each hemisphere were calculated on a voxel-by-voxel basis and merged to create the normal methionine uptake map. Scans unsuitable for inclusion into the normal map were realigned using the contralateral hemisphere and the normal uptake map for reference values, allowing the methods to be compared.

Results: Good correlation was found between uptake ratios using reference values calculated by both methods. Reference values could be reliably calculated in tumors that were previously problematic to analyze, such as those that cross the midline. Coregistration of the normal map was impaired in some cases by loss of the normal architecture, but valid reference values were obtained despite this.

Conclusion: Use of a normal uptake map may facilitate calculation of PET uptake ratios in brain tumors. Further research is required to evaluate the correlation with histological findings and the accuracy of image coregistration in the presence of distorting tumors.
Solitary fibrous tumors (SFTs) are rare spindle-cell neoplasms of mesenchymal origin that most frequently arise in the pleural cavity. More recently, this entity has been diagnosed in extrathoracic locations. In the past nine years, reports on 16 intraspinal cases have been published. We have treated two patients with spinal SFTs, one with local aggressive behavior and the other growing 30 years after local radiotherapy for a cutaneous angiomma.

Central nervous system SFTs have been reviewed in order to check similar behaviors. A 63-year-old woman was operated on with resection of a recurrent spinal SFT; histopathology showed necrosis and a high proliferative index, indicating an SFT characteristic pattern. A 36-year-old woman had a total resection of a tumor adherent and growing from the spinal cord at the T7 level, 33 years earlier, she had received local radiotherapy for a cutaneous dorsal angiomma at that level. English literature regarding spinal and intracranial SFTs has been reviewed searching for similar cases.

Results: Sixteen spinal SFT cases have been found. Only one spinal cord recurrent case after a partial resection and no spinal cases after radiotherapy, meeting radiation-induced criteria, have been found. Among intracranial SFT cases, one occipital case grew three years after radiotherapy for a pineal germ cell tumor. Of 64 intracranial cases, only three were described as aggressive.

Conclusions: Spinal solitary fibrous tumor is a rare entity. Although usually benign, an aggressive behavior cannot be discarded, and cases should be followed for recurrences after resection. Central nervous system solitary fibrous tumors can be included among tumors that can be induced after local radiotherapy for other diseases.

P245. TC-99M-MI BI NER VENT IMAGING OF INTRACRANIAL TUMORS
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Background: The increasing incidence, high mortality, and low survival rate of patients with intracranial malignancies justify the introduction of noninvasive diagnostic radiological techniques in neuro-oncology. Although single-photon emission tomography (SPECT) is not used in the initial diagnosis of brain tumors, numerous reports study its efficacy in the assessment of tumor type, recurrence, radiation necrosis, and treatment response. Recently, a number of radiopharmaceutical agents have been evaluated for the detection of primary and secondary brain tumors. Tc-99m MIBI is a small lipophilic radioligand that enters cells by diffusion and accumulates significantly more in tumor tissue compared with normal tissue because of the higher mitochondrial activity in tumor cells. Therefore, the encephalic tracer MIBI has been used to visualize primary, metastatic, and recurrent tumors.

Objective: To determine Tc-99m MIBI uptake in brain tumors and evaluate its usefulness for the differentiation of histological diagnosis.

Methods: Tc-99m MIBI SPECT was carried out in 17 patients (10 men and 7 women; mean age, 56.4 ± 7.2 years) who had untreated supratentorial primary brain tumors. Five patients had pathologically proven malignant glioma, six had meningioma, five had solitary brain metastasis, and one had an arachnoid cyst. SPECT was performed 20 and 120 min after administration of 740 MBq (20 mCi) Tc-99m MIBI. A single detector camera with a low-energy high-resolution collimator was used for image acquisition. Transverse, sagittal, and coronal views were reconstructed, and MIBI uptake index was calculated on SPECT imaging. Maximum uptake of MIBI in the lesion was expressed as a mean tumor-to-background ratio. CT and/or MRI scanning were performed, too.

Results: The MIBI index ranged from 1.9 to 3.6 (mean, 2.6 ± 1.6) in high-grade gliomas, from 3.2 to 5.4 (3.9 ± 0.2) in the pathologically proven meningiomas, and from 3.8 to 7.1 (3.4 ± 1.9) in metastatic tumors. The patient with an arachnoid cyst did not demonstrate MIBI uptake. Metastatic tumors and meningiomas have increased Tc-99m MIBI uptake compared with that of low-grade gliomas and nonneoplastic lesion.

Conclusions: Although the results seemed to be different than reported so far, particularly for brain metastasis, this preliminary study suggested that Tc-99m MIBI imaging in harmony with CT and/or MRI might be useful in differential diagnosis of brain tumors.

P246. SEVERE THROMBOCYTOPENIA AFTER FIRST CYCLE OF TEMOZOLOMIDE: WHO IS AT RISK?
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Temozolomide (TMZ) is an oral alkylating agent that has considerably improved survival of patients with newly diagnosed glioblastoma (GBM) when it is given concomitantly with radiotherapy, followed by adjuvant chemotherapy. Usually, TMZ is very well tolerated and does not cause severe toxicity. The most frequently encountered toxicity is fatigue grade II and mild nausea and vomiting, which responds to the usual antiemetic medication. However, in approximately 5% of patients, TMZ causes severe hematologic toxicity (HT), mainly thrombocytopenia often occurring very early in the treatment course as a dose-limiting toxicity. There is no evidence of cumulative myelosuppression that would be expected later, after 2 to 4 cycles. In our institution, 11 of more than 150 patients treated with TMZ within the classic five-day regimen with 200 mg/m2 developed thrombocytopenia (n = 11) and leukopenia (n = 11) after the first treatment cycle with TMZ. The patients were five men and six women, aged from 32 up to 77 years. All were chemotherapy naive before TMZ treatment. Most of the patients required hospitalization for more than a week. Two patients developed febrile neutropenia (one fatal). The thrombocytes nadir occurs mostly from day 21 to day 28 (range, 11–43 days) and recovers usually within two weeks. Thrombocytopenia grade IV was reversible in all cases, but lasted up to five weeks. Three patients of 50 treated with the concomitant regimen with 75 mg/m2 also developed thrombocytopenia WHO grade IV and leukocytopenia. Thrombocytopenia occurred from day 27 to day 30 and lasted for 21 to 30 days, respectively, and was reversible in all cases. We checked the concomitant medication (antiepileptic treatment, use of low molecular heparins for anticoagulation, steroids, etc.) and looked for common blood type. All patients had antiepileptic prevention with enzyme-inducing antiepileptic drugs such as phenytoin and were anticoagulated with low molecular weight heparin. Patients treated with low-weight heparin should be followed very carefully under TMZ. The antiepileptic medication with phenytoin should be maintained only if other AEDs are contraindicated. Cases treated with low molecular weight heparin and antiepileptic medication should be followed thoroughly—at least weekly during concomitant TMZ treatment or during the first cycle.

P247. THE PREDICTIVE VALUE OF THALLIUM SPECT SCANNING IN THE MANAGEMENT OF PATIENTS WITH SUPRATENTORIAL PRIMARY BRAIN TUMORS: AN AUDIT OF SCAN RESULTS AND HISTOLOGY
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Background: Thallium SPECT imaging is frequently used in the assessment of supratentorial primary brain tumors (SPBTs). Commonly, the scans are reported using a qualitative inspection technique and designated as “no increased uptake” or “increased uptake” of the isotope indicative of low-grade and high-grade malignancy, respectively (low grade = WHO grades I and II, and high grade = WHO grades III and IV).

Aim: To audit the predictive value of thallium SPECT in the diagnosis of SPBT.

Method: SPECT imaging was performed after i.v. injection of 100 MBq of thallium 201 (as thallous chloride) in patients with CT/MR-proven SPBT. All scans were performed on a dual-headed gamma camera (Siemens E-Cam, Siemens Medical Solutions) using low-energy, all-purpose collimation. Data were acquired in a 64 × 64 pixel matrix at each of 64 positions over a 360-degree rotation around the subject’s head. Imaging started 2 min after thallium injection and took 22 min. Tomographic reconstruction was performed on a Hermes workstation (Nuclear Diagnostics, Kent, England) using a method of filtered backprojection with attenuation correction. The data having been prefiltered by a Wiener filter. Tomographic slices, orientated to brain anatomy, were reported by a single neuroradiologist. The radiologist’s report of tumor grading was then compared with postoperative histology report of tumor grade.

Results: The results from 68 patients were audited. Five tumors reported as low grade on thallium SPECT were high grade on histology (five “false negatives”). No tumors reported as high grade on thallium SPECT werelow grade on histology (no false positives). Sensitivity = 83% specificity = 100%, +ve PV = 100%, -ve PV = 84%.

Conclusions: Thallium SPECT imaging is a reliable and helpful tool in the diagnosis of high-grade SPBT. Thallium SPECT scanning is less reliable in the diagnosis of low-grade SPBT. Further studies of the sensitivity/specificity of multimodal imaging in the prognosis of tumor grade are required.