

Abstracts

ORAL PRESENTATIONS
NEURO-IMAGING I

O.01. INFLUENCE OF P-GLYCOPROTEIN EXPRESSION ON ^{99m}Tc-TETROFOSMIN UPTAKE IN GLIOMAS

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OBJECTIVE: Multidrug resistance (MDR) remains a major obstacle to successful chemotherapeutic treatment of cancer and can be caused by overexpression of P-glycoprotein, the MDR1 gene product. P-glycoprotein (P-gp) recognizes several chemotherapeutic agents as a substrate and prevents their accumulation. Among them, both technetium-99-sestamibi and technetium-99-tetrofosmin (^{99m}Tc-TF) are single photon emission computed tomography tracers. ^{99m}Tc-MIBI uptake has been proven *in vitro* and *in vivo* to inversely correlate with P-gp levels of tumors. *In vitro* studies have shown that ^{99m}Tc-TF is influenced in a lesser degree from the P-gp expression, thus may be superior to ^{99m}Tc-MIBI for brain tumor imaging. In the present study, we evaluated *in vivo* whether ^{99m}Tc-TF uptake correlates with P-gp levels in gliomas. **MATERIALS AND METHODS:** Eighteen patients (10 males, 8 females, mean age 57.3 years) with histologically confirmed glioma were included in the study. There were 13 glioblastoma multiforme cases, 2 anaplastic astrocytomas, 2 anaplastic oligodendrogliomas, and 1 low grade astrocytoma. Brain SPECT by ^{99m}Tc-TF was performed within a week prior to surgical excision and the expression of P-gp was assessed by immunohistochemistry. Radiotracer accumulation was assessed by a semiquantitative method of image analysis, calculating the lesion-to-normal (L/N) uptake ratio. **RESULTS:** The tracer uptake ranged from faint to profound (mean L/N = 8.2, range 1.8–20). The P-gp expression ranged from 0% to 45%. Using Spearman's rho analysis we found no correlation between tracer uptake (L/N) and P-gp expression ($P = .21$, $r = .538$). **CONCLUSION:** The present data suggest that ^{99m}Tc-TF uptake is not influenced by P-gp expression in gliomas. Thus, ^{99m}Tc-TF constitute a suitable radiotracer for gliomas imaging.

O.02. PERFUSION MR IN DIFFERENTIATING BETWEEN TUMOR-PROGRESSION AND PSEUDO-PROGRESSION IN RECURRENT GLIOBLASTOMA MULTIFORME

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OBJECTIVES: The aim of this study was to evaluate perfusion magnetic resonance imaging (pMRI) for differentiation of tumor progression (PD) from pseudo-progression (Ps-PD) in patients with recurrent glioblastoma multiforme (GBM) following chemoradiation. **BACKGROUND:** The appearance of Ps-PD on brain MR following initial chemoradiotherapy is difficult to distinguish from true PD. We examined whether the technique of pMRI allows proper distinction between PD and Ps-PD in patients with recurrent GBM. **METHODS:** All files of patients with GBM with signs of radiological progression at T1-weighted gadolinium-enhanced MR scan were selected from the neuro-oncology clinic, followed by pMRI and 1 MR scan including pMRI 3 months later. Patients had received radiotherapy (60 Gy in 30 fractions) with concomitant (75 mg/m² per day and adjuvant 200 mg/m², Days 1–5, q 4 wks) temozolomide. Clinical data and MR characteristics (localization, size, rCBV) were scored at radiological progression and 3 months later. Ps-PD was defined as absence of signs of PD at re-operation, no further progression, or spontaneous improvement MR 3 months later, and no new anti-tumor therapy or any increase dexamethasone dosing. MR findings including relative cerebral blood volume (rCBV) were analyzed and compared with clinical data. **RESULTS:** In 34 patients, 82% were diagnosed as PD and 18% as Ps-PD. In 32 of 34 patients, pMRI was evaluable. After establishing a cut-off value of 2.12 for rCBV, pMRI could differentiate between the two entities with a positive predictive value of 96% for the presence of true progression (PD). Sensitivity was 88% and specificity was 83% with a negative predictive value of 63% ($P < .002$, Fisher's exact test).

CONCLUSION: pMRI seems to be a reliable technique to distinguish PD from Ps-PD in patients with recurrent GBM, and these results deserve further testing in larger sample for confirmation.

O.03. ASSESSMENT OF BEVACIZUMAB/IRINOTECAN RESPONSE IN MALIGNANT GLIOMA BY ADC MAP IMAGE ANALYSIS

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BACKGROUND: Response assessment in malignant glioma following anti-angiogenic treatment is challenging for conventional MR imaging (MRI). Despite decreased contrast-enhancement, non-enhancing parts of the tumor may continue to grow. In this retrospective study, we analyzed patients with recurrent malignant glioma during Bevacizumab/Irinotecan therapy using ADC map imaging analysis from diffusion-weighted MRI to yield ultra-structural information on cellular density and properties of the extracellular matrix in relation to the progression-free survival. **METHODS:** Fifteen patients treated with Bevacizumab/Irinotecan for recurrent malignant glioma were investigated by MRI every 2–3 months until tumor progression. Applying image segmentation, volumes of contrast-enhanced lesions on T1 and hyperintense nonenhancing T2 lesions were calculated. T2 hyperintense lesions were defined as regions of interest (ROIs) and registered to the corresponding ADC maps (T2-ADC). Histograms and cumulative histograms of the T2-ADC ROIs were calculated to quantify the apparent gray scale value distribution and were compared with progression-free survival. Software programs were used to perform segmentation (ITK-Snap), calculation of T2-ADC histograms (ImageJ), and statistical figures (SPSS). **RESULTS:** At 3-month follow-up, the overall mean contrast-enhanced T1 volume (in cm³) decreased significantly from 26.8 (± 29.43) ($P = .02$) to 5.45 (± 7.13). According to Mac Donald criteria, 12 patients showed response and 3 patients progressed. During the same period of time, the mean T2 volume (in cm³) was significantly reduced in 8 cases ($P = .005$) from 127.32 (± 59.01) to 85.61 (± 42.12) and increased in 7 cases ($P = .08$) from 140.93 (± 50.91) to 203.22 (± 126.52). T2-ADC cumulative histograms showed differences in terms of gradient and kurtosis. In 8 cases an increasing gradient and high kurtosis represented an increased amount of low ADC grey scale values that can be interpreted as an augmentation of cellular density of the tumor. These patients showed a lower chance of progression-free survival compared with patients ($n = 6$) with a decreasing slope and low kurtosis of the T2-ADC cumulative histograms. **CONCLUSION:** Changes in grey scale distribution in ADC cumulative histograms in patients with malignant recurrent glioma may be predictive for anti-angiogenic treatment response.

O.04. RADIOGRAPHIC PATTERNS OF RELAPSE IN GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM) is defined pathologically as an infiltrative glioma, and salvage therapy with bevacizumab is believed to increase the incidence of diffuse and distant invasion as assessed radiographically. **PATIENTS AND METHODS:** 80 adult patients with glioblastoma were treated with surgery followed by radiotherapy (RT) and concurrent and adjuvant temozolomide (TMZ). At first recurrence, 80 patients were treated with single agent, bevacizumab. At time of progression, 57 patients were treated with bevacizumab and a cytotoxic chemotherapy, cytotoxic chemotherapy alone, or on an investigational trial. Magnetic resonance imaging (MRI) were analyzed at four time points in each patient: at presentation, at first, second, and third recurrence. Four patterns of radiographic disease were assessed, local (unifocal disease), distant (second lesion noncontiguous with primary lesion), multifocal (>2 lesions including leptomeningeal dissemination), and diffuse. **RESULTS:** At presentation, 87.5% of glioblastoma were local, 6.25% distant, 3.75% multifocal, and 2.5% diffuse. At first recurrence following progression on RT/TMZ and before initiation of bevacizumab, 80% were local, 7.5% distant, 6.25% multifocal (including 1 with CSF dissemination), and 6.25% diffuse. At second recurrence following progression on bevacizumab, 71.25% were local, 8.75% distant, 8.75% multifocal (2 of 7 with CSF dissemination), and 11.25% were diffuse. At third recurrence (57 patients evaluable), 71.25% were local, 7.0% distant, 7.0% multifocal, and 14.0% were diffuse. Survival following progression on bevacizumab did not differ by pattern of radiographic recurrence. **CONCLUSIONS:** A majority of adult patients with GBM at diagnosis manifest MRI-defined local disease and maintain this pattern notwithstanding multiple recurrences and treatment with bevacizumab.

O.05. PREOPERATIVE ESTIMATION OF EXTENT OF RESECTION OF GLIOMAS BY DTI-FT

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DTI-fiber tracking (DTI-FT) allows the reconstruction of subcortical tracts and their relationship with tumors. This work assesses the ability of preoperative DTI-FT to predict the extent of resection achievable during surgery of gliomas performed with intraoperative brain mapping. A total of 220 patients with gliomas (mostly low grade) were included. In all patients, the cortico-spinal tract (CST), the inferior fronto-occipital (IFO), and superior longitudinalis (SLF) fasciculus were reconstructed by DTI-FT. The relationship of each of the tracts (CST, IFO, and SLF) with the tumor mass was scored by two independent observers as being unchanged, dislocated, or infiltrated. Intraoperative protocol included intraoperative language and motor mapping and monitoring (EEG, ECoG, EMG, and MEP). DTI-FT images were loaded into the neuronavigation system and available during surgery. Surgery was carried out according functional boundaries. For each patient, preoperative and postoperative MR images and DTI-FT were loaded into the neuronavigation software and image fusion was used to evaluate the extent of resection as well as the correspondence between the resection boundaries and the preoperative DTI-FT. A correlation between the preoperative score of each tract and the extent of resection (scored on FLAIR volumetric images) was then investigated. Most of the tracts were inside and infiltrated by the tumor (80%); 40% of the tumor showed more than one tract infiltration. Tract infiltration depended on tumor location and volume, being more frequently observed in rolandic and large tumors. When no tract infiltration was documented by DTI-FT, the extent of resection was total in all the cases. When one tract was infiltrated, extent of resection was total in 70% of the cases on the average, which decreased to 45% and to 33% when 2 or 3 tracts were involved, respectively. The involvement of CST and IFO was more frequently associated with a reduced chance of resection. Preoperative evaluation in DTI-FT of the level of infiltration of subcortical tracts (mainly the CST and IFO) can predict the chance of performing a total resection. When CST and IFO are infiltrated by the tumor, a total removal is rarely possible; when were outside, an extensive resection is feasible. Preoperative DTI-FT identifies those patients who will mostly benefit from surgery.

O.06. USEFULNESS OF MET-PET, FLT-PET, AND FMISO-PET FOR SURGICAL TREATMENT OF GLIOMAS

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OBJECTIVE: L-[Methyl-¹¹C]methionine (MET) positron emission tomography (PET), [¹⁸F]-fluoro-3'-deoxy-3'-l-fluorothymidine (FLT) PET, and [¹⁸F]-fluoromisonidazole (FMISO) are sensitive modalities for visualizing primary brain tumors. The objectives of this study were to evaluate the relationships between the uptake of MET, FLT, or FMISO and the histopathological grading in gliomas. **METHODS:** We examined 51 patients (22 males, 29 females; mean age: 48.7 years; range: 2–89 years; 10 diffuse astrocytomas, 1 oligodendroglioma, 3 oligoastrocytomas, 13 anaplastic astrocytomas, 1 anaplastic oligodendroglioma, 1 anaplastic ependymoma, and 22 glioblastomas) using MET–PET studies preoperatively. Moreover, 35 patients (17 males, 18 females; mean age: 51.4 years; range: 22–81 years; 6 diffuse astrocytomas, 1 oligodendroglioma, 7 anaplastic astrocytomas, and 21 glioblastomas) were examined with FLT–PET. Finally, 10 patients (6 males, 4 females; mean age: 55.8 years; range: 30–72 years; 1 diffuse astrocytomas, 2 anaplastic astrocytomas, 7 glioblastomas) were examined with FMISO–PET. MET, FLT, and FMISO uptakes were assessed by standardized uptake value of the tumor showing the maximum uptake (SUV_{max}), and the ratio of tumor tissue to the contralateral normal gray matter (T/N ratio). The tumor activity and degree of malignancy were evaluated using Ki-67 index. The correlations between SUV_{max} and Ki-67 index were determined for all subjects. **RESULTS:** All glioblastomas showed tumor uptake of MET, FLT, and FMISO. The difference in MET T/N ratio was statistically significant between grades II and IV gliomas, but not significant between grades II and III gliomas. The difference in FLT T/N ratio was statistically significant between grades III and IV gliomas, but not significant between grades II and III gliomas. The difference in FMISO T/N ratio was statistically significant between grades III and IV gliomas. FLT SUV_{max} in the tumor had a stronger correlation with Ki-67 index than MET SUV_{max}. **CONCLUSIONS:** PET studies using MET, FLT, and FMISO are useful for preoperative diagnosis in gliomas. FLT–PET seems to be superior to MET–PET in assessment of the proliferation activity on gliomas of different grades. FMISO–PET is useful for non-invasive assessment of hypoxia in malignant gliomas. Advances in molecular imaging such as PET imaging techniques will facilitate more safe and solid management and therapy for the patients with malignant gliomas.

GLIOBLASTOMA MULTIFORME AND ANAPLASTIC GLIOMA

O.07. MGMT PROMOTER METHYLATION STATUS AND EXPRESSION OF DNA MISMATCH REPAIR GENES IN PAIRED PRIMARY AND RECURRENT GLIOBLASTOMA: A TRANSLATIONAL STUDY OF THE GERMAN GLIOMA NETWORK

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INTRODUCTION: Hypermethylation of the *MGMT* promoter is strongly associated with longer progression-free and overall survival in glioblastoma (GBM) patients treated with radiotherapy and concomitant and adjuvant temozolomide. We here addressed the question whether GBM relapses are associated with changes in the *MGMT* promoter methylation status or altered expression of the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, or/and *PMS2*. **METHODS:** *MGMT* promoter methylation status was analyzed in paired primary and recurrent GBM samples of 80 patients using nonquantitative methylation-specific PCR (MSP). The vital tumor cell content of each primary and recurrent tumor specimen was histologically determined. Quantitative promoter methylation analyses using DNA pyrosequencing were performed, too, for *MGMT* in 48 patients as well as for the DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* in 42 patients. Furthermore, the levels of *MGMT*, *MLH1*, *MSH2*, *MSH6*, and *PMS2* proteins were analyzed semiquantitatively by immunohistochemistry (IHC) in 43 patients. **RESULTS:** MSP revealed *MGMT* promoter hypermethylation in 27 patients, borderline methylation in 3 patients, and no methylation in 50 patients at diagnosis. In 73 of the 80 patients, the *MGMT* promoter status of the primary tumor was retained at recurrence. In 6 patients, loss or reduction of *MGMT* promoter methylation was detected in the recurrent tumor; however, in 3 patients, this finding was explained by low tumor cell contents in the recurrent tumor specimens. In 1 patient, a change from *MGMT* borderline methylation (8% methylated alleles) to hypermethylation (50% methylated alleles) was detected. None of the investigated primary and recurrent glioblastomas showed *MLH1*, *MSH2*, *MSH6*, or *PMS2* promoter hypermethylation. However, immunohistochemical expression scores for *MLH1*, *MSH2*, *MSH6*, and *PMS2* proteins were frequently reduced in the recurrent tumor when compared with the corresponding primary tumor. **CONCLUSION:** The *MGMT* promoter methylation status does not change from the primary to the recurrent tumor in the vast majority of GBM patients. Accordingly, changes in the *MGMT* promoter methylation status are unlikely to account for acquired resistance to temozolomide. Our results further suggest that GBM recurrences often demonstrate lower *MLH1*, *MSH2*, *MSH6*, and/or *PMS2* immunoreactivity scores. However, *MLH1*, *MSH2*, *MSH6*, and *PMS2* promoter hypermethylation does not appear to account for these decreased protein levels and is appeared to be involved in not linked to GBM recurrence. Accordingly, the changes in the *MGMT* promoter methylation status are unlikely to account for acquired resistance to temozolomide.

O.08. MGMT MRNA TRANSCRIPTIONAL ACTIVITY PREDICTS CLINICAL OUTCOME IN MALIGNANT GLIOMAS AFTER RADIO-/CHEMOTHERAPY

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OBJECTIVE: Epigenetic silencing of the gene that encodes for O⁶-methylguanine-DNA-methyltransferase (*MGMT*) has been correlated with favorable clinical outcome in patients with malignant gliomas after radio-/chemotherapy using alkylating agents such as temozolomide; it has been hypothesized that *MGMT* promoter methylation leads to a decreased *MGMT* mRNA transcriptional activity and subsequent protein expression with a diminished ability of the tumor to repair chemotherapy-induced lesions. The real clinical impact of this assumption, however, still remains controversial. In the current prospective study, we analyzed the predictive impact of *MGMT* mRNA expression in malignant glioma (64 patients) after radiotherapy and/or chemotherapy with temozolomide and its correlation with the *MGMT* promoter methylation status. **METHODS:** Only vital tumor samples harvested from open

tumor resections (25 patients) and stereotactic biopsies (39 patients) were used for subsequent analyses. MGMT promoter methylation was determined by methylation-specific PCR (MSP). MGMT mRNA expression was analyzed by real-time qPCR and normalized against adequate house-keeping genes. Primary endpoint was progression-free survival (PFS). Secondary endpoints were overall survival (OS) and treatment response (TR). RESULTS: Histopathological diagnosis revealed 54 glioblastoma multiforme and 10 anaplastic astrocytomas. Thirty-two tumors were methylated and 32 tumors exhibited a low MGMT transcriptional activity (cut-off value: 0.45), respectively. Low MGMT mRNA expression turned out to be a strong predictive factor for PFS, OS, and favorable TR in univariate and multivariate models ($P < .0001$); even though the degree of MGMT mRNA expression strongly correlated with the methylation status ($P < .0001$), discordant findings were seen in 30% of the patients: Patients with a methylated tumor and high MGMT mRNA expression (15%) did significantly worse than those with low transcriptional activity ($P < .01$). Conversely, unmethylated tumors with low MGMT mRNA expression (15% of the unmethylated tumors) did better than their counterparts. CONCLUSION: Determination of MGMT mRNA expression is a powerful method for predictive evaluation of malignant glioma patients undergoing chemotherapy with alkylating agents. A substantial rate of discordant findings elucidates the fact that treatment decision in favor of chemotherapy with alkylating agents should not be based on the MGMT methylation status alone.

O.09. UPDATED RESPONSE ASSESSMENT CRITERIA FOR HIGH-GRADE GLIOMAS (HGG): REPORT FROM THE RESPONSE ASSESSMENT IN NEURO-ONCOLOGY (RANO) WORKING GROUP

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Accurate determination of response and progression is crucial to evaluate new brain tumor treatments and care for patients. Macdonald's criteria (Macdonald et al. *J Clin Oncol.* 1990; 8:1277–80), based on measuring cross-sectional enhancing tumor area, taking into account changes in steroid dose and neurological status, have been widely used. Limitations include difficulty in measuring tumors with complex shapes or indistinct borders, nontumor factors that produce imaging changes, reaction to local therapies, posttreatment changes that may mimic tumor, and lack of applicability to nonenhancing tumors. Anti-angiogenic therapies, which reduce MRI enhancement by restoring the blood-brain barrier, while nonenhancing tumor may enlarge, highlight the difficulty of assessing novel treatments. The RECIST criteria use unidimensional tumor measurements, do not consider steroids or neurological status, and have the limitations of Macdonald's criteria. The RANO group is an ongoing unofficial international multidisciplinary consensus-building effort to develop new response criteria. The now published RANO criteria for HGG measure cross-sectional enhancing and nonenhancing tumor area, and account for the changes in steroid dose and neurological status (Wen et al. *J Clin Oncol.* 2010;doi: 10.1200/JCO.2009.26.3541). Within the first 12 weeks of completing chemo-radiation, progression requires either new tumor outside the high-dose radiotherapy field or biopsy-proven tumor, to avoid "pseudoprogression." T2/FLAIR images receive more emphasis, particularly in trials on anti-angiogenic agents. Hindsight may allow a more accurate determination of progression and is now a formally described part of the outcome assessment procedure. Increase of steroids alone is still not perceived as sufficient evidence of progression

O.10. A PROSPECTIVE, RANDOMIZED, OPEN LABEL, PHASE III CLINICAL TRIAL OF NOVOTTF-100A VS BEST STANDARD OF CARE CHEMOTHERAPY IN PATIENTS WITH RECURRENT GLIOBLASTOMA

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BACKGROUND: The NovoTTF-100A device is a portable, home use, medical device which delivers low intensity, intermediate frequency, alternating electric fields (TTFields) to the brain by means of non-invasive, disposable scalp electrodes. TTFields physically interfere with cell division and assembly of organelles (Kirson et al. *Can Res.* 2004; Kirson et al. *Proc Natl Assoc Soc USA*, 2007; Salzberg et al. *Onkologie*, 2008; Kirson et al. *BMC Med. Phys.* 2009). METHODS: Adult patients with recurrent GBM were to be randomized (1:1) to either NovoTTF-100A administered continuously (20–24 hours/day, 7 day/week) or the best local standard of care (BSC) chemotherapy, at the physicians discretion, in each center. Randomization was stratified by prior surgery for recurrence and center. The number of prior treatments was not limited; a Karnofsky performance status of $\geq 70\%$ and an adequate end-organ function were required. The primary endpoint was overall survival; secondary endpoints included 1-y survival, PFS6, TTP, radiological response rate and safety. The study was powered to detect a 60% increase in overall survival (eg, 48 vs 30 weeks) with a two-tailed α of 0.05 and power of 80%. RESULTS: Between September 2006 and May 2009, 237 patients were included in 28 centers in the United States, Europe, and Israel, 120 patients were treated with NovoTTF-100A alone, and 117 patients received BSC chemotherapies including bevacizumab, nitrosureas, procarbazine, or PCV combination, temozolomide, erlotinib, irinotecan, and imatinib. Mature results will be available by September 2010, and updated complete analysis will be presented. CONCLUSIONS: This is the first phase III, controlled, clinical trial testing TTFields, an entirely novel treatment modality in cancer patients. In this trial, it was tested as a single modality in recurrent GBM, and compared with the best available chemotherapy. This treatment holds promise as monotherapy, has only very limited and local toxicity, and may be well suited for combination with standard chemotherapy.

O.11. STEM/PROGENITOR CELL FLEXIBILITY DETERMINES BOTH NORMAL BRAIN DEVELOPMENT AND BRAIN TUMORS

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How the programming and reprogramming of stem/progenitor cells regulate normal brain development and brain cancer is still not well known. One of the tools that we have chosen to investigate stem/progenitor cell regulation is the neuronal transcriptional repressor, RE1-silencing transcription factor (REST). REST is expressed in most nonneural cells, including neural stem/progenitor cells (NSCs), but is absent in most neuronal cells. Previously, we found that countering REST function by forced activation of REST target genes in NSCs, and even muscle progenitor cells, was sufficient to cause neuronal differentiation, indicating an enormous cellular flexibility of stem/progenitor cells. Although REST is normally not expressed in most neural cells, we previously found that approximately 50% of human medulloblastomas, a malignant pediatric brain tumor, express REST and that this abnormal expression of REST causes medulloblastoma-like cerebellar tumors by maintaining "stemness" of NSCs. We then found that REST maintains self-renewal and pluripotency in ES cells and this process is regulated by extracellular matrix components. Our recent work indicates that REST regulates self-renewal of normal NSCs and its expression is diminished as NSCs differentiate. Interestingly, a subset of glioblastoma patient sample-derived cancer-initiating cells (glioblastoma stem-like cells or GSCs) expresses abnormal levels of REST. The role of REST in the GSCs is to maintain stemness through a microRNA-mediated mechanism. We are currently working on examining whether REST could function as a therapeutic target in these glioblastoma tumors. Taken together, the results of our studies indicate that stem/progenitor cells are more flexible than previously believed and that simple alteration of transcriptional regulators in these cells can affect both normal brain development and brain tumors, such as glioblastoma.

O.12. EFFICIENT ENGRAFTMENT OF MGMP140K GENE-MODIFIED CD34+ CELLS FOLLOWING NONMYELOABLATIVE BCNU CONDITIONING IN PATIENTS WITH GLIOBLASTOMA

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BACKGROUND: Glioblastoma, the most common adult primary malignant brain tumor, confers poor prognosis (median survival of 15 months) notwithstanding aggressive treatment. Combination chemotherapy including carmustine (BCNU) or temozolomide (TMZ) with the MGMT inhibitor O⁶-benzylguanine (O6BG) has been used, but has been associated with dose-limiting hematopoietic toxicity. **OBJECTIVE:** To assess safety and efficacy of a retroviral vector encoding the O6BG-resistant MGMP140K gene for transduction and autologous transplantation of hematopoietic stem cells (HSCs) in MGMT unmethylated, newly diagnosed glioblastoma patients in an attempt to chemoprotect bone marrow during combination O6BG/TMZ therapy. **METHODS:** Three patients have been enrolled in the first cohort. Patients underwent standard radiation therapy without TMZ followed by G-CSF mobilization, apheresis, and conditioning with 600 mg/m² BCNU prior to infusion of gene-modified cells. Posttransplant, patients were treated with 28-day cycles of single dose TMZ (472 mg/m²) with 48-hour intravenous O6BG (120 mg/m² bolus, then 30 mg/m²/d). **RESULTS:** The BCNU dose was nonmyeloablative with ANC <500/ μ L for \leq 3 d and nadir thrombocytopenia of 28,000/ μ L. Gene marking in pre-infusion colony forming units (CFUs) was 70.6%, 79.0%, and 74.0% in Patients 1, 2, and 3, respectively, by CFU-PCR. Following engraftment, gene marking in white blood cells and sorted granulocytes ranged between 0.37–0.84 and 0.33–0.83 provirus copies, respectively, by real-time PCR. Posttransplant gene marking in CFUs from CD34-selected cells ranged from 28.5% to 47.4%. Patients have received 4, 3, and 2 cycles of O6BG/TMZ, respectively, with evidence for selection of gene-modified cells. One patient has received a single dose-escalated cycle at 590 mg/m² TMZ. No additional extra-hematopoietic toxicity has been observed thus far and all three patients exhibit stable disease at 7–8 months since diagnosis. **CONCLUSIONS:** We believe that these data demonstrate the feasibility of achieving significant engraftment of MGMP140K-modified cells with a well-tolerated dose of BCNU. Further follow-up will determine whether this approach will allow for further dose escalation of TMZ and improved survival.

MOLECULAR MARKERS I

O.13. MOLECULAR MALIGNANT PROGRESSION IN GLIOMAS; ELUCIDATING THEIR GENETIC “LIFE STORY”

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Up till now, typing and grading of diffuse gliomas is based on histopathological features. However, because of, especially, lack of unequivocal criteria and sampling, the robustness of this classification is suboptimal, and more objective tools are needed for more reliable assessment of their biological behavior (eg, nearly all low-grade diffuse gliomas eventually progress to high-grade malignancy); however, time to progression varies considerably and there is currently no valid parameter that unambiguously predicts how rapidly malignant progression will occur. Over the last decades it has become increasingly clear that molecular genetic markers are helpful in recognizing more uniform subgroups of gliomas (eg, loss of chromosome 1p and 19q is reported to predict longer survival and better response to (chemo)therapy whereas methylation of the MGMT gene predicts chemosensitivity to alkylating agents). Furthermore, several genes were reported to be involved in malignant progression of gliomas; however, detailed information about their “timing” and cooccurrence in the course of molecular progression is relatively lacking. We therefore evaluated in a spectrum of over 300 diffuse gliomas the (co-)occurrence of copy number changes involving chromosomes 1p and 19q, CDKN2A, PTEN, and EGFR(vIII) as detected by Multiplex Ligation-dependent Probe Amplification (MLPA). Our results show that high malignancy grade is associated with particular copy number changes and the cooccurrence of these changes. Consequently, also in cases that are histopathologically still diagnosed as a low-grade glioma, such changes may indicate aggressive tumor behavior. Based on our findings we propose a scheme for the timing of the different events in the course of molecular progression, molecular malignancy being characterized by the cooccurrence of multiple changes and their exact

malignant character (hemi- < homozygous loss; low-level gain < (high-copy) amplification). Interestingly, different types of 1p/19q aberrations were identified, some of them being indicative for molecular malignancy (partial or isolated losses) which warrants an accurate identification, separating them from those indicative of a favorable biological behavior (codeletion of complete 1p and 19q). We are currently evaluating the clinical relevance of identification of molecular malignancy as proposed, and preliminary results indicate that this scheme will be helpful for establishing a risk-profile for individual glioma patients and thereby ultimately aid in therapeutic decision-making.

O.14. COMPARISON OF MS-PCR, METHYLIGHT, PYROSEQUENCING, MS-HRM, AND IMMUNOHISTOCHEMISTRY FOR MGMT ANALYSIS

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MGMT status is a predictive factor of response to standard treatment of newly diagnosed glioblastomas involving temozolomide (TMZ) and radiotherapy, and is used as a prognostic factor in anaplastic gliomas. Since it is becoming a crucial biological marker in new clinical glioma trials, and is beginning to be used in routine clinical practice, there is a strong need to determine the best technique for MGMT analysis. In a French multicenter study, we compared 5 techniques: classical MS-PCR, MethyLight, pyrosequencing (PYR), MS-HRM, and immunohistochemistry (IHC). Each technique was centrally performed at an expert center. To analyze the reproducibility of MGMT methylation assays, 3 primary cell lines (GB2/GB3 and GBM2) were tested in 10 separate series. GB2 and GB3 were never methylated (Meth) with either MethyLight or MS-HRM, but were Meth in 7 of 10 and 9 of 10 cases with MS-PCR, respectively, while mean methylation levels were 5% and 9% for PYR, with reproducibilities of 11% and 6%, respectively. GBM2 was always Meth with MS-HRM and MS-PCR, methylation levels being 42% and 77% for MethyLight and PYR, with reproducibilities of 72% and 7%, respectively. A good linearity was observed for each technique (after sequential mixing of 100% and 0% methylated samples) with detection of levels as low as 2.5%. For IHC, slides from two selected blocks were immunostained and analyzed in 6 different series. The number of positive cells ranged from 8% to 60% (mean 31%) in 1 case and from 3% to 20% (mean 8%) in the other. Following tests on 99 samples from patients with newly diagnosed GBM treated with standard treatment (radiation and TMZ), the best predictive values for overall survival were obtained by PYR ($P < .0001$ /cut off 9%), MS-PCR ($P < .0001$), and IHC ($P < .001$ /cut off 25%). MethyLight ($P = .09$) and MS-HRM ($P = .03$) yielded disappointing results in this series of patients. Contrary to recent studies, MGMT expression assessed by IHC was well correlated with overall survival. As observer variability is a reported problem with this technique, which could account for the poor reproducibility observed in this study, stained slides are currently evaluated by 2 other pathologists to validate the result. For MGMT promoter methylation analysis, PYR appears to be a very robust technique and a good alternative to classical MS-PCR, which is not well adapted to clinical practice. The next step of the project aims at implementing these techniques in different laboratories.

O.15. TRANSCRIPTIONAL INACTIVATION AND PROMOTER HYPERMETHYLATION OF THE TUMOR SUPPRESSOR GENE NDRG2 IN HIGH-GRADE OLIGODENDROGLIAL TUMORS

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BACKGROUND: The *NDRG2* gene is a member of the N-myc downstream-regulated gene family that is located on chromosome 14q11.2. It has been proposed that the *NDRG2* gene is a candidate tumor suppressor gene (TSG), which expression *in vitro* inhibits the proliferation of tumoral cells and promotes cell differentiation. Consistent with its potential function as a TSG, downregulation

of *NDRG2* expression is found in several human cancer cell lines and tissues, including meningiomas and glioblastomas (GBs). AIM: A gene expression profiling analysis to identify differentially expressed genes between high- and low-grade oligodendrogliomas (OGs) revealed that *NDRG2* was consistently down-regulated in high-grade OGs. Therefore, to analyze the potential role of *NDRG2* as a TSG in gliomas, we performed mRNA expression and promoter hypermethylation of *NDRG2* in a series of 78 primary glioma tumors. MATERIALS AND METHODS: The human glioma samples consisted of 19 GBs (WHO grade IV) and 59 oligodendroglial tumors (OTs), including 19 WHO grade II oligodendrogliomas (OGs), 16 WHO grade III OGs, 11 WHO grade II mixed oligoastrocytomas (OAs), and 13 WHO grade III OAs. mRNA expression levels were measured by quantitative real-time reverse transcription polymerase chain reaction (PCR) analysis. Promoter hypermethylation was determined by sodium bisulfite-modified treated DNA followed by methylation-specific PCR. RESULTS: Low mRNA expression levels relative to non-tumoral brain tissue were detected in 50% (5 of 10) of high grade OTs, and 92.3% (12 of 13) of GBs. In contrast, only 7.1% (1 of 14) of the low grade OTs showed *NDRG2* reduced mRNA expression levels. Promoter hypermethylation was detected in 38.5% (10 of 26) of high-grade OTs, as well as in 58.8% (10 of 17) of GBs, while none of the low-grade OTs showed *NDRG2* promoter hypermethylation. Likewise, there was a significant correlation between the low RNA expression levels and/or the promoter hypermethylation of *NDRG2* and high-grade OTs ($\rho = 0.459$; $P < .01$). CONCLUSION: Taken together, our results suggest inactivation of *NDRG2* in high-grade OTs through hypermethylation of its promoter region, which points out its role as a TSG. In addition, *NDRG2* inactivation may be a useful biomarker to predict a more aggressive behavior in OTs.

O.16. A FOUR-GENE SIGNATURE ASSOCIATED WITH CLINICAL OUTCOME IN HIGH-GRADE GLIOMAS

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Molecular studies of high-grade gliomas (HGGs) have highlighted the heterogeneity of these tumors, and have linked molecular signatures to their natural history and to differences in survival rates. The ability to identify such molecular subtypes of tumors is essential for guiding therapeutic advances. We report the development and validation of a robust risk-score model highly associated with the outcome of patients with newly diagnosed HGG. We considered a supervised approach to account for the WHO grade of malignancy when deriving gene biomarkers associated with outcome. We performed a meta-analysis of HGGs microarray data sets (267 patients) to identify such biomarkers from a robust signature related to tumor aggressiveness. These biomarkers were used to construct a risk-score equation based on a Cox proportional hazards model. The model best associated with overall survival (OS) and with good discrimination (C-statistic) was based on the expression of 4 genes. Patients were ranked according to their risk score and stratified into 2 groups. Low-risk score patients had a median OS longer than high-risk score patients (46.6 vs 11.7 months, $P < .001$). These results were validated on an independent microarray study of 59 patients. We performed RT-qPCR validation on an independent set of HGGs (194 patients) and compared the performances of our risk-score model with the prognostic value of currently admitted clinical and molecular risk factors. Two multivariate models were built, including age, treatment, grade, RTOG RPA classes, *MGMT* methylation status, and *IDH1* mutational status; one with and one without the 4-gene expression risk score. These models were used to estimate the prognostic value of the gene expression risk score for 176 patients with complete data for all variables and for a subset of 105 patients treated with temozolomide chemoradiation. This analysis confirmed that both the mutations of *IDH1* and the presence of *MGMT* promoter methylation were associated with a survival benefit ($P < .01$ in the whole cohort and $P < .05$ in the subset). It also showed that the 4-gene risk score was strongly associated with OS in these two groups, independently from clinical and molecular risk factors ($P < .01$). Each time, the model discrimination improved significantly with the addition of the 4-gene risk score (0.816 vs 0.846, $P < .001$ and 0.792 vs 0.822, $P < .001$, respectively), showing that it added beyond standard clinical parameters and beyond both the *MGMT* methylation status and the *IDH1* mutational status.

One explanation for the association between the 4-gene signature and clinical outcome is that it may detect the molecular fingerprints inherent to glioma aggressiveness. These results suggest the importance of this 4-gene signature as a stratification factor for future comparative therapeutic trials, though it needs to be further investigated in a prospective clinical study.

O.17. EXPRESSION OF TELOMERASE REVERSE TRANSCRIPTASE (hTERT) IN HUMAN GLIOBLASTOMA SPECIMEN IS ASSOCIATED WITH SHORTER PATIENT SURVIVAL AND IS A PREREQUISITE FOR IN VITRO IMMORTALIZATION

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hTERT, the catalytic subunit of human telomerase, contributes to cancer cell immortalization by telomere stabilization. The aim of this study was to characterize hTERT expression in gliomas and the respective primo-cell cultures with a focus on glioblastomas (GBMs) and to investigate its relation with disease progression *in vivo* and tumor cell immortalization *in vitro*.

Since 2001 primary cell cultures have been established from 272 tumor tissues histologically verified according to WHO criteria as low-grade glioma WHO I/II ($n = 23$) and anaplastic astrocytoma WHO III ($n = 14$), GBM WHO IV ($n = 226$), gliosarcoma ($n = 7$) and giant cell glioblastoma ($n = 2$). hTERT mRNA expression was investigated in all primary cell cultures and additionally in GBM tumor tissues ($n = 96$) by RT-PCR and calculated relatively to GAPDH mRNA. Data were verified in subgroups by real-time RT-PCR. Telomerase enzyme activity was assessed using the Telomeric Repeat Amplification Protocol (TRAP) of the TRAPeze Telomerase Detection Kit (Chemicon). hTERT expression levels were compared with overall survival of GBM patients using SPSS software. Twenty-nine percent (79 of 272) of the analyzed cell cultures displayed hTERT gene expression. Out of these the vast majority (87%) of primo-cultures, representing exclusively high-grade gliomas (WHO III/IV), developed into stable, immortal cell lines whereas hTERT-negative cells failed to grow *in vitro* or ceased growth between passages 1 and 10. In contrast, all low-grade gliomas (WHO I/II; $n = 23$) were negative with respect to both hTERT expression and the ability for extended *in vitro* cultivation. In parallel to primo-cell cultures, hTERT expression was analyzed in 96 GBM tumor samples. Forty-seven of 96 (49%) showed detectable hTERT expression. Kaplan-Meier survival estimates revealed a borderline significant survival benefit for patients whose tumors lacked hTERT expression with a median survival of 20.1 vs 14.3 months for patients whose tumors expressed hTERT. All long-term survivors ($n = 13$; >40 months) were low/negative in telomerase. Summing up our data show that telomerase activity is essential for successful *in vitro* propagation of glioma cell models implicating that *in vitro* studies on GBM generally focus exclusively on the hTERT-positive patient subgroup. Lack of hTERT expression in the tumor tissue is associated with longer overall survival in glioma patients, indicating that hTERT might have quality as prognostic biomarker predicting tumor aggressiveness.

O.18. IDH1 MUTATIONS IN GLIOMAS: CORRELATION WITH GENOMIC PROFILE AND PROGNOSIS

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Recently, *IDH1* codon 132 mutations (mostly Arg132His) have been found in gliomas, resulting in the loss of normal isocitrate dehydrogenase activity and the acquisition of an alpha-ketoglutarate reductase activity. Rarely mutations can also affect the mitochondrial isoform *IDH2*. Using direct sequencing and new PCR approaches such as COLD PCR (coamplification at lower denaturation temperature-PCR) combined with high-resolution melting (HRM), we investigated the mutational status of *IDH1* and *IDH2* gene in 2272 tumors of central nervous system including 1821 gliomas (1238 when considering initial surgery), and correlated *IDH1/IDH2* status with histology, genomic profile, *MGMT* status, and outcome. We identified 831 *IDH1* mutations (mutation rate 36.6%) and only 35 *IDH2* mutations (mutation rate 2.5%). In grade II-IV gliomas, *IDH1/2* mutations were inversely correlated with grade, affecting 234 of 347 (67%) grade II, 165 of 341 (48%) grade III, and 45 of 550 (8%) grade IV gliomas. The *IDH1* mutation was tightly related to the 1p19q codeleted group and *MGMT* methylation, but mutually exclusive with *EGFR* amplification (2.5% *IDH1* mutated 4 of 177). Strikingly, all the 1p19q codeleted gliomas were mutated on *IDH1* (92%) or *IDH2* (8%). *IDH1* mutation was associated with a better outcome in grade II (143.0 vs 92.0 months, $P = .002$),

grade III (109.0 vs 20.0 months, $P < .0001$) and grade IV gliomas (27.0 vs 14.0 months, $P = .0002$). After adjustment for grade, age, MGMT status, genomic profile, and treatment, multivariate analysis confirmed that *IDH1* mutation is a favorable prognostic marker in the entire population of gliomas (HR = 0.6; 0.42–0.84). Then, combining innovative PCR approaches and biochemical dosages, we were able to determine the IDH status in the biological fluids of a subset of these patients. In order to determine whether *IDH1* status may predict response to treatment, we are now investigating the response to radio- and chemotherapy of glioma cells expressing mutated vs wild-type *IDH1*. Updated results will be presented.

BRAIN AND LEPTOMENINGEAL METASTASIS

O.19. PROSPECTIVE MULTICENTRIC VALIDATION OF A SURVIVAL PROGNOSTIC INDEX IN LEPTOMENINGEAL CARCINOMATOSIS: ANALYSIS INTERIM OF THE ACME STUDY

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INTRODUCTION: Leptomeningeal carcinomatosis (LC) represents a devastating complication of systemic cancer. Although median survival is short despite therapy, up to 10–20% of patients can achieve 1-year survival with treatment. To decide which patients should be treated is difficult. To help in the decision of which patients are best candidates to be treated, a survival prognostic index (PI) was recently proposed. The ACME study is conducted to validate this PI. The aim of this report was to present the preliminary results. **PATIENTS AND METHODS:** The enrollment was initiated on June 2008 and recruitment period will finish on June 2010, participating 29 Spanish centers. LC diagnosis is based on the presence of positive cytology on cerebrospinal fluid (CSF) or CSF biochemical abnormalities associated with suggestive clinical and magnetic resonance. Clinical data, CSF parameters, tumor-related characteristics, and treatment information are recorded. PI is composed by 3 prognostic variables: Radiation Therapy Oncology Group (RTOG) neurological scale ≤ 2 , glucose level in CSF ≥ 2.7 mmol/L, and presence of infratentorial symptoms. Each variable is scored as 0 or 1, according to the absence or presence. Group of unfavorable PI includes patients who score < 2 points. The impact of single parameters on overall survival is determined by both univariate and multivariate analysis. **RESULTS:** Up to now, 73 patients are enrolled. Data from the first 46 patients included with complete follow-up was analyzed. Thirty-four patients received intrathecal with or without systemic chemotherapy. Univariate analysis identified female sex, breast cancer, Karnofsky Performance Status, negative CSF cytology, PI, and treatment (intrathecal with/without systemic chemotherapy) having a prognostic influence on survival. However, multivariate analysis revealed that breast cancer (HR: 3.03; 95% CI: 1.18–7.69, $P = .021$), negative CSF cytology (HR: 3.85; 95% CI: 1.33–11.11, $P = .012$), treatment (HR: 7.14; 95% CI: 2.5–20, $P < .001$), and PI (HR: 2.77; 95% CI: 1.1–7.14, $P = .031$) were associated independently with longer overall survival in LC patients. **CONCLUSION:** Preliminary results confirm PI as useful prognostic score in LC patients. Moreover, breast cancer and a negative cytology on CSF also emerge as independent good prognostic factors.

O.20. NEOPLASTIC MENINGITIS: VALUE OF MRI AND PROTEIN ANALYSIS AND PATTERNS OF LYMPHOMATOUS CYTOMORPHOLOGY

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INTRODUCTION: Neoplastic meningitis (NM) from lymphoma or leukemia, although a well-known and relatively frequent complication of aggressive lymphoma and leukemia, is still difficult to detect. With cytomorphology, one key diagnostic procedure, neoplastic lymphocytes are difficult to distinguish from inflammatory lymphocytes. We evaluated here whether specific morphological criteria can improve this differentiation. Moreover, we assessed the sensitivity of MRI and protein analysis for

the detection of all kinds of NM in comparison with CSF cytology. **PATIENTS AND METHODS:** To establish cytomorphological criteria, 42 cytospin preparations of CSF from patients with confirmed CSF involvement by aggressive lymphoma or acute leukemia were compared with 26 samples of inflammatory diseases. CSF cytology was analyzed morphologically for preselected parameters of cell, cytoplasm, and nucleic appearance and the presence of mitoses or apoptoses. For the comparison of cytology and MRI, 38 patients with leukemic or lymphomatous meningitis were evaluated retrospectively for MRI signs of neoplastic meningitis and for CSF protein abnormalities (total protein, oligoclonal bands, lactate, and ferritin). **RESULTS:** As expected, none of the cytomorphological parameters sharply discerns neoplastic and inflammatory changes. However, neoplastic cells were significantly larger than inflammatory lymphocytes with a mean of 3.0 as opposed to 1.8 times the size of normal small lymphocytes ($P = .0001$). Moreover, irregular shape, pointed borders of the cytoplasm, and deep notches in the nucleus were significantly more often found with neoplastic than with inflammatory lymphocytes. The total cell count was elevated in 68% of cases of lymphomatous meningitis. While cytomorphology was comparable with MRI in solid neoplasms, it could also achieve approximately 90% sensitivity for the detection of NM. In hematological neoplasms, spinal and/or cranial MRI detected only 71% of cases with normal and 52% with elevated cell counts. Total protein was elevated in 77% of cases, lactate in 55%, and ferritin in 48%. Oligoclonal IgG was found in 11% isolated in the CSF and in 18% in CSF and serum identically. In approximately 95% of all cases of NM, at least one of the analyzed laboratory tests was pathological. **CONCLUSIONS:** CSF cytology is more sensitive than MRI for the detection of NM from hematological and comparable in solid neoplasms, but application of both methods clearly enhances the sensitivity by at least 10%. No single cytomorphological pattern is sufficient to detect neoplastic lymphocytes. Considering a combination of cell size and irregular shape of cell and nucleus may improve the diagnostic accuracy of CSF dissemination by aggressive hematological malignancies.

O.21. CASE SERIES OF FRAMELESS LINEAR ACCELERATOR-BASED STEREOTACTIC RADIOSURGERY TO THE POSTOPERATIVE RESECTION CAVITY FOR BRAIN METASTASIS: AN INITIAL REPORT OF OUTCOMES AND PATTERNS OF FAILURE

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BACKGROUND: Whole-brain radiotherapy (WBRT) is the standard of care following resection of a brain metastasis. Lack of a survival benefit and concern regarding possible neurocognitive effects with this approach has led to stereotactic radiosurgery to the resection cavity being investigated as an alternative treatment strategy. Radiosurgery is likely to offer effective local control while preserving WBRT for use as a salvage therapy for distant brain failures. We report our initial experience using a linac-based frameless SRS system. **METHODS:** We retrospectively reviewed the treatment outcomes, patterns of failure and the image-guided setup accuracy of the first 15 consecutive cases treated at Brigham and Women's Hospital using image-guided (ExacTrac by Brainlab) linear accelerator-based radiosurgery with a relocatable stereotactic frame and aquaplast mask (BrainLAB Mask System). The target volume was the resection cavity without a margin, excluding the surgical track. Median number of brain metastasis per patient was 1 (range 1–3). Median planning target volume was 3.3 cm² (range 0.53–10.8 cm²). Median prescribed dose was 18 Gy with normalization ranging from 69 to 81%. Ninety-nine percent of the PTV was covered by the prescribed dose in all cases. Mean conformity index was 1.62 (range 1.41–1.92). **RESULTS:** At a median follow-up of 8.2 months (interquartile range 7.3–12.1 months), local control was achieved in 13 of 14 cases. True local recurrence occurred in 1 case. No marginal failures occurred. Distant brain failure occurred in 5 of 14 cases. Median time to any failure was 7.5 months. Salvage therapy was administered to 5 patients (3 received WBRT, 1 received further radiosurgery, and 1 received systemic therapy only). No grade 3 or higher toxicity was recorded. Factors associated with lack of CNS failure (local or distant) included a single brain metastasis and long interval between initial cancer diagnosis and the development of brain metastasis. The frameless image-guided radiosurgery was delivered with submillimeter accuracy. The mean residual setup error was 0.45 mm (SD = 0.19 mm) and the mean intrafraction motion was 0.57 mm (SD = 0.31 mm). **CONCLUSIONS:** Image-guided frameless stereotactic radiosurgery to the resection cavity following surgery for a brain metastasis is a safe and accurate technique that offers durable local control and postpones the use of WBRT in selected patients. This technique should be tested in larger prospective studies.

O.22. TREATMENT OF BRAIN METASTASES FROM NON-SMALL CELL LUNG CANCER WITH WHOLE-BRAIN RADIOTHERAPY (WBRT) IN COMBINATION WITH GEFITINIB (GFT) OR TEMOZOLOMIDE (TMZ): A RANDOMIZED MULTICENTER PHASE II TRIAL OF THE SWISS GROUP OF CLINICAL CANCER RESEARCH (SAKK #70/03)

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BACKGROUND: Patients with BM rarely survive >6 months and are commonly excluded from clinical trials. We aimed at improving outcome by exploring 2 combined modality regimens with at the time novel agents for which single-agent activity had been shown. **METHODS:** NSCLC patients with multiple BM were randomized to WBRT (10 × 3 Gy) and either GFT 250 mg p.o. daily or TMZ 75 mg/m² p.o. daily ×21/28 days, starting on Day 1 of RT and to be continued until PD. Primary endpoint was overall survival, a Simon's optimal 2-stage design was based on assumptions for the 3-month survival rate. Cognitive functioning and quality of life were also evaluated. **RESULTS:** Fifty-nine patients (36 M, 23 F; 9 after prior chemo) were included. Median age was 61 years (range 46–82), WHO PS was 0 in 18 patients, 1 in 31 patients, and 2 in 10 patients. All but 1 patients had extracranial disease; 33 of 43 (TMZ) and 15 of 16 (GFT) had adenocarcinoma histology. GFT arm was closed early after stage 1 analysis when the prespecified 3-mo survival rate threshold (66%) was not reached, causes of death were not GFT related. Main causes of death were PD in the CNS 24%, systemic 41%, both 8%, and toxicity 10% [intestinal perforation (2 patients), pneumonia (2), pulmonary emboli (1), pneumonitis NOS (1), seizure (1)]. We summarize here other patients' characteristics for the 2 trial arms: TMZ (*n* = 43)/GFT (*n* = 16); median treatment duration: 1.6/1.8 mo; Grade 3–4 toxicity: lymphopenia 5 patients (12%)/0; fatigue 8 patients (19%)/2 patients (13%). Survival data for TMZ/GFT arms: 3-month survival rate: 58.1% (95% CI 42.1–73)/62.5% (95% CI 35–85); median OS: 4.9 months (95% CI 2.5–5.6)/6.3 months (95% CI 2.2–14.6); median PFS: 1.8 months (95% CI 1.5–1.8)/1.8 (95% CI 1.1–3.9); median time to neurop. progr.: 8.0 months (95% CI 2.2–X)/4.8 (95% CI 3.9–10.5). In a model to predict survival time including the variables' age, PS, number of BM, global QL, total MMSE score, and subjective cognitive function, none of the variables accounted for a significant improvement in survival time. **CONCLUSIONS:** The combinations of WBRT with GFT or TMZ were feasible. However, in this unselected patient population, survival remains poor and a high rate of complication was observed. Four patients died as a result of high-dose corticosteroids. Preliminary evaluation of cognitive function and QL failed to show significant improvement. Indications and patient selection for palliative treatment should be revisited and careful monitoring and supportive care is required. Research and progress for this frequent clinical situation is urgently needed.

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O.23. FREQUENCY, PATTERNS OF CARE, AND OUTCOME OF NEOPLASTIC MENINGITIS (NM) FROM SOLID TUMORS IN THE REGIONE PIEMONTE, ITALY: A PROSPECTIVE SURVEY FROM A CANCER NETWORK

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BACKGROUND: Neoplastic meningitis (NM) is a devastating neurologic complication of cancer whose frequency and patterns of care are not well known. We investigated in a prospective survey, the frequency, patterns of care, and outcome of NM from solid tumors in a Community Hospital-based regional cancer network. **METHODS:** Clinical forms to collect information on history, neurological symptoms/signs, MRI patterns, CSF cytology, treatment options, and outcome were sent to 29 neurologic and 42 medical oncology Services of the Regione Piemonte (Italy). Data were centrally reviewed in a University Hospital to confirm the diagnosis and make the final analysis. **RESULTS:** From January 2008 to December 2008, we enrolled 68 patients with suspected NM. Diagnosis was confirmed in 59 patients (87%). Diagnosis was pathologically confirmed in 27 of 59 (46%) patients while was clinico-radiological in 32 of 59 (54%). There were 39 females and 20 males with a median age of 59 years (range 38–80). The site of primary tumor was: breast in 25 of 59 (42%), lung in 18

of 59 (31%), unknown in 5 of 59 (8%), gastrointestinal tract in 4 of 59 (7%), skin (melanoma) in 3 of 59 (5%), miscellaneous in 4 of 59 (7%) patients. The systemic disease at the time of diagnosis of NM was progressive in 55 of 59 (93%) and absent/under control in 4 of 59 (7%) patients. Brain metastases were concomitant in 26 of 55 (47%) patients. The median latency between first symptom and NM diagnosis was 4 weeks (range: 0–26 weeks). Treatment for NM consisted in intrathecal chemotherapy with liposomal cytarabine (14 of 59 patients), radiotherapy (WBRT or focal RT on bulky disease; 12 of 59 patients), RT + intrathecal chemotherapy (2 of 59), surgical removal of spinal bulky disease (1 in 59), whereas 30 in 59 patients (51%) underwent supportive care only. Median survival was 6.8 weeks. In a multivariate analysis, the only parameter that influenced the prognosis was Karnofsky >60 (*P* < .0042). **CONCLUSIONS:** This is the first Community Hospital-based regional study and highlights that the prognosis is poor compared with specialized University Hospitals and that half of the patients are candidates only to aggressive therapy.

O.24. STEM CELL TRANSPLANTATION FOR CNS RECURRENCE OF SYSTEMIC NHL: AN INTERNATIONAL PRIMARY CNS LYMPHOMA GROUP (IPCG) PROJECT

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BACKGROUND: Prognosis is poor in patients with relapsed lymphoma with central nervous system (CNS) localization. In chemosensitive-relapsed systemic lymphoma without CNS localization, autologous stem cell transplantation (ASCT) is the treatment of choice and is able to increase the long-term survival rate, especially when combined with rituximab. Small retrospective series of transplanted patients have shown that this treatment is feasible in selected cases with CNS recurrence, but no prospective data are available. Given the rarity of the disease, an international collaboration within the IPCG was formed to obtain data on patients from a variety of countries. **METHODS:** From affiliated and interested centers performing ASCT, all patients with a CNS localization of systemic lymphoma at first recurrence or progression potentially eligible for ASCT were selected from local databases. Anonymized data were collected on primary disease, recurrence or progression, treatment of recurrence or progression, result and toxicity of this treatment, and survival. **RESULTS:** From 6 centers in 5 countries, 72 patients were identified. Initial treatment varied but contained intrathecal treatment or prophylaxis in 13 patients, and systemic rituximab in 32. Initial symptoms of the relapse were of CNS disease in 50 patients, of systemic disease in 7, and of both in 14. Within the CNS, 44% of patients had a brain parenchyma lesion only, 36% had a leptomeningeal localization with or without a parenchymal lesion. Patients initially treated with rituximab had an increased risk of CNS parenchymal relapse: 74% compared with 44% in patients who were rituximab naive (*P* = .014, χ^2 test). Treatment of the recurrence was not uniform, but 93% of patients was treated with HD-MTX or HD-cytarabine containing regimens. Twenty-four patients were not eligible for transplantation because of age, prior transplantation, or unknown reasons. Of the remaining 48 patients, 17 (35%) received ASCT. Median survival from the time of CNS relapse in all patients was 8 months, and that in transplanted patients >49 months. Survival at 1 year after transplantation was 81%. **CONCLUSIONS:** Significantly more patients initially treated with rituximab had a CNS parenchymal lesion rather than leptomeningeal localization only. Only 35% of patients potentially eligible for transplantation was transplanted; those reaching transplantation had favorable survival following transplantation.

CELL BIOLOGY/IMMUNOTHERAPY

O.25. BONE MARROW-DERIVED CELLS DYNAMICALLY INTERACT WITH GLIOMA CELLS DURING TUMOR INVASION AND ANGIOGENESIS

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Hematopoietic progenitor cells (HPCs), but also mature blood cells, are increasingly investigated regarding their role for tumor angiogenesis, with

conflicting results for brain tumors. Moreover, their role for brain tumor invasion is not defined. We therefore aimed to investigate the kinetics of recruitment, the spatiotemporal interaction with tumor vessels and tumor cells, and the migratory pattern of intravenously applied cells from the bone marrow of β -actin eGFP transgenic mice: (a) lineage depleted hematopoietic precursor cells (lineage negative = CD5⁻, CD11b⁻, CD19⁻, CD45R⁻, Ly-6G⁻, TER119⁻); (b) hematopoietic stem cells (lin⁻, Sca-1⁺, cKIT/CD117⁺), and (c) mature macrophages (lin⁺, CD115⁺). To achieve this aim, multiphoton laser scanning microscopy (MPLSM) in combination with a chronic cranial window was used to image both red-fluorescent U87 glioma cells and eGFP-expressing cell populations (a–c) within the glioma micromilieu. Blood vessels were highlighted by yellow fluorescence.

After i.v. injection, all three cell populations showed a specific homing into the glioma, with a characteristic kinetic for each of them, ranging from immediate (maximum day 1) to late (maximum day 7) homing. The bone marrow-derived cell lines displayed distinct morphological features after extravasation into the glioma. Incorporation into glioma vessels happened only occasionally and in a pericyte-like position; however, bone marrow-derived cells showed the ability to proliferate over time and become part of the vascular wall. Interestingly, a very small subset of U87 glioma cells migrated throughout the tumor. Tumor cell migration exclusively occurred in close vicinity to bone marrow-derived cells, suggesting a potential role for tumor invasion. In conclusion, our study provides the first *in vivo* investigation of dynamic interactions of brain tumor and hematopoietic cells. We could identify specific actions that support a role of hematopoietic cells in glioma progression.

O.26. NOTCH PATHWAY BLOCKADE AFFECTS THE MIGRATORY AND DIFFERENTIATING CAPACITY OF BRAIN TUMOR INITIATING CELLS

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BACKGROUND: The high-grade glioma, glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults, and confers a grave prognosis. Effective treatment remains a significant therapeutic challenge. The majority of GBM's is often difficult to operate, because of their location and infiltrative growth pattern, and nonsurgical treatments (chemo- and radiation therapy) are often ineffective. As such, relapse is almost certain and new treatment modalities are urgently needed. Brain tumor initiating cells (bTICs) are a population of neural stem cell (NSC)-like cancer cells reported in GBM. bTICs are increasingly being assigned a central role in brain tumor initiation, progression, treatment resistance, and relapse and are suggested as a novel target in glioma therapy. The Notch signaling pathway is important in maintaining an undifferentiated pool of normal NSC and in determination of cell fate. Components of the Notch pathway are often found aberrantly expressed in GBM and recent results demonstrate that active Notch signaling is important for the maintenance and growth of GBM-derived bTICs. Thus, the Notch signaling pathway might be an appropriate target for GBM therapy targeting bTICs. **AIM:** We investigated the functional role of Notch signaling in bTICs by examining the effect of Notch inhibition on tumorigenicity and stem cell-like properties. **RESULTS:** Primary neurosphere cultures were established from xenografts originally derived from human primary GBM. All cultures were enriched in cells with NSC-like characteristics and the majority of cultures, moreover, exhibited high Notch expression and activation. Notch inhibition by the γ -secretase inhibitor DAPT led to reduced primary neurosphere formation. Established GBM neurosphere cultures treated with DAPT, furthermore, displayed reduced expression of the NSC marker Nestin and increased expression of markers of the 3 neural lineages, suggesting increased differentiation. When neurosphere cells were induced to differentiate during DAPT treatment, they showed an altered differentiation pattern, in accordance with the established role of Notch during cell fate decisions. Finally, the Notch signaling pathway was demonstrated to play a role in the *in vitro* tumorigenic potential of the GBM neurosphere cultures, as displayed by inhibition of cell migration, in a modified Boyden chamber, upon Notch blockade. The overall effect of DAPT treatment was more pronounced in cultures exhibiting high Notch expression and activation, compared with cultures with low Notch expression and activation. **CONCLUSION:** On the basis of these results, we suggest that Notch signaling contributes to the stem cell-like character and tumorigenic potential of bTICs, when these display dysregulated Notch pathway activation, and that it might be possible to target bTICs in human GBM through the Notch signaling pathway.

O.27. NG2 PROMOTES RESISTANCE TO IONIZING RADIATION BY ELEVATED PEROXIREDOXIN-1 AND DNA DAMAGE RESPONSE IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBMs) are lethal cancers that respond poorly to radiotherapy and the mechanisms may involve stem/progenitor cells. Several studies proclaimed that brain tumors enriched in CSCs were preferentially resistant to ionizing radiation and chemotherapy as a result of altered checkpoint and DNA repair pathways compared with conventional tumor cells. Others have claimed that these cells are associated with increased reactive oxygen species and that this is an additional mechanism for radiation resistance. Since the glial progenitor marker NG2 has been shown to regulate tumor response to chemotherapy, we examined whether it also affected response to radiotherapy. Quantification of NG2 expression in 96 patient GBM biopsies revealed that high expressers had shorter survival outcomes than low expressers, $P = .02$. Two-dimensional (2D) proteomics of 11 of these biopsies showed that peroxiredoxin-1 (PRDX-1) was upregulated in the shortest surviving patients, and was associated with reduced oxidative damage. Furthermore, NG2 expressing GBMs were highly resistant to ionizing radiation (IR) *in vitro* and *in vivo* and increased PRDX-1 levels in a dose-dependent manner. shRNA-mediated NG2 knockdown sensitized the tumor cells to IR and attenuated dose-dependent induction of PRDX-1. Moreover, NG2 expressing cells rapidly induced DNA damage response signaling as indicated by phosphorylation of H2AX, ATM, and Chk2 proteins compared with NG2-negative cells. PRDX-1 knockdown transiently slowed tumor growth rates *in vivo* and partially sensitized the tumors to ionizing radiation *in vitro*. These data demonstrate a novel role for NG2 in mediating radioresistance in human GBMs by interaction with PRDX-1 and DNA damage response machinery.

O.28. CD44 LOSS OF FUNCTION IMPEDES GLIOMA PROGRESSION IN A SPONTANEOUS MURINE MODEL

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CD44 is a transmembrane receptor for hyaluronan that coordinates intracellular signaling and cytoskeleton rearrangements in response to cues from the extracellular matrix. As brain tumors develop in a hyaluronan-rich environment, overexpression of CD44 can lead to the enhancement of proliferation, migration, and survival facilitated by CD44. We have developed a murine model of gliomas that is uniquely suited for CD44 loss of function studies. Malignant gliomas were induced in mice by transfecting plasmids encoding SV40LgT and NRasG12V into the lateral ventricle of wild-type (CD44^{+/+}) and knockout (CD44^{-/-}) mice. Tumor progression was monitored weekly using bioluminescent imaging and directly correlated with tumor burden. Grade 3–4 gliomas developed in CD44^{+/+} mice within 1 month of oncogene delivery. These tumors advanced rapidly as assessed by steadily increasing bioluminescent imaging and a median survival of 39 days. Two-color immunohistochemistry (IHC) was developed against CD44 and SV40LgT to detect CD44 expression within the bulk tumor and the infiltrative glioma cells. IHC studies have shown remarkably similar phenotypes of CD44 overexpression in both mouse and human tumor specimens. In addition, CD44-positive tumor cells can be found infiltrating into the perivascular space in the normal brain of tumor bearing mice. In contrast to CD44^{+/+} rapid tumor growth, CD44^{-/-} tumors have a significant delay in progression (median survival = 50 days). Importantly, a subset of tumors in CD44^{-/-} mice spontaneously regress as measured by bioimaging. CD44 loss of function was rescued by expressing murine CD44 cDNA in *cis* on the NRasG12V plasmid. The significant extension of survival in CD44^{-/-} mice is abolished when CD44 expression is rescued exclusively in the tumor cells. Therefore, glioma cells require CD44, rather than the brain microenvironment, to facilitate tumor initiation and progression. Our results demonstrate that loss of CD44 impedes the development of malignant gliomas. Furthermore, the spontaneous regression of CD44^{-/-} tumors suggests that CD44 may be crucial to maintaining a niche supportive of tumor cell self-renewal and survival. Ongoing studies will look at CD44 modulation of multidrug transporter activity and sensitivity to chemotherapeutic agents because of loss of function of CD44.

O.29. THERAPEUTIC TARGETING OF THE NG2 PROTEGLYCAN WITH MAB 9.2.27 AND ADOPTIVELY TRANSFERRED NK CELLS Lyses HUMAN GLIOBLASTOMA MULTIFORME IN VIVO

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Glioblastoma multiforme (GBM) is a lethal subgroup of intracranial tumors with a median survival of 14.6 months, despite multimodal treatment. We have previously shown that several treatment resistant tumors aberrantly express the neural progenitor marker NG2. We therefore aimed to target NG2 in GBMs using the 9.2.27 mAb and adoptively transferred autologous natural killer (NK) cells and to determine the mechanisms of anti-tumor effect. The NK cells and mAb were infused intratumorally by convection-enhanced delivery (CED) in rat brains transplanted with human GBM xenografts, as well as syngeneic rat gliosarcoma. Magnetic resonance imaging was used to longitudinally monitor the tumor growth characteristics. Combined NK + mAb targeting resulted in significantly longer survival times compared with the vehicle, and monotherapy controls (U251-NG2: log-rank test, $P = .0081$); (U87: log-rank test, $P = .0003$). Histological analyses revealed strong presence of MPO, granzyme, and IFN γ -expressing granulocytic cells in focal areas of strong necrosis/apoptosis in the NK + mAb- and mAb-treated groups. Moreover, double-labeling revealed the greatest numbers of M1-type macrophages that were ED1/CD8 positive that penetrated deep into the tumor of the NK + mAb-treated animals. Flow cytometric analysis revealed less M2 phenotype macrophages in this group compared with the monotherapy controls. Animals treated with NK cells recruited only uniformly double ED1/CD8-positive cells that were less abundant and remained at the tumor brain boundary. MRI revealed tumor growth arrest in many NK + mAb-treated animals, whereas mAb-treated tumors displayed reduced contrast enhancement and necrosis. In conclusion, combined 9.2.27 mAb and NK cell therapy strongly affected tumor growth and vascular parameters and prolonged survival. Thus, adoptive cell therapy with NK cells may be an amenable therapy for treatment-resistant GBMs.

O.30. PHASE III ANTI-EGF-RECEPTOR ANTIBODY (OSAG-101) FOR NEWLY DIAGNOSED GLIOBLASTOMA: SAFETY AND CURRENT STATUS

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The epidermal growth factor receptor, EGF-R, is considered a highly relevant therapeutic target for glioblastoma resulting in a wide spectrum of approaches directed against the intercellular signaling pathway, the ligand-binding capacity of the receptor or the specific immunogenicity of the vIII splice variant. Because of promising preclinical and early clinical findings, the evaluation of the therapeutic effect of a monoclonal antibody against the EGF-R (nimotuzumab) which has a lower affinity than cetuximab, thus binding more specifically to highly overexpressing cells was undertaken in a phase III design. Nimotuzumab (OSAG-101) was tested in an open-label, randomized, multicenter phase III trial in patients with newly diagnosed glioblastoma. OSAG-101 is administered by i.v. infusion (weekly infusion of 400 mg) in addition to the current standard therapy with concomitant radiochemotherapy using temozolamide followed by biweekly infusions of 400 mg temozolamide thereafter. Nimotuzumab administration in this trial was to continue until progression. Patients with histologically confirmed glioblastoma were included without specification of resection status. Patients under the age of 18 and over 70 years were excluded. Primary endpoint was time to progression as determined by centralized review of standardized MRI and a prespecified evaluation protocol. Overall survival was chosen as a secondary endpoint with quality of life and safety as additional parameters. Between August 2008 and March 2010, 145 patients were enrolled in 10 sites with 165 subjects. All except one patient were GBM on central histopathological review. Just <50% of the patients had a gross total resection with no residual contrast enhancement whereas the larger group had partial resections with residual contrast enhancement, including patients with biopsy only. The observed adverse reaction pattern was the same in both study arms and both strata and reflecting the event pattern of the disease and its standard treatment. Specifically, no rash, conjunctivitis, or mucositis as known for anti-EGF-R reagents were reported. We conclude from the trial so far that the intravenous administration of OSAG-101 for newly diagnosed glioblastoma is safe and free of

additional toxicity to the standard radiochemotherapy regimen. Seventy-five patients have reached their primary endpoint at this point and an interim analysis is currently conducted to provide first indications for efficacy. Accrual is expected to be complete by the end of March 2010.

MENINGEOMA AND PEDIATRIC BRAIN TUMORS

O.31. THE EFFECT OF EDEMA ON HEALTH-RELATED QUALITY OF LIFE IN WHO GRADE I MENINGIOMA PATIENTS

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BACKGROUND: Studies on the associations between pre- and postoperative cerebral edema and quality of life in WHO grade I meningioma patients are virtually lacking. In patients with other types of brain tumors, associations between cerebral edema and clinical symptoms have been shown. Edema may contribute to the deficits in neurological and cognitive functioning, and consequently to aspects of patients' quality of life. **AIM OF THE STUDY:** To determine the effects of pre- and postoperative cerebral edema on health-related quality of life in WHO grade I meningioma patients. **METHODS:** Twenty-five WHO grade I meningioma patients who underwent surgery were individually matched to 25 healthy controls for age, sex, and educational level. We determined functional status and HRQOL at least 1 yr post-operatively. Furthermore, we determined the volume of cerebral edema on pre- and postoperative (3 months) MRI scans. The contribution of cerebral edema on HRQOL was investigated by correlational analysis. **RESULTS:** No significant differences were found in HRQOL between meningioma patients and healthy controls. However, 76% of patients reported a high level of fatigue; 32% reported to be depressed. Both pre- and postoperative tumor-related edema volume were found to be a significant predictor of the patients' physical and social functioning, and bodily pain. **CONCLUSIONS:** The present study suggests an important role for cerebral edema in HRQOL in meningioma patients. Meningioma patients with a significant amount of cerebral edema seem to be at risk for developing psychological problems and should therefore be screened neuropsychologically. Further research should be focused on the effect of treatment of cerebral edema on the one hand, and the impact of neuropsychological interventions on the other hand on HRQOL in meningioma patients with edema.

O.32. HYPOFRACTIONATED STEREOTACTIC RADIOTHERAPY OF THE OPTIC NERVE SHEATH MENINGIOMAS: AN EFFECTIVE OPTION

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OBJECTIVE: The management of primary Optic Nerve Sheath Meningiomas (ONSMs) is still controversial. Surgery easily leads to a complete blindness of the affected eye. On the other hand, the role of conventional radiotherapy remains uncertain and literature lacks of data to confirm its usefulness. The aim of this study was to evaluate the level of effectiveness and safety, according to our experience, of CyberKnife® (Accuray Incorporated) robotic radiosurgery as first-choice treatment for optic nerve sheath meningiomas. **METHODS:** In the period between May 2004 and June 2008, we treated 21 patients affected by an ONSMs, with the frameless CyberKnife system. The age ranged from 36 to 73 years (average 54 years). The M/F ratio was 4/17. The prescribed dose was 25 Gy prescribed to the 70%–85% isodose line. All patients were treated with a Stereotactic Radiotherapy treatment; particularly, they underwent a 25-Gy treatment in 5 fractions. Before the treatment, 3 patients had a conserved visual function whereas 11 presented a deficit of the sight or of the visual field. Seven patients were blind. Patients were evaluated both for the tumor growth control and the visual function. **RESULTS:** The mean follow-up period was 21 months (range 7–36 months). All patients well tolerated the procedures. Only 1 patient developed a mild optic neuropathy (remitted after a systemic steroid therapy). No others' acute or late radiation induced toxicities were observed. The median of tumor volume was 2.8 cc (range 0.3–23 cc). No patients showed a progression disease at MRI

controls. Two patients (9%) had a partial response. No patients had a worsening of the visual function. If we considered only the patients with a partial deficit of the sight or visual field, 60% showed an improvement. CONCLUSIONS: ONSM frameless stereotactic radiotherapy was found to be safe and effective. Tumor growth control was complete and visual function was maintained or improved. If the long-term follow-up will sustain the preliminary results, stereotactic radiotherapy could become the new first-choice treatment.

O.33. SURVIVAL AND FUNCTIONAL OUTCOME OF MENINGIOMA PATIENTS: LONG-TERM DISABILITY AND POOR SURVIVAL

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INTRODUCTION: The 5-yr control rates of WHO grade I meningioma as reported in the literature is ~90% after complete resection and ~55% after a non-radical resection. The role of adjuvant radiotherapy (RT) is debated. Although survival is generally described as favorable, we found earlier that cognition and quality of life are impaired in many long-term meningioma survivors. **OBJECTIVE:** In this retrospective study of a large neurosurgical series, we report long-term results in terms of survival, tumor recurrence, and functional outcome. **METHODS:** Analysis of all 212 patients after a neurosurgical resection for WHO grade I benign intracranial meningioma between 1985 and 2003; 159 females (70%) and 63 males (30%) with an average age of 53 (± 13.9) years at first diagnosis. Thirty-five (16.5%) patients received RT. Mean follow-up was 11.4 (± 5.1) years. Long-term functional outcome was assessed by a mailed questionnaire to the general practitioner. Statistical analysis including Cox' multivariate analysis was performed with SPSS. The age- and gender-specific survival was calculated by applying Dutch life-table statistics to each individual patient for the individual duration of follow-up. **RESULTS:** The overall survival at 5-, 10-, 15-, and 20 years was 93%, 81%, 63%, and 54%. This is substantially lower than the expected age- and sex-specific survival for this specific group, calculated at 94%, 86%, 79%, and 66%, respectively. In a multivariate analysis, survival was better with a lower Simpson grade ($P = .018$) and a lower age at diagnosis ($P < .0001$). Tumor progression rates at 5-, 10-, and 15-yr were 17%, 26%, and 32%. Lower Simpson grade ($P = .002$), Karnofsky performance score >70 before surgery ($P = .05$), and a lower age at diagnosis ($P = .033$) were associated with a lower recurrence rate. Eight patients (4%) had adjuvant RT; 27 patients (13%) received salvage RT for recurrent disease. RT was associated with a worse survival ($P = .08$) and a higher recurrence rate ($P < .0001$). After first surgery, 27 (13%) patients did not show any remission and 46 (22%) had more symptoms. **CONCLUSION:** Despite the benign histology of meningiomas, the long-term survival is severely challenged by the tumor and its complications; one-third of patients has stable or progressive symptoms. The role of radiotherapy remains unclear, since radiotherapy was mostly reserved for salvage treatment of recurrent disease.

O.34. SECOND-LOOK SURGERY (SLS) FOR EPENDYMOMA: THE ITALIAN EXPERIENCE

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INTRODUCTION: Complete resection of ependymoma is associated with better PFS/OS; smaller residues are even associated with better prognosis than "bulky" residues. According to experienced neurosurgeons, a truly complete excision of an infratentorial ependymoma is not feasible without serious risks whenever tumor arises from the floor of the fourth ventricle. SLS on smaller tumors can be associated with less dangerous anesthesiologic conditions and reach complete tumor removal. In this view, there is a possible, still uncertain, role for neo-adjuvant chemotherapy in preparing further surgical approaches. **METHODS:** From 1994 up to now, we have adopted two subsequent protocol for intracranial ependymomas: in both a phase of

adjuvant chemotherapy was prescribed for children with surgical residues, before radiotherapy, in view of possible SLS before it. In the first protocol, that accrued a total of 63 children, 9 were submitted to more than one surgical act: 4 after the 1st excision and 5 after surgery and chemotherapy: 3/4 plus 3/5 were rendered CR without additional sequelae, and their prognosis both for PFS and for freedom from local relapse was comparable to that of children operated once. In the subsequent protocol the efforts toward complete resection were improved. **RESULTS:** Of 95 patients accrued from 2001 to 2009, 29 had measurable disease after 1st surgery and/or adjuvant chemotherapy. Twenty-two were re-operated: 5 after 1st surgery, 15 after chemotherapy, and 2 soon after radiotherapy; 2 children had 3 and 1 had 4 excisions. Eleven of 22 patients obtained CR: one only had a neurologic worsening. We compared the outcome of the 58 patients CR after one surgical act with those 11 obtaining CR after more acts: both groups had 3- and 5-year PFS of 66% and 3-year freedom from local relapse was 82% and 87%, respectively. **Discussion:** SLS demonstrated feasible without major morbidity and results improved during time. Local tumor control because of SLS was comparable in patients with one and multiple resection.

O.35. THIRTY-FIVE YEARS OF SURGICALLY TREATED PEDIATRIC MENINGIOMAS IN THE NETHERLANDS: A DESCRIPTIVE EPIDEMIOLOGICAL CASE STUDY

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OBJECTIVE: To review and describe the epidemiology and the clinical, radiological, pathological, and management profile of all pediatric meningiomas surgically treated during the last 35 years in the Netherlands. **MATERIAL AND METHODS:** All pediatric patients (≤ 18 yr of age) with the diagnosis meningioma, treated at one of the neurosurgical centers in the Netherlands during the last 35 years, were identified in the PALGA database, the nationwide network, and registry of histo- and cytopathology. Data were retrieved from clinical records, radiological findings, operative reports, and pathological examinations. **RESULTS:** In total, 115 registries of meningioma histology in pediatric patients were identified in the PALGA database. Forty-six cases were excluded because either the histological diagnosis was a revision and confirmation of the original histology, or the original histological diagnosis was changed after revision. Thus, 69 patients (37 males, 54%) with meningiomas were included, making this the largest study of its kind. **Clinical presentation:** the most common symptom was raised intracranial pressure (30%). Mean age at diagnosis was 11.7 yr (0.3–18.8). **Location:** most frequently on the convexity (22%). **Etiology:** 11 patients (16%) had neurofibromatosis type 2 and 4 patients received prior radiotherapy. **Histology:** 42 patients (61%) had a WHO grade I, with meningotheliomatous meningioma as most common histological subtype. Five patients (7%) had a WHO grade III tumor. **Surgery:** macroscopic total resection was accomplished in 31 patients (42%) and subtotal in 19 patients (30%). Simple decompression was used in 5 patients (7%). **Resection grade** was missing in 14 patients (21%). **Additional treatment:** 15 patients (22%) received radiotherapy postoperatively. **Follow-up:** mean follow-up was 4.9 yr (0–27.8). A total of 22 radiological confirmed recurrences were diagnosed in 13 patients (19%) within a mean period of 3.9 yr (0.1–26.3). Eleven tumors recurred after macroscopic total resection. Nine patients with a recurrent tumor were first diagnosed with a WHO I (69%), none with a WHO III tumor. Six patients (9%) died as a result of their meningioma. **CONCLUSION:** Pediatric meningiomas are extremely rare. This is the first-single country study and one of the largest childhood series on this tumor. The presentation and biological behavior is different compared with meningiomas in adults. A high recurrence rate is observed and outcome tends to be worse.

O.36. DOSE CONSTRAINT MODEL TO PREDICT NEUROCOGNITIVE OUTCOMES IN YOUNG PATIENTS WITH LOW-GRADE BRAIN TUMORS TREATED WITH STEREOTACTIC CONFORMAL RADIOTHERAPY

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BACKGROUND: To analyze the effect of radiotherapy (RT) dose levels on various brain structures as a model to preserve neurocognition in young patients with low-grade brain tumors treated prospectively with stereotactic conformal radiation therapy (SCRT). **MATERIALS AND METHODS:** Thirty-five patients (median age 13 yr) with low-grade and benign residual/progressive brain tumors (craniopharyngioma, cerebellar astrocytoma, choroid plexus papilloma, hypothalamic glioma, other low-grade glioma) were

treated with SCRT to a dose of 54 Gy/30 Fr/6 weeks with 2- and 3-year follow-up were analyzed. Prospective neuropsychological assessment were done at baseline pre-RT and at subsequent follow-up with an age-appropriate neuropsychological battery of tests. The change in intelligent quotient (IQ) scores was correlated with various factors and dose volumes to normal brain structures using logistic regression analysis. RESULTS: One-third of patients had >10% drop in FSIQ over baseline. Comparison of dosimetric data in patients showing a significant drop (>10%) in IQ with patients with maintained IQ revealed that patients receiving >42.5 Gy (80% of the prescribed dose) to >13% of volume ($P = .048$) and >27 Gy (50% of the prescribed dose) to >50% of the volume of left temporal lobe were the ones to show significant drop in FSIQ ($P = .06$). Doses to the hippocampus are being analyzed and show a positive trend toward its association and a decline in cognition. CONCLUSION: Our detailed dosimetric data show a possible dose constraint model for high-precision RT planning to prevent a decline in neuro-cognition. Doses to left temporal lobe and hippocampus appear to be the most important dose-limiting structures.

MOLECULAR MARKERS II

O.37. CI-PERINOMS: CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY OUTCOMES MEASURES STUDY
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Chemotherapy-induced peripheral neuropathy (CIPN) is a major, frequent, and potentially dose-limiting adverse event of a wide variety of chemotherapeutic agents. Despite its relevance, no formally validated instruments to assess the occurrence and the severity of CIPN have been described so far. The aims of CI-PERINOMS are (i) to evaluate through a multi-center, international collaboration among experienced neurologists and oncologists the best method(s) available to assess and monitor CIPN, (ii) to determine the validity and reproducibility of the proposed outcome measures, and (iii) to produce a CIPN-specific disability sum score based on the selection of relevant questions from a large database. The study started in January 2009 and it is planned that patients' enrollment will be completed by December 2010 or at the recruitment of 300 patients. So far 20 European/US centers (*) received EC approval and 151 patients have been enrolled at 16 centers. According the study protocol, inter and intra-observer comparison and test-retest studies are to be performed by two investigators in each participating center on patients with a stable CIPN. The scales/instruments used in the study are: TNSc = Total Neuropathy Score, clinical version; VAS = visual analogue pain scale; PI-NRS = 11-point pain intensity numerical scale; C-ODSS = calibrated-overall disability sum score; NCI-CTC = National Cancer Institute-Common Toxicity Criteria, version 3; QLQ-CIPN20 EORTC = quality of life questionnaire for CIPN; QLQ-C30 = EORTC 30-item questionnaire for cancer patients; QoL-PS = quality of life personal score; and mISS = modified INCAT sensory sum-score. A small battery of nerve conduction studies is proposed to each patient, in order to compare the neurophysiologic results with those obtained with clinical methods. We are convinced that the results of this study will improve the knowledge on CIPN and will be useful in designing future studies to prevent or ameliorate CIPN.

*W. Grisold, D. Psimaras, S. Koeppen, A. Argyriou, H. Kalofonos, B. Frigeni, F. Lanzani, C. Briani, R. Plasmati, F. Pastorelli, L. Padua, A. Pace, A. Mazzeo, A. Toscano, A. Schenone, T. Ros, J. Bruna, Marta Penas Prado, Iñigo Rojas-Marcos, T.J. Postma, H. Heimans, W. Boogerd, I.S.J. Merkies, C.G. Faber, D.R. Cornblath, and S. Dorsey.

O.38. GLUT1/SLC2A1 IS CRUCIAL FOR DEVELOPMENT OF BRAIN MICROVASCULATURE WITH BLOOD-BRAIN BARRIER (BBB) PROPERTIES IN VIVO: POTENTIAL CLINICAL IMPLICATIONS
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Glucose transporter 1 (Glut1) is expressed at high levels in the capillary endothelial cells of barrier tissues such as the blood-brain barrier (BBB). In normal brain of human, rodent and zebrafish Glut1 is selectively present in the cerebral capillary endothelium. The BBB is composed of tightly linked cerebral endothelial cells sealed by tight junctions (TJ) and adherens junction (AJ). Because Glut1 and the TJ/AJ proteins are concomitantly downregulated in human high-grade gliomas and some other situations in which

BBB breakdown has taken place, we hypothesized that this molecule may play a significant role in the development of cerebral capillaries with BBB properties. The homologue Glut1 amino sequence in zebrafish is highly similar to that of humans and, therefore, the zebrafish is eligible as a model organism for the investigation of the human Glut1 gene. In our zebrafish model of Glut1 knockdown, the development of the cerebral microvasculature appeared to be interrupted with reduced expression of the TJ/AJ proteins and induced vasogenic brain edema. The data provide the first functional assessment of the role of Glut1 in the development of the cerebral capillary endothelium *in vivo* and suggest a crucial role of this molecule in the development of the cerebral microvasculature and formation of the BBB. Therefore, modulation of Glut1 expression and function may well have important clinical implications for the development of new therapeutic avenues toward recovering BBB disruption in devastating brain disorders.

O.39. TIMP-1 AND THE TIMP-1 INTERACTING CELL SURFACE PROTEIN CD63 IN CULTURED ASTROCYTOMA DERIVED SPHEROIDS: EXPRESSION AND CO-EXPRESSION WITH STEM CELL MARKERS
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In previous studies, we have shown that the immunohistochemical expression of TIMP-1 (tissue inhibitor of metalloproteinases-1) and CD63 increases with tumor grade in astrocytomas grade II-IV. Moreover, we demonstrated that a high TIMP-1 protein expression in glioblastomas was associated with a shorter overall patient survival. Recent studies have suggested that TIMP-1 can prevent chemo-induced apoptosis and in a study, using human breast epithelial cells (MCF10A), it was demonstrated that the interaction of TIMP-1 and CD63 is necessary for the TIMP-1 anti-apoptotic pathway. The aim of the present study was to investigate the expression of TIMP-1 and CD63 in cultured organotypic multicellular spheroids (OMS) and cell line spheroids (CLS) derived from astrocytomas in order to assess spheroid models for future studies involving TIMP-1, CD63, and chemo-resistance. By investigating the spheroids immunohistochemically, we wanted to elucidate whether TIMP-1 and CD63 are co-expressed within the spheroids and whether they are expressed by tumor stem like-cells, since these cells are known to be more resistant to chemotherapeutic treatment. In the present study, OMS from 9 astrocytomas and CLS from 3 commercial astrocytoma cell lines were included as well as the glioblastoma tumor stem cell line SJ-1 made in our laboratory. In general, high levels of CD63 protein were detected in all the original tumors, whereas TIMP-1 was moderately expressed. TIMP-1 and CD63 expression in OMS was similar to the expression in the original tumors. TIMP-1 was expressed at low-to-moderate levels in CLS, whereas CD63 was expressed by all tumor cells in all spheroids. TIMP-1/CD63 double immunofluorescence staining was performed on selected OMS and CLS showing tumor cells expressing both proteins. Furthermore, double staining was performed with TIMP-1 and the stem cell markers CD133, nestin, and Sox2, demonstrating that a population of the tumor stem-like cells expressed TIMP-1. In conclusion, this study shows that spheroid models can be used in future studies investigating the role of TIMP-1 and CD63 in chemo-induced apoptosis. Furthermore, these results indicate that a fraction of the tumor stem-like cells could be protected by TIMP-1-CD63 interaction.

O.40. MYELODYSPLASIA IN PRIMARY BRAIN TUMOR PATIENTS TREATED WITH ALKYLATING AGENTS
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BACKGROUND: Treatment-related myelodysplastic syndrome (t-MDS) and acute myelogenous leukaemia (t-AML) represent rare secondary events in patients with primary tumors of the nervous system. The emergence of temozolomide as an effective alkylating agent with little acute toxicity or cumulative myelosuppression has led to protracted courses of chemotherapy for many patients with malignant and even low-grade infiltrative gliomas. In the absence of solid incidence data, we were interested in reviewing the published experience of t-MDS/t-AML in this patient population. METHODS: Case reports and small case series of patients with t-MDS and t-AML during or after treatment with alkylating chemotherapy for a primary brain neoplasm were identified through a comprehensive search of the PubMed

database of the US National Library of Medicine. We recorded type of alkylating and other chemotherapy agents used, dose, concomitant or sequential irradiation, genetic predisposition, type of myelogenous tumor, cytogenetic findings, latency between completion of chemotherapy and diagnosis of t-MDS/t-AML, treatment, and outcome. **RESULTS:** We identified 39 cases fulfilling eligibility criteria. There were 17 male and 16 female patients (gender not listed in 6) with a median age of 20 years [range 0.25–69 yr]. The most common primary tumor was anaplastic astrocytoma (9) followed by medulloblastoma, low-grade astrocytoma (6 each), glioblastoma (5), and choroid plexus papilloma (3). Twenty-eight patients developed t-MDS. Of those, 12 progressed to t-AML. In 11 patients, t-AML was the first hematologic diagnosis. Median interval between completion of chemotherapy and diagnosis of t-MDS/t-AML was 17 months (range 0–29 months). Patients received lomustine, carmustine, nimustine, procarbazine, temozolomide, cyclophosphamide, or nitrogen mustard as part of their brain tumor therapy. Thirty patients in addition received partial, whole-brain, or craniospinal irradiation. In 3 patients, a genetic tumor predisposition syndrome might have played a role in developing t-MDS/t-AML. **CONCLUSION:** Albeit rare, the occurrence of t-MDS/t-AML underlines the importance of properly designed clinical studies as the basis for the implementation of novel treatment paradigms. Evolution of a secondary neoplasm reflects a complex pathogenetic process dependent upon genetic susceptibility, environmental factors, and treatment (exposure to ionizing radiation and mutagenic chemotherapeutic agents). Studies regarding the individual leukemogenic potential of these factors are lacking and their individual contribution and possible synergisms remain unsolved.

O.41. CHEMOTHERAPY-INDUCED POLYNEUROPATHY SCORE (CIPS): A NEW TOOL IN THE DIAGNOSIS OF CHEMOTHERAPY-INDUCED POLYNEUROPATHY (CIPN)

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INTRODUCTION: Chemotherapy-induced polyneuropathies (CIPN) are representing a therapy-limiting factor in the treatment of different oncological diseases. Early detection and diagnosis of CIPN is important, in order to prevent patients from neurotoxicity induced loss of neurological function. The total neuropathy score (TNS) is currently the most frequently used score to assess CIPN. However, evaluation of CIPN by means of the TNS is rather time consuming, and needs to be done by neurological trained personnel. Therefore, practical application of the TNS for everyday clinical use is difficult. The purpose of the study was to design a simple, practicable questionnaire (CIPS), which can easily be used in the clinical setting. **METHODS:** The CIPS was created from elements of the validated TNS and clinic-neurological experience. In this consecutive prospective study, 21 chemo-naïve patients with colorectal carcinoma and adjuvant oxaliplatin chemotherapy were included. All patients were treated and tested at the Oncology Department of the KFJ-Hospital in Vienna. Patients were examined with the TNS and the study questionnaire CIPS at baseline, at the 4th and at the 6th cycle of chemotherapy. **RESULTS:** Of 21 included patients, 4 patients were drop-outs. From 17 remaining study participants, 15 (88%) developed a CIPN and 9 (60%) study participants an acute oxaliplatin-induced neurotoxicity. The results showed a significant correlation of the TNS and the CIPS to all 3 scheduled dates of examination, as well as over time. Gender and age had no influence on the development of CIPN.

O.42. THE POTENTIAL ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN RADIATION NECROSIS OF THE BRAIN, FROM THE PATHOLOGICAL CONSIDERATION OF HUMAN SURGICAL SPECIMEN

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PURPOSE: With the advancement of high-dose radiation technologies for brain tumors, radiation necrosis has become a great problem. Here, we describe the potential role of vascular endothelial growth factor (VEGF) in radiation necrosis (RN) of the brain from a pathological and immunohistochemical standpoint. Also let us advocate the strategy to treat RN depending on the pathological findings and from literature.

MATERIALS AND METHODS: From June 2004 to July 2009, we treated 27 cases of symptomatic RN in the brain. These cases included different tumor histology, such as glioblastoma, metastatic brain tumor, and malignant meningioma, and were treated with different radiation modalities. Follow-up medical treatment included mainly oral steroids, anticoagulants, vitamin E, and others for at least 1-month duration. For 18 patients who were refractory to these medical treatments, we performed surgical excision of the necrotic mass. The surgical specimens were analyzed histopathologically with hematoxylin and eosin (H&E) staining and anti-VEGF immunohistochemistry. **RESULTS:** In all surgical specimens, irrespective of original tumor histology and radiation modalities, H&E staining showed marked angiogenesis and reactive astrocytosis at the boundary between the apparent necrotic area and the normal brain. We described this border zone as the “peri-necrotic” area. The most prominent vasculatures in this area consisted of a thin endothelium, mimicking venules, which is identified as telangiectasis. Immunohistochemistry indicated that VEGF was produced mainly in the reactive astrocytes in this peri-necrotic area. There was no evidence of marked immunoreactivity of VEGF either in the center of the necrotic tissue nor in the intact brain. Clinically, all RN cases treated by bevacizumab and removal of necrotic tissue showed the rapid shrinkage of the pre-lesional edema. **DISCUSSION:** These findings suggest that VEGF in the peri-necrotic area might be a cause of angiogenesis and the subsequent peri-lesional edema typically found in radiation necrosis of the brain. **CONCLUSION:** During the early phase of RN, there is a highly characteristic fibrinoid necrosis of the small venules and arterioles that is followed by necrosis of the surrounding brain parenchyma. Thus, in this early phase of RN, anticoagulants may be effective for maintaining microcirculation by preventing such small venules and arterioles from the thrombotic obstruction. However, in the later advanced phase of RN, medical treatment with anti-VEGF antibody, bevacizumab or surgical removal of the necrotic tissue and associated peri-necrotic area may serve to decrease this edema and provide immediate symptomatic improvements, because of the effect of reducing VEGF in this area.

NEURO-IMAGING II

O.43. HOT SPOTS IN ¹⁸FET-PET DELINEATE MALIGNANT TUMOR PARTS WITHIN SUSPECTED WHO GRADE II GLIOMA

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OBJECTIVE: This prospective study correlates metabolic maps of intratumoral [¹⁸F]fluoroethyltyrosine (FET) uptake kinetics with detailed histopathology and molecular genetic profiling in untreated adults with magnetic resonance imaging (MRI)-based suspicion of a WHO grade II glioma. Special attention was set on diagnostic accuracy of FET-PET in noninvasive delineation of an anaplastic focus. **METHODS:** Individual maps of FET uptake kinetics were generated and metabolic hot spots were outlined three dimensionally. Novel ¹⁸FET-PET-guided serial stereotactic biopsy procedures were found suitable for stepwise histopathological and molecular genetic evaluation. Histopathology was done according WHO criteria by independent observers. O⁶-methylguanine-DNA methyltransferase (MGMT) promoter methylation was determined by methylation-specific polymerase chain reaction/sequencing and isocitrate dehydrogenase (IDH1/2) mutations by immunohistochemistry analysis, respectively. **RESULTS:** A total of 373 biopsy samples from 55 consecutive patients were analyzed. In 24 patients, a malignant glioma was diagnosed. Homogeneous metabolic kinetics was significantly linked to histopathological homogeneity in 40 patients. In 15 patients, a heterogeneous FET uptake kinetic was found throughout tumor volumes and a precise stepwise topographic correlation with grade II and III histopathology was confirmed. FET-PET analysis reached a sensitivity of 92% and specificity of 82% in determination of an anaplastic focus. Eleven out of 14 tumors with heterogeneous histopathology were MGMT methylated and 9 tumors showed IDH1/2 mutations. Both molecular markers were homogeneously distributed throughout each tumor irrespective of an anaplastic focus. **CONCLUSION:** Homogeneous or heterogeneous glioma histology can be precisely delineated by dynamic FET-PET evaluation; an anaplastic focus can be reliably identified. This finding has implications for prognostic evaluation, biopsy planning, and individualized treatment strategies.

O.44. LANGUAGE MAPPING FINDINGS AND CORRELATION WITH DTI–FT DATA DURING SURGICAL REMOVAL OF LESIONS INVOLVING LANGUAGE AREAS OR PATHWAYS

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Surgical resection of lesions involving language areas or pathways requires intraoperative identification of functional cortical and subcortical sites to effectively and safely guide resection. Diffusion tensor imaging (DTI) and fiber tractography (FT) are magnetic resonance (MR) techniques based on the concept of anisotropic water diffusion in myelinated fibers, which enable three-dimensional reconstruction and visualization of white matter tracts, and provide information about the relationship of these tracts with the tumor mass. We have routinely used DTI–FT to reconstruct various tracts involved in the language system [superior longitudinalis (SLF), inferior fronto occipitalis (IFO), inferior longitudinalis (ILF), uncinate (UNC), premotor fibers] in a series of 305 patients with gliomas (mostly low grade) involving the language areas or pathways. DTI–FT information was loaded into the neuronavigational system and combined intraoperatively with those obtained with direct electrical stimulation applied at subcortical level, with bipolar electrical stimulation (DES). In this work, we report the results of such experience, describing the findings for each tract and discussing technical aspects of the combined use. Large part of SLF was located inside the tumor mass and highly infiltrated by it, and can be safely resected because not functional. IFO was a discrete fasciculus and, when identified intraoperatively, was functional in most of the cases. ILF was located at the lateral portion of temporal tumors and identified as a separate tract and functional in small tumors. The temporal portion on UNC could be removed, whereas the frontal portion had to be preserved to keep language functional integrity. Premotor face fibers must be reconstructed, identified, and preserved intraoperatively to avoid anarthy. Tract identification was associated with a 79% chance to develop early postoperative deficits. The rate of definite deficits (at 1 mo) was <2%. The rate and speed of recovery in the postoperative period correlated with the subcortical organization of some of the tracts, such as the SLF, and could be predicted preoperatively. The combined use of DES and DTI–FT allowed to effectively and safely trace language tracts, reduced duration of surgery, patient fatigue, and kept a high rate of patient functional integrity

O.45. USEFULNESS OF NMR-BASED METABOLOMICS (METABOLOME) USING THE ANALYSIS OF WATER AND LIPID SOLUBLE METABOLITES AS THE PREDICTIVE FACTORS OF MALIGNANT-TYPE MENINGIOMAS

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PURPOSE: In meningiomas which are considered to be benign brain tumors, there are malignant-type tumors. Most of these malignant-type meningiomas are histologically diagnosed anaplastic or atypical ones. However, some of malignant-type meningiomas show poor clinical courses, although histological diagnoses are benign. It is difficult to distinguish this specific group from usual benign-type meningioma. Therefore, we tried to gain characteristic extraction by the metabolite expression profiling using nuclear magnetic resonance (NMR)-based metabolomics (comprehensive metabolite analysis). **METHODS:** We extracted water and lipid soluble metabolites from recent frozen surgical specimens which are 31 meningiomas, including 2 anaplastic-, 1 atypical-, and 2 malignant-type cases, and measured ¹H-NMR spectra. Then, we did analysis by data-processing software Alice2 for metabolome™ ver1.0 (JEOL DATUM) and ADOMEWORKS/ModelBuilder™ ver3.1 (Fujitsu). Finally, we searched for the parameters which characterized malignancy in loading plot. **RESULTS:** *Water soluble metabolites:* Surgical specimens were distributed to almost 2 domains (grade 1 and grade 2/3 domains). Two anaplastic and 1 atypical meningiomas were distributed in the same domain, and 2 malignant-type meningiomas were distributed over extremely near location in the grade II/III domain. *Lipid soluble metabolites:* Malignant-type meningiomas were distributed near location in the grade III domain. However, grade II domain was isolated. **CONCLUSIONS:** This study suggests that NMR-based metabolomics are very useful for prediction of malignant-type meningiomas that were histologically benign.

O.46. INTRA-OBSERVER AND INTER-OBSERVER AGREEMENT IN VOLUMETRIC ASSESSMENT OF GLIOBLASTOMA MULTIFORME RESECTION

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OBJECTIVE: The aim of this study was to analyze intraobserver and interobserver agreement of manual segmentation as a method for volumetric assessment of glioblastoma multiforme (GBM) resection. **METHODS:** Three observers performed volumetric assessment of preoperative tumor volume (PreTV) and postoperative tumor volume (PostTV) by manual segmentation on contrast-enhanced T1-weighted magnetic resonance imaging (MRI) data sets of 8 patients. Measurements were repeated after an interval of minimally 2 weeks. Intraobserver and interobserver agreement for PreTV, PostTV, and residual tumor volume percentage (RTV) were expressed in intraclass correlation coefficients (ICC). **RESULTS:** Intraobserver agreement is high for PreTV (ICC = 0.99), PostTV (ICC = 0.73 – 0.94) and RTV (ICC = 0.89 – 0.94). Interobserver agreement is high for PreTV (ICC = 0.97), but low for PostTV (ICC = 0.54) and RTV (ICC = 0.52). **CONCLUSION:** Volumetric assessment of GBM resection seems to offer high intraobserver agreement, but low interobserver agreement. The results of this study suggest that using absolute RTV values to relate the extent of tumor resection with survival may be unreliable. More research is needed before this method can be used as a valid endpoint for clinical studies.

O.47. ¹⁸F-FLUOROTHYIMIDINE (FLT)–POSITRON EMISSION TOMOGRAPHY TO DETERMINE THE PROLIFERATIVE TUMOR VOLUME IN HIGH-GRADE GLIOMA AND CORRELATION WITH SURVIVAL

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INTRODUCTION: ¹⁸F-fluorothymidine (FLT) is a tracer for positron emission tomography (PET) depicting tumor cell proliferation. Quantitative analysis by calculating the maximum standardized uptake value (SUV_{max}) has been shown to correlate with the Ki-67 index, time to progression, and overall survival. For estimating the proliferative tumor volume (PTV), different PET segmentation methods can be used. The aim of this study was to identify the method that best predicts overall survival. **MATERIALS AND METHODS:** From July 2007 to August 2008, 26 consecutive patients with suspected high-grade glioma underwent a preoperative computed tomography (CT) and FLT–PET scan. The SUV_{max} of all tumors was calculated after manual delineation of the PTV on the co-registered CT and FLT–PET scans. Three different segmentation methods for estimating the PTV were used. The 50% isocontour of the SUV_{max} signal was used to estimate PTV_{50%}. The signal-to-background (SBR) ratio was used as an adaptive threshold delineation (PTV_{SBR}) method. The iterative background-subtracted-relative threshold level method was used to estimate PTV_{RTL}. The Cox proportional hazard regression model was used to assess the significance of the SUV_{max} and the different PTVs on survival. Receiver-operating-characteristic (ROC) curve analysis was used to identify the threshold for patients with longer survival. Kaplan–Meier analysis and log-rank statistical test were used to test the power of FLT–PET for predicting survival. **RESULTS:** Twenty-two patients had a diagnosis of glioblastoma multiforme, 2 of anaplastic oligodendroglioma, 1 of anaplastic ependymoma, and 1 of anaplastic astrocytoma. The tumor was resected in 17 patients and 9 patients received a biopsy. The mean age was 52 years (range 35–67 years), and 20 patients were male. The mean overall survival was 411 days (min. 51 days, max. 881 days, SD 262) and 19 patients died during the follow-up period. The PTV_{SBR} was associated with a significant better survival ($P = .03$) compared with the PTV_{RTL}, PTV_{50%}, and SUV_{max}. ROC analysis found a threshold volume for the PTV_{SBR} of 11.4 cc (sensitivity 68%, specificity 71%). Kaplan–Meier analyses showed a significant discrimination between short and long survival ($P = .04$, log rank) for this threshold. **DISCUSSION AND CONCLUSION:** The proliferative tumor volume as determined by FLT–PET is associated with survival in high-grade malignant gliomas. SBR is the best method to estimate the PTV.

O.48. EARLY PROGRESSION BETWEEN SURGERY AND ADJUVANT CHEMO-RADIOTHERAPY IN GLIOBLASTOMA

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BACKGROUND AND PURPOSE: The assessment of early progression after surgery and before adjuvant treatment in glioblastoma (GBM) may (i)

offer the opportunity for customized target definition for radiotherapy, (ii) allow to modify the therapeutic program also by the patient enrollment into experimental trials, and (iii) permit to monitor more precisely the response to therapy. However, data on early progression in GBM are still lacking. Herein, the incidence and the methods to identify this phenomenon were investigated. **MATERIALS AND METHODS:** Thirty-seven patients with newly diagnosed GBM were retrospectively analyzed. Early post-operative magnetic resonance (EPMR) was compared with 1-mo postoperative examination to evaluate the extent of surgery and to identify possible new areas of contrast enhancement (CE). To classify these areas of CE as suggestive of tumor growth or surgical effect, 4 different magnetic resonance imaging (MRI) approaches were compared: (i) EPMR diffusion, (ii) T2-weighted imaging, (iii) 1-mo diffusion, and (iv) 1-mo perfusion. **RESULTS:** Based on EPMR, 67% of cases were classified as incompletely resected. Seventeen out of 37 (46%) patients showed an increased area of CE between surgery and chemo-radiation. By EPMR diffusion, in 3 of 17 patients these new CE corresponded to areas of reduced diffusion and therefore indicative of postsurgical infarct; in the other 14 of 17 patients, they were indicative of tumor progression or a combination of progression and infarct. Comparing T2-weighted imaging EPMR vs 1-mo, 4 of 17 patients showed an increase of edema, suggestive of tumor progression. In the new areas of CE, by 1-mo diffusion, 2 of 17 patients showed the coexistence of reduced diffusion. Finally, by 1-mo perfusion, 11 of 17 patients showed the coexistence of hyperperfusion. Considering EPMR diffusion and 1-mo perfusion, they provided the most similar classification with an agreement in 11 of 17 patients. It is noteworthy that the extent of resection does not seem to influence the rate of tumor progression: 33% of the patients that performed gross total surgery vs 40% of those partially resected, experienced disease progression. **CONCLUSIONS:** Our findings suggest that early progression frequently occurs in GBM between surgery and the beginning of adjuvant treatment. EPMR diffusion, identifying post-surgical ischemic areas, and perfusion, detecting neo-angiogenesis, seemed to be the more reliable approaches.

SUPPORTIVE CARE

O.49. IDENTIFYING CLINICAL DEPRESSION IN PATIENTS WITH BRAIN TUMORS

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BACKGROUND: Depression is under-recognized in patients with brain tumors. A standardized psychiatric interview is the “gold standard” method of diagnosing clinical depression. We studied the frequency, course and clinical associations of DSM-IV major depressive disorder (MDD) in adults with glioma. **METHODS:** This was a prospective, twin-centre, longitudinal cohort study of adults with a new histological diagnosis of primary cerebral glioma. All subjects had a structured clinical interview to diagnose or exclude MDD. Data are presented from the first two time-points: T1 (shortly after starting radiotherapy, or equivalent) and T2 (3 months later). **RESULTS:** We examined 155 patients at T1 and 108 at T2. Fifty-seven percent patients were male. Mean age was 54 years, 86% had high-grade glioma, 78% received radical radiotherapy, and the median KPS was 90. We diagnosed MDD in 20 patients at T1 (12.9 ± 5.3%) and in 16 cases at T2 (14.8 ± 6.7%). Of the 20 patients with MDD at T1, 2 had recovered by T2; 7 continued to have MDD and 11 dropped out of the study. Nine new patients developed MDD by T2. These changes underlay the overall tendency for the point prevalence of MDD to increase over time ($P = .065$, McNemar test). We found univariate associations (all χ^2 , $P < .05$) between MDD and functional impairment (KPS ≤ 70), current steroid use, a family history of depression, male gender, current antiepileptic drug prescription and/or high emotional distress (NCCN distress thermometer score $\geq 4/10$). In multivariate analysis, MDD was independently associated with functional impairment and high emotional distress (logistic regression χ^2 , $P < .001$, $R^2 = .294$). **CONCLUSIONS:** This is the first longitudinal study of depression in glioma patients using clinical interview. Major depression afflicted nearly 1 in 5 individuals during the study period. MDD persisted at reassessment after 3 months, and new cases occurred over time. Those with MDD were difficult to retain in the longitudinal study. Functional impairment and emotional distress were independent risk factors. Clinicians should screen for depression in patients with glioma who report high distress on the NCCN distress thermometer, or who are functionally impaired (KPS ≤ 70). They may also consider screening those with epilepsy, who are an easily identifiable group. The lack of association with any tumor-related variables suggests that in glioma, MDD could be more representative of a psychological reaction to loss than a “direct”

tumor disruption of neuronal emotional networks. However, more research on this question would be required.

O.50. PSYCHOLOGICAL FACTORS OF THE RETARDATION OF MENTAL DEVELOPMENT IN CHILDREN WITH RECURRENT BRAIN TUMORS

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BACKGROUND: The survivors of recurrent childhood cancer achieve fewer milestones than their peers with respect to autonomy development, social development, and psycho-sexual development or achieve, the milestone when they are older than their peers. There is the evidence of retardation of mental development in children with brain tumors as the result of not only complex consequences of the brain tumors and their treatment but the psychosocial situation of development, social situation. This is the challenge for the complex use of the multidisciplinary psychological methods – neuropsychological, clinical psychology, psycho-somatic, family psychology. **METHODS:** The results of the complex testing of 50 children of the age 10–16 years old and their mothers were examined (with spinal brain tumors $n = 10$; with head brain $n = 40$). The Luria’s method of complex neuropsychological diagnostics (for children), projective drawings, Children Apperceptive Test; questionnaire of Parental Attitude, Spilberger test, and questionnaire of Posttraumatic Growth (for mothers) were used as well as interview. **RESULTS:** The most sensitive for the brain functions disorders in children is neuropsychological testing of visual-spatial and visual-constructive functions (in 40% of children with head brain tumors). The parental attitude to the child, actual mothers’ emotional state, intention to continue education, opportunity of social contacts are the most significant prognostic factors of the mental development and compensation of the neuropsychological disorders excluding visual-spatial and some other. **CONCLUSIONS:** There is the need of special educational and psycho-social programs for the children with recurrent brain tumors and their families.

O.51. A HADS DEPRESSION SUBSCALE SCORE ≥ 8 CAN HELP SCREEN FOR DEPRESSION IN ADULTS WITH PRIMARY GLIOMA

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BACKGROUND: No study has validated a screening tool for the purposes of diagnosing depression in adults with glioma. We examined whether the hospital anxiety and depression scale (HADS) discriminates between depressed and nondepressed glioma patients compared with a structured psychiatric interview. **METHODS:** This was a prospective, two-center, longitudinal cohort study of adults with newly diagnosed primary cerebral glioma. All subjects completed the 7-item depression subscale of the HADS (HAD-D, score range 0–21) and received a “gold-standard” structured interview to diagnose or exclude major depressive disorder (MDD). Data are presented from the first two time-points: T1 (shortly after starting radiotherapy) and T2 (3 months later). Analysis was done by receiver operating characteristic curves. **RESULTS:** We examined 133 patients at T1 and 90 at T2. Of them, 57% were male. Baseline sample characteristics were: mean age 54 years, 84% had high-grade glioma and 80% received radical radiotherapy. The HAD-D showed good discrimination both at T1 (AUC = 0.93) and at T2 (AUC = 0.98). At T1, a cut-off of ≥ 7 had 0.93 sensitivity and 0.89 specificity for MDD (PPV = 54%; NPV = 99%, LR⁺ = 9.1). With a cut-off ≥ 8 , sensitivity was 0.73 and specificity was 0.92, but predictive values were similar (PPV = 55%; NPV = 96%; LR⁺ = 9.6). At T2, a cut-off of ≥ 8 had 0.92 sensitivity and 0.92 specificity for MDD (PPV = 67%; NPV = 99%; LR⁺ = 11.8). **CONCLUSIONS:** Depression in glioma is potentially treatable. The HAD-D may be a useful screening tool. It discriminated well between depressed and nondepressed patients in our cohort of 133 well-functioning adults with primary glioma. A cut-off of ≥ 8 may be favored because this threshold functioned well at either time-point studied. This score is also consistent with established cut-offs in other populations of medically ill patients and may be more familiar to clinicians. The higher PPV at T2 may represent a lower “background” level of distress as patients adjust to their diagnosis. The HAD-D can be completed easily by most glioma patients receiving treatment, but does have practical limitations. The paper is not easily completed by patients with dysphasia, hemianopia, neglect, or hemiparesis. The impact of cognitive impairment and the reproducibility and internal consistency of the

scale remain to be studied. However, our data suggest a useful role for the HADS depression subscale in improving the standard of supportive and psychological care of adults with glioma.

O.52. SCREENING FOR COGNITIVE IMPAIRMENT IN PRIMARY BRAIN TUMOR: IS THE MONTREAL COGNITIVE ASSESSMENT A MORE SENSITIVE TOOL THAN THE MINI MENTAL STATE EXAMINATION?

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BACKGROUND: The cognitive sequelae of primary brain tumor (PBT) and its associated treatments are well established. Although the mini mental state examination (MMSE) is widely used as a screening measure to detect cognitive impairment, it has limitations. This study aimed to compare a newer and potentially more sensitive screening tool, the Montreal Cognitive Assessment (MoCA), to the MMSE in a neuro-oncology population. **METHODS:** A series of 40 patients with histologically confirmed PBT from Liverpool Hospital, Sydney, were recruited over a 7-month period. Both the MMSE and MoCA screening tools were administered to all patients to assess the presence of cognitive impairment. Clinical and socio-demographic data were also documented. **RESULTS:** There were 21 males and 19 females, with mean age of 51.3 years (SD = 15.9) and median time since diagnosis 13 months (IQR 4.0–22.8). The tumor diagnoses were: high-grade $n = 11$ (28%), low-grade $n = 11$ (28%), and benign tumors, including pituitary adenoma and meningioma, $n = 18$ (44%). A total of 16 patients underwent resection only, 11 patients received both surgery and radiotherapy (RT), while $n = 9$ were treated with a combination of surgery, RT, and chemotherapy. Thirteen patients (33%) had a history of epileptic seizures and 14 (35%) were taking anti-epileptic medications at time of assessment. Only 8 (20%) patients were taking prescribed steroids. In the assessment of cognitive function, 28 patients (70%) were deemed impaired using the MoCA, compared with only 6 (15%) using the MMSE. Of the 34 patients with a normal MMSE, 22 of 40 (55%) demonstrated cognitive impairment according to the MoCA. There were no patients with a normal MoCA score who also had an impaired MMSE score. Notably, of the 28 patients with an abnormal MoCA, 22 of 28 (79%) had a normal MMSE. **CONCLUSIONS:** These preliminary results suggest that the MoCA has demonstrated utility as a brief cognitive screening tool and is more sensitive in detecting cognitive impairment than the MMSE in a population of primary brain tumor patients.

O.53. HOW TO BEST MEET THE NEEDS OF PATIENTS WITH A GLIOBLASTOMA AND THEIR FAMILIES: SCREENING FOR PSYCHOSOCIAL DISTRESS OR A STANDARD CONSULTATION WITH A SOCIAL WORKER AS PART OF THEIR MEDICAL TREATMENT

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PURPOSE: The aim of the study was to evaluate the need of integrated psychosocial counseling as part of the treatment plan in patients with a high-grade glioma and to investigate whether standard counseling leads to improved psychosocial support for the patients and their family. **MATERIALS AND METHODS:** Patients with a glioblastoma multiforme (GBM) are potentially faced with more physical, psychological, and social problems because of the nature of their illness. Patients are aware of the fact that they have an aggressive tumor with a limited lifespan. Owing to its effect on the cerebral function, not only the patient can experience a great loss of health and functioning, but the disease also has an obvious social impact on family life. The family often needs to take on new responsibilities and has to face at the same time the fear of losing their loved one. Since 2003, in MAASTRO clinic, all patients are screened on the level of psychosocial distress using a short validated questionnaire, the screening inventory of psychological problems (SIPP). Despite the resulting advice, not all patients make use of the psychosocial support. To improve psychosocial care, two strategies are followed: all patients are selected for counseling

using the SIPP. Patients with a GBM are offered a counseling session with a social worker specialized in oncology as part of the treatment plan. The purpose of a standard counseling session is to check how the patient and his family are coping with the new situation (distress), to give psycho-education, emotional support, and practical advice and explain what the social worker can mean for them during and after treatment. **RESULTS:** Of 123 patients treated for a primary GBM between 2006 and 2010, 101 (82%) patients were referred for standard counseling at the beginning of the radiotherapy. Of the 101 referred patients, 43 (42.6%) were further actively accompanied, whereas for 58 (57%) patients there was no further necessity of psychosocial support. Nevertheless, the baseline counseling was experienced in a positive way. Follow-up counseling ranged between 4 and 14 sessions during and after radio/chemotherapy per patient depending on the needs. Patients with devastating effects of the brain tumor and an accompanying large impact on their personality and abilities need of continued support. **CONCLUSIONS:** If counseling is integrated into the treatment plan, the hurdle of seeking advice is lower and support is easier accepted (majority of 82%). There is a large need for psycho-social counseling for patients harboring a GBM and should therefore be part of the overall treatment plan to improve quality-of-life for patients and their families.

O.54. EPILEPSY AT THE END OF LIFE IN MALIGNANT GLIOMAS

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Epilepsy is common in patients with brain tumors. Frequently, an epileptic seizure is the presenting sign of the cerebral lesion, but late seizures may also occur. More than one-third of cases with primary brain tumors present with epileptic seizures, and the incidence at diagnosis varies with histological type and location. Another 10%–30% of patients develop epilepsy later in the course of their disease. Patients who present with seizures as the first sign of a malignant glioma are at increased risk of recurrent seizures despite treatment with antiepileptic drugs. However, little is known about the incidence of epilepsy in the last stage of disease and at the end of life of brain tumor patients. We retrospectively analyzed the incidence of seizures in the last months of life in a series of patients affected by malignant gliomas assisted at home during all the course of disease until death with a comprehensive neuro-oncological home care program. One hundred fifty-seven patients affected by malignant gliomas were included in this study. Epilepsy at onset was present in 52 (33.1%) patients; 33 (21%) patients presented late onset epilepsy (during the course of disease and before the last months of life); 58 patients (37.6%) presented epilepsy in the last month before death. In 9 cases, seizures in the last month of life occurred in patients not presenting previous epilepsy. The incidence of seizures in the last month of life was higher in patients presenting late onset epilepsy (25 of 33, 75.8%) with respect to the group of patients presenting early onset (25 of 52, 48.1%). In the group of patients not presenting previous epilepsy (72 of 157, 46%), the incidence of epilepsy at the end of life was 12%. In this group, 47 patients received anticonvulsant prophylactic treatment and 25 not. The occurrence of seizures was not correlated with anticonvulsant treatment. Anticonvulsant treatment at the end of life of brain tumor patients presents particular concerns with respect to other stages of disease, given the frequent difficulty to swallow oral therapy in patients dying. Oral treatment needs to be changed in advance with intramuscular or subcutaneous administrations. More studies should be addressed to the issue of epilepsy at the end of life in brain tumor patients.

GLIOMA

O.55. INF- β SENSITIZES GLIOMA CELLS TO TEMOZOLOMIDE IN AN MGMT- AND P53-INDEPENDENT MANNER

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The alkylating agent temozolomide is the first drug to significantly prolong progression-free and overall survival when used in combination with surgery and radiotherapy in newly diagnosed glioblastoma. Glioma cell sensitivity to temozolomide is strongly associated with promoter methylation of the O⁶-methyl guanine transferase (MGMT) gene. Further, *in vitro* studies

have demonstrated that the restoration of wild-type p53 activity also sensitizes glioma cells to temozolomide. On the basis of prior reports that demonstrated a sensitization of glioma cells to temozolomide by interferon- β (INF- β), we aimed at characterizing the underlying mechanisms in more depth. We report that human glioma cells uniformly express mRNA for interferon receptors I and II whereas only interferon receptor II protein is consistently detected by flow cytometry at the cell surface. INF- β strongly sensitizes glioma cells to temozolomide-induced reproductive cell death. This sensitization is independent of p53 as glioma cells with an siRNA-mediated silencing of the p53 gene become more susceptible to temozolomide after prior exposure to INF- β , too. Further, MGMT-negative glioma cells and transfectants genetically engineered to overexpress MGMT can be equally sensitized to temozolomide by INF- β . This effect of INF- β is observed in acute cytotoxicity assays and in clonogenic survival assays. Most importantly, there is also a sensitization of stem-like glioma cells to temozolomide by INF- β . In summary, we find that the INF- β -mediated sensitization to temozolomide is neither mediated by p53 nor limited to MGMT-negative or -positive cell lines, suggesting that INF- β might be a powerful adjuvant to increase the benefit from temozolomide chemotherapy in glioblastoma.

O.56. RELATIONSHIP TO DIFFERENT CELLS OF ORIGIN PREDICTS THE TGF- β RESPONSIVENESS OF GLIOBLASTOMA CANCER STEM CELLS

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CD133⁻ TGF- β -susceptible adult neural stem cells (NSCs) and CD133⁺ TGF- β -resistant fetal forebrain NSCs are cell populations that may transform into glioblastoma cancer stem cells (CSCs). This prompted us to compare TGF- β responsiveness of CSCs and their relationship to adult or fetal NSCs. TGF- β modulated SMAD2 phosphorylation, proliferation, migration, and tumorigenicity in 3 of 9 CSC lines. Six CSC lines resisted TGF- β partially because of low TGF β 2 expression. The transcriptional profile of the CSC lines proved that the relationship to either adult or fetal NSCs predicted the susceptibility towards TGF- β . Fetal NSC-like, CD133⁺, neurosphere-like growing CSCs were resistant to TGF- β while adult NSC-like, mainly CD133⁻, adherently growing CSCs responded to TGF- β . Together, TGF- β susceptibility delineates two different types of CSC and thereby points toward different cells of origin.

O.57. SUNITINIB MALATE AS A SINGLE AGENT OR COMBINED WITH LOMUSTINE (CCNU) IN PATIENTS WITH RECURRENT, TEMOZOLOMIDE REFRACTORY HIGH-GRADE GLIOMA

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BACKGROUND: Receptor tyrosine kinase signaling causes profound neo-angiogenesis in high-grade gliomas (HGGs). The KIT, PDGFR- α , and VEGFR2 genes are frequently amplified and expressed in HGGs and represent a molecular target for therapeutic inhibition by the small molecule kinase inhibitor sunitinib malate. **PATIENTS AND METHODS:** A first cohort of patients with progressive HGGs following prior RT and temozolomide received a daily dose of 37.5 mg sunitinib until progression or unacceptable toxicity (2-stage phase II design). Following the first stage, the study was amended to recruit a second cohort of patients with secondary glioblastoma (sGB), treated with a daily dose of 25 mg sunitinib (28 out of 42 days) and CCNU (80 mg/m² on day 15). T1 \pm Gd and T2-weighted MRI images were obtained to evaluate tumor response in both cohorts. In the first cohort MRI-based and dynamic susceptibility contrast (DSC)-enhanced perfusion measurements were performed before and during therapy; cerebral blood volume (CBV) and cerebral blood flow (CBF) lesion-to-normal-white matter ratios were measured to evaluate the antiangiogenic effects of sunitinib single agent. **RESULTS:** Twenty-one patients were recruited in the first cohort. The most frequent grade ≥ 3 adverse events were skin toxicity, neutropenia, thrombocytopenia, and lymphocytopenia. None of the patients achieved an objective response, whereas a decrease in CBV and CBF within the lesion when compared with the normal brain was documented in 4 out of 14 (29%) patients evaluable for DSC-enhanced perfusion measurements. Median time-to-progression and overall survival were 1.6 (95% CI 0.8–2.5) and 3.8 (95% CI 2.2–5.3) months, respectively. No correlation could be established between VEGFR2, PDGFR- α , and KIT gene copy numbers or

protein expression and the effects of sunitinib. Three patients with an sGB experienced a regression of their glioblastoma following CCNU administration at the time of progression on sunitinib (PFS >6 months in 2 patients). Recruitment to the second cohort is ongoing (4 patients have been recruited at present). **CONCLUSIONS:** Single agent sunitinib at 37.5 mg/day demonstrated insufficient activity to warrant further investigation in recurrent HGG. Investigation of the activity of sunitinib in combination with CCNU is ongoing, updated results will be reported at the meeting.

O.58. NON-R132 MUTATIONS IN IDH1 IDENTIFY A NOVEL SUBGROUP OF LOW-GRADE GLIOMAS WITH DISTINCTIVE LOCATION, INFILTRATIVE BEHAVIOR, DISMAL OUTCOME, AND UNIQUE MOLECULAR PATTERN

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INTRODUCTION: Chromosomes 1p and 19q deletions and TP53 mutations represented the 2 main genetic alterations described in low-grade gliomas (LGGs). Interestingly, TP53 mutations and 1p19q codeletion were found to be exclusive. The predictive impact of these two genetic alterations on outcome in LGG is still source of controversies. However, LGGs harboring 1p19q deletion and no TP53 mutations have been reported to have a better prognosis than tumors with TP53 mutation and no losses on 1p and 19q. Intriguingly, no data are available on the intermediate group of LGGs harboring a “null” phenotype (no TP53 mutation and no 1p19q codeletion). Recently, mutations of isocitrate dehydrogenase enzyme isoforms 1 (IDH1) and 2 (IDH2) have been found in a large proportion of LGGs. To date, few data are available regarding the prognostic impact of IDH1 and 2 mutations in a homogenous LGG population. We address here, for the first time, a comprehensive analysis of the segregation of non-R132 mutations in IDH1 in distinct molecular subtypes of LGGs and report the clinical outcome and radiological features of this novel subgroup of tumors. **METHODS:** Patients (48) treated at Timone University Hospital, Marseille, France, between 2002 and 2008 were selected from the following criteria: histologic diagnosis of WHO grade II glioma; available paraffin-embedded tissue; available magnetic resonance imaging data at diagnosis; clinical and follow-up data from the database; and written informed consent. The histology of all tumors was centrally reviewed by two independent neuropathologists. Complete physical and neurologic examinations, KPS score, and MRI scan data were collected at the time of diagnosis. MRI data assessed by two neuroradiologists included tumor size, midline mass effect, heterogeneity, infiltration, contrast enhancement, and location. MRI-based extent of surgery was assessed at 3 months post-op. **RESULTS:** Sex ratio was 1.29 (27 men and 21 women) and median age 39.8 years (range, 22–71 years). A total of 41 mutations in IDH1 were identified (85.4%) and 2 mutations in IDH2. Five-year overall survival was 86.6 vs 60 months in patients with R132 IDH1 and non-R132 IDH1 mutated tumors, respectively ($P < .01$). Furthermore, non-R132 IDH1-mutated tumors had a no mutation in TP53 and no codeletion of 1p19q in 71.4% of cases compared with 8.3% in IDH1-mutated tumors ($P < .001$). Finally, 7 of 7 (100%) of the non-R132 IDH1-mutated tumors were paralingual and displayed an infiltrative radiological phenotype compared with 9 of 41 (21.9%) patients of R132 IDH1-mutated tumors ($P < .0001$). **CONCLUSION:** Non-R132 mutations in IDH1 identify a novel subgroup of LGGs with distinctive topography, radiological aspect, and dismal outcome. Furthermore, non-R132 mutations in IDH segregate in a distinct molecular subtype of LGGs.

O.59. DYNAMIC HISTORY OF LOW-GRADE GLIOMAS TREATED WITH FIRST-LINE PCV CHEMOTHERAPY

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The objective of this retrospective study was to evaluate the impact of first-line PCV chemotherapy on low-grade glioma (LGG) growth. For this purpose, the mean tumor diameter (MTD) of 21 LGGs was evaluated on serial magnetic resonance images before ($n = 13$), during and after PCV onset ($n = 21$). During PCV, a decrease of the MTD was observed in all patients. After the end of PCV, though at a slower rate, a continuing decrease of the MTD was observed in 20 out of 21 patients. Median duration of this

persistent decrease was 2.7 years (0–7 years). According to MacDonald's criteria, the rates of partial and minor responses were 44% at the end of PCV (6% partial and 38% minor responses), but 75% at the time of maximal tumor response, after a median of 3.4 years following PCV onset (43% partial and 32% minor responses). A persistent and prolonged decrease of LGGs volume (>2 years) was observed in 60% of the patients despite no more chemotherapy was administered. These results challenge the current view that in LGGs a prolonged chemotherapy treatment is necessary to achieve a prolonged response and also to raise the issue of the mechanisms involved in the persistent tumor decrease once chemotherapy is stopped.

O.60. WHICH MOLECULAR SIGNATURES PREDICT PROGRESSION-FREE SURVIVAL AFTER SURGERY ALONE IN PATIENTS WITH LOW-GRADE GLIOMAS?

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PURPOSE: *TP53* mutations, 1p/19q codeletions, *O*⁶-methylguanyl-methyltransferase (MGMT) promoter methylation, and isocitrate dehydrogenase (*IDH*)-1/2 mutations have been proposed to be associated with outcome in patients with low-grade gliomas. Here we sought to clarify whether these molecular signatures reflect a favorable natural course or a favorable response to radiotherapy or chemotherapy. **Experimental Design:** Ninety-three patients with diffuse astrocytoma WHO grade II (A-II, *n* = 42), oligoastrocytoma (OA-II, *n* = 24), or oligodendroglioma (O-II, *n* = 27), who had not received radiotherapy or chemotherapy after their first operation, were monitored until the end of follow-up (*n* = 34) or until the first progression (*n* = 59), with a median follow-up of 6.1 years. Tumor tissues were analyzed for *TP53* mutations, 1p/19q status, MGMT promoter methylation, and *IDH*-1/2 mutations. **RESULTS:** The estimated median progression-free survival (PFS) was 3.9 years (95% CI 2.9–4.9). Fifty-nine patients progressed and 17 patients died. The relative frequencies were 27.0% (24 of 89) for *TP53* mutations, 41.1% (37 of 90) for 1p/19q codeletions, 43.8% (39 of 89) for MGMT promoter methylation, and 81.5% (75 of 92) for *IDH*-1/2 mutations. *TP53* mutations were uncommon in patients with 1p/19q codeletions. None of the molecular markers was prognostic for PFS, using multivariate adjustment for histology, extent of resection, age, and gender. Similarly, none of the parameters predicted survival from first progression. Solely *IDH*-1/2 mutations were associated with prolonged overall survival. **CONCLUSIONS:** None of the studied parameters is a sensitive prognostic biomarker in patients with grade II gliomas who do not receive radiotherapy or chemotherapy after surgery. *IDH*-1/2 mutations were confirmed to predict longer survival.

O.61. A COMPREHENSIVE STUDY OF THE ASSOCIATION BETWEEN THE *EGFR* AND *ERBB2* GENES AND GLIOMA RISK

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BACKGROUND: Glioma is the most common type of adult brain tumor and glioblastoma, its most aggressive form, has a dismal prognosis. Receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR, ERBB2, ERBB3, and ERBB4) family, and the vascular endothelial growth factor receptor (VEGFR), play a central role in tumor progression. We investigated the genetic variants of *EGFR*, *ERBB2*, *VEGFR*, and their ligands, *EGF* and *VEGF*, on glioma and glioblastoma risk. In addition, we evaluated the association of genetic variants of a newly discovered family of genes

known to interact with *EGFR*: *LRIG2* and *LRIG3* with glioma and glioblastoma risk. **METHODS:** We analyzed 191 tag single nucleotide polymorphisms (SNPs) capturing all common genetic variation of *EGF*, *EGFR*, *ERBB2*, *LRIG2*, *LRIG3*, *VEGF*, and *VEGFR2* genes. Material from 4 case-control studies with 725 glioma patients (329 of whom were glioblastoma patients) and their 1610 controls was used. Haplotype analyses were conducted using SAS/Genetics software. **FINDINGS:** Fourteen of the SNPs were significantly associated with glioma risk at *P* < .05, and 17 of the SNPs were significantly associated with glioblastoma risk at *P* < .05. In addition, we found that one *EGFR* haplotype was related to increased glioblastoma risk at *P* = .009, odds ratio [OR] = 1.67 (95% confidence interval [CI]: 1.14, 2.45). The Bonferroni correction made all *P*-values nonsignificant. One SNP, rs4947986 next to the intron/exon boundary of exon 7 in *EGFR*, was validated in an independent data set of 713 glioblastoma and 2236 controls, OR = 1.42 (95% CI: 1.06, 1.91). **INTERPRETATION:** Previous studies show that regulation of the EGFR pathway plays a role in glioma progression, but the present study is the first to find that certain genotypes of the *EGFR* gene may be related to glioblastoma risk. Further studies are required to reinvestigate these findings and evaluate the functional significance.

QUALITY OF LIFE

O.62. COGNITIVE DEFICITS IN PATIENTS WITH GLIOMAS IN ELOQUENT AREAS BEFORE AND AFTER AWAKE SURGERY

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INTRODUCTION: Cognitive performance is an important outcome measure of brain tumor treatment, as neuropsychologic deficits have an impact on quality of life. Previous neuropsychologic studies found that the majority of glioma patients have deficits in one or more cognitive domains such as language, executive functions, verbal and nonverbal memory, and processing speed. The presence and severity of cognitive disorders is one of the main factors in the decision procedure about awake operations. However, the effect of surgery on these cognitive deficits still needs further investigation. In the present exploratory study, a comprehensive assessment of cognitive functioning was performed before and after awake craniotomy. **METHOD:** Cognitive functioning of patients with gliomas in language or motor areas (*n* = 29) before and 3 months after awake craniotomy was assessed with Aachener aphasia test; Boston Naming Test; Verbal (Letter and Category) Fluency; WordMemory; Stroop Colour-Word Test; Trail Making Test (TMT) A,B; Orientation and Clock drawing. Within 4 days after surgery, a neurologic examination was carried out to screen for functional deficits. Change in cognitive performance was related to the pathology (WHO grade), type, volume, and location of the tumor. **RESULTS:** Before surgery, results on BNT, WordMemory, Verbal Fluency, Stroop Test, and TMT deviated from performance of healthy adults (*P* < .01). Three months after surgery, the same profile of deficits was found. Compared with preoperative assessment, performance slightly decreased on letter fluency (*P* = .015), category fluency (*P* = .036) and TMT B (*P* = .044). Patients with clinically observed language deficits in the direct postoperative stage (62%) consistently declined more on these tests than patients with no direct postsurgical language impairment. The pathology, type, volume, and localization of the tumor did not affect the change of cognitive performance. **Discussion:** Data about cognitive functioning of patients with gliomas in eloquent areas before and after awake surgery are scarce. The results of our study indicate that the global cognitive profile of glioma patients does not considerably change after surgery. The further decline of 3 months after surgery on some language and executive tasks appears to be related to the occurrence of language deficits in the direct postoperative stage. To observe the long-term cognitive outcome, a 1-year follow-up is performed. Furthermore, a larger group of patients will be investigated with a protocol containing more nonverbal tests, to disentangle the influence of language and other cognitive functions (eg, memory, executive functions) on performance of this patient group.

O.63. QUALITY OF LIFE IN HIGH-GRADE GLIOMA PATIENTS AND THEIR RELATIVES IN THE END-OF-LIFE PHASE

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INTRODUCTION: Despite intensive treatment with surgery, chemotherapy, and radiotherapy, patients with high-grade glioma (HGG)

eventually experience tumor recurrence up to a point that no further curative treatment options are available. From that moment on, only supportive treatment is given. In this end-of-life phase, maintaining acceptable quality of life (QOL) as long as possible is the main goal. Previous studies demonstrated that symptom burden increases as death approaches and it is assumed that symptom burden negatively affects QOL of both patients and their relatives. However, until date, no quantitative information regarding QOL in the end-of-life phase is available. The aim of our study was to describe QOL toward the end of life in HGG patients and their relatives. **METHODS:** We identified a cohort of 148 deceased HGG patients diagnosed in 3 Dutch hospitals in 2005 and 2006. Physicians and relatives of patients in this cohort were approached for the study and asked to fill in a questionnaire regarding the end-of-life phase of the specific patient. In this study, the end-of-life phase was divided in the last 3 months before death and the last week before death. Physicians of 93 patients (63%) participated in the study and answered questions concerning symptoms in the end-of-life phase. Relatives of 127 patients could be traced, and 68 relatives (54%) participated in the study. The questionnaire for relatives covered questions regarding symptoms and QOL issues of the patient as well as questions about the relatives' QOL in the last 3 months of the patients' life. Data were recorded descriptively. **RESULTS:** Both physicians and relatives reported loss of consciousness (34%–45%), confusion (53%–75%), incontinence (31%–55%), headache (31%–45%), and seizures (38%–40%) as most important symptoms in the last 3 months of life. Symptom burden increased in the last week of life. According to their relatives, 90% of HGG patients were limited in social activities and would probably have rated their general QOL as poor. QOL of the relatives in the last 3 months of the patients' life was also compromised; 85% of relatives were limited in social activities and 65% felt burn-out. Moreover, in 60% of the cases, the disease disrupted family life and, in 20% of the cases, the disease perturbed the relationship between the patient and his/her proxy. **CONCLUSION:** Symptom burden is high and QOL is rated poor in the end-of-life phase of HGG patients. Informal caregivers report relatively low QOL for themselves as well as high levels of stress. This knowledge could be used to develop specific protocols and interventions to improve the QOL of glioma patients and their relatives.

O.64. MALIGNANT GLIOMA PATIENT AND CAREGIVER CONGRUENCE IN QUALITY OF LIFE REPORTING

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BACKGROUND: Assessing quality of life (QOL) in patients with malignant gliomas (MGs) is often complicated by the progression of neurocognitive impairment and the tendency of patients to minimize aspects of QOL. Accordingly, caregiver reports of a patient's QOL are particularly valuable. The purpose of this study was to investigate QOL concordance between patient and caregiver, to identify relevant discrepancies. **METHODS:** Patients with MG within 6 months of diagnosis or relapse were eligible for this study if they had an involved caregiver. The Functional Assessment of Cancer Therapy-Brain (FACT-Br) was given to patients and caregivers at baseline and then on the day magnetic resonance imaging (MRIs) were done for tumor assessment and continued until tumor progression. Patients were asked to fill out the FACT-Br and their caregiver was asked to fill out the same questionnaire as they perceived the patient would respond. MRI was done approximately every 2 months and questionnaires were given prior to disclosure of MRI results. **RESULTS:** Seventy-two pairs of FACT-Br scales were collected over the course of the study from 25 patient-caregiver pairs. A consistent discrepancy between patient and caregiver was seen. Patients reported their overall QOL to be better than perceived by their caregivers by an average of 6.8 points on the 200-point scale ($P = .01$). This difference was observed similarly for patients with newly diagnosed and recurrent MG and their caregivers. Significant differences were found within subscales of physical ($P = .03$), emotional ($P = .01$), and functional ($P = .02$) well-being. Social well-being was the only subscale where a significant discrepancy was not noted. The average score for the FACT-General was 77.4 in this sample of MG patients, lower than the national average of 80.1 across all cancers. **CONCLUSIONS:** The results indicate patients consistently report their QOL to be more favorable than perceived by their caregivers. This finding underscores the importance of including caregivers in clinical assessments to obtain a comprehensive view of patient QOL and functional status. Physician-caregiver communication is essential to ensure quality care for patients with MG.

O.65. PREDICTORS OF QUALITY OF LIFE OF PARTNERS OF HIGH-GRADE GLIOMA PATIENTS

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Although primary brain tumors account for only 2% of all cancers, they may severely disrupt a patient's daily life activities because of the multiplicity and severity of the symptoms associated with this disease. The physical, cognitive, and behavioral symptoms caused by the tumor and its treatment do not only affect the patient, but also the ones living with the patient. In their new role as caregivers, partners of these patients may experience a great deal of stress and caregiver burden, negatively affecting their quality of life (QOL) and thus their ability to cope with their new caregiver tasks. The present study aims at (i) evaluating QOL of partners of high-grade glioma patients and (ii) determining which partner and patient-related factors affect partners' QOL. Forty-eight patient-caregiver dyads participated in this study. Most patients were diagnosed with a GBM ($n = 32$). The remainder had anaplastic oligodendrogliomas ($n = 9$), anaplastic astrocytomas ($n = 2$), or WHO grade III oligoastrocytomas ($n = 2$). Partners were somewhat more often female ($n = 29$) than male. Mean age of the partners was 51.0 years ($SD = 11.2$). All partners filled out extensive questionnaires concerning their QOL (SF36), feelings of depression and anxiety (HADS), and caregiver mastery (CMS). Additionally, partners reported their perceptions of patients' health-related quality of life (HRQOL) (SF36), neurological functioning (BCM20), and cognitive functioning (MOS). Compared with general population controls, matched for age, sex, and educational level, caregiving partners reported better physical functioning ($P = .000$), but poorer mental functioning ($P = .002$). Expectantly, partners' feelings of caregiver mastery ($P = .000$) and feelings of anxiety and depression ($P = .000$) strongly predicted their mental functioning. In addition, HRQOL ($P = .002$) and neurological functioning ($P = .015$), but not cognitive functioning ($P = .342$) of the patient, were predictive of mental functioning of the partners. Neither patient nor partner variables predicted the physical functioning of the partners. Our study demonstrates that partners of high-grade glioma patients experience poorer mental functioning. These limitations in mental functioning are most strongly predicted by their own feelings of depression and anxiety and feelings of caregiver mastery. Health-related quality of life of the patient and their neurological functioning also predicted the mental functioning of the partners, however, to a lesser extent. These results suggest that partners, in their new role as caregivers of high-grade glioma patients, might benefit from psychological interventions aimed at the enhancement of their quality of life.

O.66. GLIOBLASTOMA IN ELDERLY PATIENTS: HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN A RANDOMIZED TRIAL COMPARING 6-WEEKS OF TADIOTHERAPY (RT) VS HYPOFRACTIONATED RT OVER 2 WEEKS VS TEMOZOLOMIDE CHEMOTHERAPY (TMZ)

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BACKGROUND: Despite advances in treatment, survival of patients with GBM over 60 years is still often less than 1 year. In the perspective of a short expected survival, the quality of the remaining life and the effects of therapy on health-related quality of life (HRQoL) should be given special emphasis when recommending treatment for the individual patients. Several studies have focused on survival of the elderly, but few data are available on HRQoL for different treatments. In a randomized trial, we compared survival and HRQoL for 3 treatment options, 6 weeks of RT, vs hypofractionated RT, or chemotherapy with TMZ. **MATERIALS AND METHODS:** Newly diagnosed GBM patients, age

≥60 years with PS 0–2, were randomized to either standard RT (60 Gy in 2-Gy fractions over 6 weeks), hypofractionated RT (34 Gy in 3.4-Gy fractions over 2 weeks), or 6 cycles of chemotherapy with TMZ (200 mg/m² day 1–5 every 28 days). QoL was determined by the EORTC QLQ 30 questionnaire and the Brain Cancer Module at inclusion, before start of therapy, at 6 weeks, 3 months, and 6 months after start of treatment. Patients were followed until death. The primary study endpoint was overall survival (OS) and secondary objectives were HRQoL, neurologic symptom control, and safety. RESULTS: A total of 342 patients were included and 292 patients were randomized between the 3 treatment options and 50 patients between hypofractionated RT and TMZ. Median age was 70 years (range 60–92) with 58% being male. Performance status was 0–1 for 75% of patients and 73% had undergone surgical resection. CONCLUSION: The results from the HRQoL analysis of this trial will be presented together with survival data at the upcoming EANO meeting.

MISCELLANEOUS

O.67. HAVE CLINICAL FEATURES AND TREATMENT OUTCOME OF 166 PATIENTS WITH NEUROLYMPHOMATOSIS (NL) CHANGED OVER A PERIOD OF 36 YEAR? ASSESSMENT OF A CONTEMPORARY INTERNATIONAL PRIMARY CNS LYMPHOMA COLLABORATIVE GROUP (IPCG) SERIES AND LITERATURE CASE REVIEW

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Neurolymphomatosis is a rare clinical entity characterized by infiltration of peripheral nerves, nerve roots, plexus, or cranial nerves by malignant lymphocytes. The IPCG retrospectively analyzed 50 patients (Group A) assembled from 12 centers in 5 countries over a 16-year period. As 70% of patients in this series were diagnosed during the last 8 years, we tried to compare the contemporary series with literature review. The latter included case reports of 44 patients published from 2001 to 2008 (Group B) which corresponds to the period of diagnosis of the greater fraction of our patients, and 72 patients (Group C) identified earlier during a 28-year period (1972–2000). Median age (55.5 years) and male preponderance (60%) in our series were similarly noted in Groups B and C. NL presented as the first manifestation of malignancy in 26% and 29% of Groups A and B, respectively. The predominant malignant cell type was B-cell, whereas T-cell type occurred in 10%, 25%, and 5% of Groups A, B, and C. In our series, NL affected more than 1 anatomic structure in 58% of patients with peripheral nerves being the most frequently involved site (60%) while spinal, cranial nerve, and neural plexus infiltration occurred at a similar rate (40%–48%). Similar observations were noted for Group C. Painful neuropathy was frequent (76%, 57%, 47% for Groups A, B, and C) with sensorimotor type being the most common. The yield of imaging studies was high with positive MRI reported in >70%. FDG-PET was performed in 40 patients (Groups A and B) and suggested the diagnosis in 84% and 90%, respectively. CSF cytology was positive in 40% across series and biopsy (76 patients) confirmed the diagnosis in 88%, 90%, and 80% in Groups A, B, and C. NL was diagnosed only at autopsy in 46% of Group C patients as opposed to Groups A and B where it diagnosed 8% and 5% of patients. Treatment for NL was given to 124 patients with response rate ranging between 46% and 72%. High-dose methotrexate was used more often in our series while intra-CSF therapy was given to almost 40% of the treated patients in all series. Survival was not reported previously and in our series the median overall survival was 10 months with 12 and 36 months survival proportions of 46% and 24%, respectively. In conclusion, NL is a challenging diagnosis but contemporary imaging techniques frequently detect the relevant neural invasion. An aggressive multimodality therapy can prevent neurological deterioration and is associated with a prolonged survival in a subset of patients.

O.68. EFFICACY OF TAILORED TREATMENT FOR HIGH- AND LOW-RISK MEDULLOBLASTOMA IN ADULTS: A LARGE PROSPECTIVE PHASE II TRIAL

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PURPOSE: To assess the efficacy of treatment of medulloblastoma (MB) in adults (>18 years). METHODS: Ninety-five MB patients were enrolled a prospective phase II trial conducted between 1/1989 and 2/2009; 30 low-risk (LR) patients (T1, T2, T3a, M0, without postoperative residual disease) underwent radiotherapy (36 Gy) to the craniospinal axis, supplemented by a local tumor dose (18.8 Gy; total, 54.8 Gy), and 65 high-risk (HR) patients (T3b–T4, or postoperative residual tumor) received 2 cycles of “up-front chemotherapy” (cisplatin 2.5 mg/m²/daily for 4 days, etoposide 40 mg/m²/daily for 4 days, and cyclophosphamide 1,000 mg/m² on day 4; every 4 weeks) before the same radiation therapy, followed by maintenance chemotherapy if M1, M2, or M3 disease was present. RESULTS: Progression-free survival at 5 and 10 years (PFS-5y and PFS-10y) were 78% (62–93) and 46% (23–70) in LR vs 50% (37–62) and 36% (23–49) in HR ($P = .009$ and $P = .03$, respectively) patients. Survival at 5 and 10 years (OS-5y and OS-10y) was 92% (81–100) and 65% (43–7) in LR vs 58% (46–71) and 45% (31–8) in HR ($P = .002$ and $P = .02$, respectively). Five-year and 10-year PFS was 68% (50–85) and 54% (34–74) in M0 patients vs 35% (18–51) and 19% (2–35) in M1–2–3 patients ($P = .007$ and $P = .006$). OS at 5 and 10 years were 71% (54–88) and 62% (43–81) in M0 vs 47% (29–65) and 29% (11–47) in M1–2–3 patients ($P = .09$ and $P = .04$); residual disease had no significant impact on 10-year PFS or 10-year OS. There were no deaths from toxicity, which was mainly hematological and successfully managed with dose reductions and supportive care. CONCLUSIONS: Since the incidence of MB in adults is extremely rare, data appearing in literature on this condition have been reported in small retrospective series. The findings made in the present prospective study on a large series of patients, the first of its type to appear in literature, clearly indicate the standard of care in MB in adults, and should constitute a benchmark for further studies.

O.69. PLASMA IGE LEVELS CORRELATE WITH THE DIAGNOSIS AND PROGNOSIS OF GLIOMA PATIENTS

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BACKGROUND: Previous studies have shown that glioma patients report allergies less frequently, and have lower IgE levels than controls. To evaluate its potential as a surrogate biomarker for glioma, we measured plasma IgE levels in glioma patients and healthy controls, and correlated them with clinicopathological factors and the patients' outcome. METHODS: We used enzyme-linked immunosorbance assay (ELISA) to determine the plasma IgE levels of 25 normal subjects and 232 glioma patients (85 grade II glioma patients, 40 grade III glioma patients, and 107 GBM patients). We also collected longitudinal plasma samples from 70 patients with GBM and compared the plasma IgE levels before operation, 1 week after operation, in the middle of radiotherapy, after 2 cycles of chemotherapy, and after recurrence. We determined the correlation between plasma IgE levels and the outcomes of the patients. RESULTS: Plasma IgE levels were significantly lower in glioma patients ($P = .004$), low-grade glioma patients have lower IgE levels than high-grade glioma patients do ($P = .029$). Oligodendroglial tumors have higher IgE level than astrocytic tumors and mixed tumors both in grade II ($P = .014$) and grade III ($P < .001$) glioma patients. In 24 patients with paired preoperational and 2 cycles chemotherapy plasma samples, IgE levels increased after successful removal of the tumor ($P = .002$), and the increase correlated with the patients' survival (increase >100 vs ≤100 ng/mL, 127.5 vs 62.3 weeks. $P = .012$, log-rank). Plasma IgE level increase of >100 ng/mL has 80% specificity and 78% sensitivity to predict the patients' long survival (>18 months). CONCLUSIONS: Plasma IgE levels correlate with clinical and pathological factors in glioma patients. Our results suggest that plasma IgE levels have the potential to become a biomarker for glioma patients.

O.70. ANTI-ANGIOGENIC TREATMENT IN A CLINICALLY RELEVANT GLIOBLASTOMA MODEL REDUCES BLOOD FLOW AND INCREASES TUMOR CELL INVASION

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INTRODUCTION: Glioblastoma (GBM) is a highly vascularized tumor, and endothelial cell proliferation is one of the neuropathologic hallmarks of the disease. Therefore, the concept of interfering with the generation of new blood vessels is an attractive strategy against GBM. Recent clinical trials have shown good response rates with bevacizumab, an antibody against vascular endothelial growth factor (VEGF). Yet, the effect is short lived and the physiologic mechanisms of bevacizumab action and the biologic consequences are poorly understood. **MATERIAL AND METHODS:** Here, we assessed the response to bevacizumab of highly angiogenic and invasive GBM xenografts, obtained by serial passaging of human GBM biopsies in nude rats. Animals with orthotopic tumors received weekly i.v. injections of 10 mg/kg bevacizumab for 3 weeks. Before sacrifice, animals were analyzed by magnetic resonance imaging (MRI) on a 7T Pharmascan (Bruker). MRI protocols included T1- and T2-weighted sequences to assess tumor morphology and edema, MR spectroscopy to assess key metabolite concentration, diffusion-weighted imaging to assess cellularity, and dynamic contrast enhancement MRI to assess tumor perfusion, and vascular permeability. After sacrifice, tumors were harvested for transcriptomic, proteomic, and metabolomic studies as well as histology and immunohistochemical analysis. **RESULTS:** In agreement with clinical data, we found that bevacizumab reduces vessel permeability and leakiness of the blood-brain barrier as reflected by the loss of contrast enhancement and reduced K_{trans} and V_e parameters. Interestingly, bevacizumab reduced blood flow and blood volumes, while spectroscopy data point at increased lactate concentration in treated tumors. A reduction in vessel number was observed while the extent of cell infiltration in the brain parenchyma was significantly increased. Key angiogenesis-related genes were upregulated after treatment. **CONCLUSION:** Bevacizumab treatment in GBM leads to a reduction of blood vessels and increased tumor hypoxia which is accompanied by increased cell invasion. A novel model of tumor cell plasticity involving a metabolic switch will be discussed.

O.71. THE QUEST FOR HUD-SPECIFIC T CELLS: ATTEMPTS TO GENERATE A HUD-SPECIFIC T-CELL LINE

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In the Hu paraneoplastic syndrome, a T-cell-mediated immune response targeted against the intracellular protein HuD is thought to be the cause of neurologic disease and neuronal destruction in patients with HuD expressing tumors. However, HuD-specific T cells are rare, and extremely difficult to detect. Moreover, no HuD-specific T-cell lines are available to validate possible test strategies. Therefore, we tried to generate a HuD-specific T-cell line using two different approaches: (i) in vitro induction of HuD-specific T cells and (ii) selection and expansion of HuD-specific T cells from peripheral blood of Hu-PNS patients. In the first experiment, peripheral blood mononuclear cells (PBMNC) were drawn from 3 healthy CMV seronegative subjects and divided over 5 parallel cultures. Unloaded dendritic cells (DCs), and DCs loaded with HuD protein, HuD peptide fragments mix (protein-spanning, overlapping 15-mers), PP65 protein, or PP65 peptide mix were added to the subsequent cultures. Readout by intracytoplasmatic IFN- γ production and addition of loaded DCs was performed at day 11, 21 and 31. In 3 of 3 subjects, a positive response to PP65 peptide mix, and in 2 of 3 a positive response to PP65 protein was found. However none of 3 patients showed a significant response to the HuD protein or HuD peptide mix. In the second experiment, PBMNCs were drawn from 4 patients with a definitive diagnosis of Hu-PNS and divided in to 4 parallel cultures. These cells were stimulated with IL-2, and peptide-loaded autologous PBMNCs were added every 2 weeks using the same peptides as in experiment 1, except PP65 protein. Readout was performed every 2 weeks by flowcytometric intracellular IFN- γ and TNF- α staining. This regimen was continued 8–12 weeks. None of the 4 patients showed positive results to HuD protein or peptides. One of the patients was CMV seropositive, and indeed showed IFN- γ production upon stimulation with PP65 mix. These experiments show that, although our methods were successful in the context of cytomegalovirus infection, these seem not suited for production of an HuD-specific T-cell line, nor detection of HuD-specific T cells. Either the culture strategy does not stimulate HuD-specific T cells properly, or our readout method is not sensitive enough. Therefore, we recently started using an autologous feeder system, lowered the interval of adding stimulator cells to 1 week, and additionally performed readouts using flowcytometric CD

107a and CD 137 staining as markers for degranulation and T-cell activation. If successful, HuD-specific T-cell lines would enable us to validate the methods used so far to detect HuD specific T-cell, and would offer an unique opportunity to study HuD-specific T-cell function in vitro.

O.72. COMBINED USE OF MONOPOLAR AND BIPOLAR MOTOR MAPPING FINDINGS AND CORRELATION WITH DTI DATA DURING SURGICAL REMOVAL OF LESIONS INVOLVING MOTOR PATHWAYS

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Surgery of lesions involving motor areas or pathways requires their intraoperative identification to guide resection and preserve functional integrity. The brain-mapping technique allows performing such a identification. DTI-FT reconstructs tracts, including CST, and SMA. This work reports the correlation of neurophysiologic findings obtained during removal of lesions located in or within M1, SMA or CST, and DTI data. The correlation was performed on 280 patients with gliomas. The neurophysiology includes mapping, with direct electrical stimulation (DES, bipolar, and monopolar), and monitoring (EEG, ECoG, and MEP) procedures. Motor responses were evaluated by multichannel EMG. DTI-CST (3T DTI maps) was available in all patients, and in 40 also with different FA thresholds. DTI-CST and SMA was loaded onto the neuronavigation system and available intraoperatively for correlation with DES data. Motor responses were registered in all patients. They appeared as focal when CST was stimulated close to the surface, or affecting multiple muscles with deep stimulation. The near-cortical stimulation induced overt movements, and deep subcortical stimulation induced muscle activations detected by EMG. In more than 95% of patients, a high level of correlation was observed between DTI-FT data and DES findings. When CST was highly infiltrated, DTI-FT failed to show fibers in the upper part of CST, where DES induced responses. In such cases, bipolar stimulation did not evoke responses, which were induced by monopolar stimulation. Monopolar stimulation evoked responses in a large cortical and subcortical area, even faraway from CST: indeed, bipolar stimulation allowed a more precise localization of CST, well correlated with DTI-CST. Monopolar stimulation was useful in patients with a long seizure history to successfully localize the CST and decrease the occurrence of intraoperative seizures. FA varied among the same area of the tumor and in its deep portion, accordingly with the degree of infiltration of tract, and results varied according to the stimulation modality. DTI-CST data showed a good correlation with DES findings. DES techniques, bipolar and monopolar, evoked motor responses in all patients. The combined use of bipolar and monopolar DES and DTI-FT allowed to effectively and safely trace the tract.

POSTER PRESENTATIONS

[Poster numbers marked with * will also be presented orally in a Poster Session]

CELL BIOLOGY AND SIGNALING

P.001*. PROTEIN TYROSINE PHOSPHATASES IN GLIOMA BIOLOGY

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Receptor tyrosine kinases (RTKs) such as EGFR, PDGFR, and MET are well known to have an important role in oncogenic signaling in gliomas. Phosphorylation of tyrosine residues on proteins through such RTKs can be counteracted by protein tyrosine phosphatases (PTPs). An important role for PTPs as “flip side of the coin” for RTK activity in glioma oncogenesis is therefore to be expected. Although the PTP PTEN is clearly functioning as a tumor suppressor in high-grade gliomas, the role of other PTPs is still largely unknown. To elucidate the relevance of PTPs in glioma biology, we first performed an in depth literature search that yielded information on 15 out of the 107 PTP genes present in the human genome to be potentially implicated in glioma biology. Besides PTEN, overexpression of PTPRZ is clearly associated with these tumors, although its exact function in oncogenesis is not clear at present. Also inactivating mutations, including

homozygous microdeletions, in PTPRD have been reported. Furthermore, some interesting PTPs that can counteract receptor tyrosine kinases, including TCPTP (dephosphorylates EGFRvIII), PTPRJ (counteracts PDGFR, VEGFR2, and MET), and several PTPs that influence cell migration are on this list of PTPs that may regulate outgrowth of glioma cells. To extend our knowledge on the role of PTPs in glioma biology, we performed expression profiling (Affymetrix U133 Plus 2 platform) and evaluated mRNA expression levels in glioma biopsies. RNA extracted from >70 glioma samples was hybridized to Affymetrix U133 Plus 2 arrays and data were imported in the dCHIP software program. Comparing different groups of glioma (eg, oligodendroglioma vs GBM, normal vs amplified EGFR), several PTPs were identified that displayed differential expression profiles. We further analyze the relevance of these candidates for glioma biology by exploiting overexpression and/or knockdown experiments in relevant orthotopic glioma xenograft models. Altogether, increasing evidence suggests that certain PTPs play a fundamental role in glioma biology. Interference with such PTPs may complement the current therapeutic approaches and thereby contribute to the improvement of the prognosis for patients for these so far incurable tumors.

P.002* . DOWNREGULATION OF MEMBRANE PROTEIN UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR ASSOCIATED PROTEIN MAKES GLIOMA CELLS IMMOBILE AND CAN BE A TARGET FOR NOVEL GLIOMA THERAPY

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The aim of this study was to identify a new target molecule that can be utilized for glioma anti-invasion therapy. In the present study, we have identified 4 candidate genes that express higher in glioma tissues compared with normal brain control by cDNA microarray analysis. Among the 4 genes identified, we focused on a membrane protein; urokinase-type plasminogen activator receptor associated protein (uPARAP), which is one of the members of urokinase plasminogen activator system since previous reports discussed its relationship to cancer metastasis in breast cancer. uPARAP protein was expressed 4 of 4 (100%) glioma samples regardless of its World Health Organization grade, but did not express in normal brain control. Introduction of 2 independent small-interfering RNAs targeted uPARAP into 2 different glioma cell lines (KNS42 and KNS81), resulted in downregulation of uPARAP. Boyden chamber migration and invasion assay showed that downregulation of uPARAP decreases both invasiveness and migration property in vitro. Phalloidin staining showed that in uPARAP knocked-down glioma cells, polymeric actin became organized in stress fibers and the lamellipodia disappeared. On the basis of our findings, we suggest that RNA interference-mediated downregulation of uPARAP decreases invasion and migration property in glioma cells in vitro. The inhibition of invasion and migration property was mediated via reorganization of the actin cytoskeleton. Downregulation of uPARAP could be a novel anti-invasion therapeutic strategy for malignant gliomas.

P.003* . METABOLOMIC CHARACTERIZATION OF STEM-LIKE GLIOBLASTOMA CELL LINES

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INTRODUCTION: The biology of glioblastoma multiforme (GBM) is poorly understood, but there is currently great interest in the metabolomic make-up of this cancer type. **METHODS:** Cell lines derived from human GBM tissue were cultured under serum-free conditions following the Cambridge Protocol, which enriches the culture with tumor competent, self-renewing cells. We used ¹H-NMR to analyze the concentrations of metabolites in cell extracts and cell media for 4 stem-like GBM cell lines both before and after they had been induced to differentiate by mitogen withdrawal and addition of serum. **RESULTS:** Using principal component analysis, it was possible to determine the differences between the metabolic profiles of the 4 cell lines tested, and to detect significant changes in their metabolomic profile after cell differentiation. Most of the metabolites contributing to these changes have now been identified. Further data mining by carbon flux analysis, which quantifies the changes, shows that they are consistent between all 4 cell lines. **CONCLUSION:** Our data suggest that myo-inositol, which is present in the stem-like state, is reduced to undetectable levels by differentiation. Also several amino acids show different secretion and consumption patterns in the differentiated state compared with the initial stem-like state.

P.004* . REVERSAL OF EFFECT OF U87 DERIVED MICRO-VESICLES ON BIOLOGICAL PROCESSES OF GLIOBLASTOMA MULTIFORME

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Micro-vesicles, also known as exosomes, are extracellular, membrane-bound vesicles derived from the intraluminal membranes of multivesicular bodies (MVBs) of the endocytic pathway. These MVBs fuse with the plasma membrane, which causes the release of vesicles into the extracellular environment. Various cell types, including tumor cells, have been shown to produce micro-vesicles, which are believed to play a role in signal transduction. Recently, glioblastoma-derived micro-vesicles have been shown to contain proteins and RNA. Since micro-vesicles from different tumor cells appear to be involved in several biological processes, we set out to investigate the influence of U87 micro-vesicles on multiple biological processes, including angiogenesis and proliferation. After that, we treated U87 cells with interferon- β and isolated their micro-vesicles. These micro-vesicles were used to assess effects on the above-mentioned biological processes. We found that micro-vesicles derived from untreated, but not from treated, U87 cells stimulate angiogenesis of glioblastoma cells. Micro-vesicles from both cells promote proliferation. Since we found that exosome production was unchanged, we studied the fusion capacity of these micro-vesicles as well as their protein and RNA content. Differences between the untreated and treated exosomes were observed. In conclusion, U87 micro-vesicles influence the behavior of glioblastoma cells. This can be reversed by treating the cells with interferon- β .

P.005* . TARGETING THE RELAPSE-INDUCING CELL POPULATION OF GLIOBLASTOMA

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OBJECTIVE: Residual glioblastoma (GBM) cells that persist in the surrounding parenchyma after complete macroscopic resection represent one of the major driving forces of mortality in GBM. While exposed to post-surgical therapy, little is known on their biology. It was the goal of this study to isolate and profile these potentially relapse-inducing cells. **PATIENTS AND METHODS:** Paired tissue specimens were obtained from 33 GBM patients. Residual GBM cells were derived from experimental biopsy of the resection margin after completion of standard neurosurgery. A second tissue sample, used as an internal reference, represented the routinely resected tumor core. Tumor cells were isolated, expanded under controlled in vitro conditions, and profiled (histology, growth kinetics, invasion studies, self-renewal analysis, qPCR, combined SNP/FISH analysis, FACS, in vitro drug-response assays, and xenotransplantation) in direct comparison. **RESULTS:** Sample analysis revealed residual cells as distinct malignant subentities in GBM. They fulfill the functional criteria of (rapidly proliferating, highly invasive) transit amplifiers rather than to represent (multipotent, self-renewing) stem cells. Stem-like GBM cells were almost exclusively detected in the routinely resected tumor core (71% of the center vs 14% of the periphery samples). Expression analysis revealed in 52 of 72 comparative measurements that mRNA levels of PDGFR-A/B, TGFB-2, TGFR-1, VEGFR-2, VEGF-A, CD44, L1CAM, CD133, and/or uPAR transcripts varied more than 50% between core and residual cells of the same GBM patient. Also, in 16 of 25 comparative measurements, different in vitro responses to radio- and/or chemotherapy (CCNU, Temozolomide) were noted. We have furthermore obtained multiple specimens from tumor core vs resection margin regions to closely assess the degree of intragroup heterogeneity. These experiments similarly revealed residual cells as clearly distinguishable from GBM core cells in every patient investigated ($n = 5$). Ongoing studies focus on the question whether these dissimilarities arise from distinct environmental cues in situ or whether GBM residual cells represent a truly independent cellular subentity. **CONCLUSION:** Residual cells are unique cellular targets in GBM. They could be responsible for the recurrence of disease. Thus, characterization of residual tumor cells at the time of initial surgery may open new avenues for future diagnosis and treatment of GBM.

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P.006*. CARBON ION IRRADIATION WITH OR WITHOUT THE ADDITION OF CILENGITIDE IS AN EFFECTIVE INHIBITOR OF INTEGRINE-MEDIATED TUMOR CELL MIGRATION

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BACKGROUND: Multiple extracellular matrix proteins have been described to promote glioma cell motility accounting for infiltrative growth. Fibronectin (Fn) and vitronectin (Vn) have recently been targeted by cilengitide (CGT), a cyclic peptide known to inhibit $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins that interact with Vn ($\alpha_v\beta_3/\alpha_v\beta_5$) and Fn ($\alpha_v\beta_3$ /others). Implemented in most glioma treatment regimes, radiotherapy (RT) was also shown to alter integrin signaling. We recently demonstrated that photon irradiation enhances tumor cell migration at low doses, thus, possibly promoting tumor cell infiltration into the adjacent healthy brain. In the present study, we analyzed the effects of carbon ion irradiation on glioma cell migration \pm the addition of CGT. **METHODS:** Twenty-four hours before migration experiments and FACS analyses, U87 glioma cells were irradiated with single photon doses of 1, 2, and 10 Gy using 6 MV photons at a linear accelerator. Particle radiotherapy was applied with an extended Bragg peak ($E = (128 \pm 7) \text{ MeV/u}$, $LET = (91.5 \pm 1.5) \text{ keV}/\mu\text{m}$) at single carbon ion doses of 0.5 and 3 Gy at the Heidelberg Ion Therapy Center (HIT). The migration chambers were separated by 8- μm pore size polycarbonate membranes coated with Fn and Vn. CGT was added to the upper well. After 5 hours of migration, the membranes were stained and analyzed microscopically by an investigator blinded to experimental setup. Quantitative FACS analysis of integrin expression was performed with a B&D FACScan using PE- and FITC-labeled antibodies directed against $\alpha_v\beta_3$ and $\alpha_v\beta_5$. **RESULTS:** Expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ was not altered by CGT. In migration assays, CGT inhibited transmigration through Vn- but not Fn-coated membranes. Photon irradiation increased migration on both Fn and Vn at low doses of 2 Gy. Addition of CGT to photon-irradiated cells decreased transmigration through Vn- but not Fn-coated membranes. FACS analyses revealed an increased expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ following low-dose photon irradiation, which was still detected after single doses of 10 Gy. On the contrary, carbon ion irradiation alone significantly inhibited both Vn- and Fn-based transmigration and fully abrogated any migration if combined with CGT. Accordingly, expression of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ was decreased following carbon ion doses of 0.5 and 3.0 Gy. **CONCLUSION:** Low doses of photons, such as applied in fractionated RT, bear the risk of promoting glioma cell migration on Vn and Fn. CGT may additionally be administered to counteract photon-induced hypermigration toward Vn; however, Fn-based migration is not affected by CGT. Carbon ion irradiation achieves strong inhibition of migration on both Vn and Fn, which is further increased by combination with CGT. Therefore, local infiltration of glioma cells may be prevented efficiently by using carbon ion RT in regimes combined with molecular targeted therapies.

P.007*. IGF/IGFBP SIGNALING IN MERLIN-DEFICIENT TUMORS

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All schwannomas, 50%–60% of meningiomas, 29%–38% of ependymomas, and all tumors as part of the inherited tumors disease. Neurofibromatosis 2 (NF2) are caused by loss of merlin. Current therapies for merlin-deficient tumors especially in NF2 are insufficient, leaving patients with severe morbidity. There is a need for new therapies. We focused on schwannomas as they are a hallmark of NF2 and serve as serve as a model for merlin-deficient tumors. We aim to define therapeutic targets for schwannoma treatment. Using our *in vitro* model for human schwannoma, we showed the overexpression/activation of platelet-derived growth factor receptor- β (PDGFR- β) and ErbB2/3 in schwannoma leading to strong activation of extra-cellular signal-regulated kinase 1/2 (ERK1/2) and AKT and increased proliferation which we successfully inhibited by Sorafenib, AZD6244, and Lapatinib. Basal proliferation was partly dependent on PDGFR- β and was independent on ERK1/2 and ErbB2. Increased adhesion of schwannoma was also PDGFR- β independent. These data suggest the involvement of additional factors. We have in this study investigated insulin-like-growth-factors-I/II (IGF-I/II) as they are important for Schwann cells, regulate adhesion, proliferation, and survival via ERK1/2/AKT/FAK basally activated in schwannoma and are upregulated in cancers. IGF-binding proteins (IGFBPs) are also upregulated in cancer-regulating cell proliferation, differentiation, and survival. We show here that IGF-I/II and IGFBP-1 are overexpressed/released from schwannoma cells and increase proliferation and adhesion. IGF-I receptor is also overexpressed and activated in schwannoma cells. We suggest that IGF/IGFBP system is involved in schwannoma development. Targeting IGF/IGFBP

system together with PDGFR- β and possibly ErbB2/3 pathways would be an excellent approach in schwannoma treatment. We show dissection of respective pathways that seem crucial for any educated drug therapy being it mono or combinational therapy.

P.008. DOES BIPARTITE CLUSTER OF 7 + 46 MICRORNAS ON CHROMOSOME 14Q32.31 PLAY A ROLE IN GLIOMAGENESIS? I. Lavon, A. Granit, O. Einstein, and T. Siegal; Hadassah Hebrew University Medical Center, Jerusalem, Israel

BACKGROUND: We demonstrated that gliomas exhibit a microRNA (miRNA) expression profile that is reminiscent of neural precursor cells (NPCs) (Lavon et al. Neuro-Oncology, 2010). There are obvious similarities in the self-renewal capacity of stem cells and cancer cells but the tumorigenic role of miRNAs that display similar expression profile in gliomas and nontransformed NPCs have not been established yet. The bipartite cluster of 7 + 46 miRNAs on chromosome 14q32.31 was uniformly downregulated in all gliomas tissues as well as in NPCs. This region is frequently deleted, or genetically altered, in gliomas and in other haematopoietic and solid malignancies. Therefore, we assumed that it might represent a large tumor suppressor miRNA cluster. **OBJECTIVE:** To study the function of individual miRNAs from chromosome 14q32.31-miRNA cluster in gliomas. **METHODS:** To evaluate the role of the investigated miRNAs, we cloned the pri-microRNA into a lentivirus-based vector under the control of CMV promoter. This vector is co-expressing green fluorescent protein as a reporter gene, permitting tracking of infected cells. U87MG glioma cell line was transduced with either a lentivirus-based vector that contains one of the miRNAs on 14q32.31 or with an empty vector. Expression of the mature miRNAs was evaluated by real-time RT-PCR. The effect of each miRNAs on cell proliferation was tested by cell titer blue assay. **RESULTS:** Enforced expression of individual miRNAs from the miRNA cluster on chromosome 14q32.31 was achieved by retroviral infection of U87MG glioma cell line. Overexpression of 14q32 miR1 reduced the proliferation rate of the U87MG cell line in a dose-dependent manner. Overexpression of 2 of the tested miRNAs (14q32miR1 and 14q32miR2) induced spheroid-like cell morphology. **CONCLUSIONS:** miRNA members derived from the miRNA cluster in chromosome 14q32.31 might play a role in the proliferation rate and morphology of gliomas. Further investigation is needed to uncover their role in gliomagenesis. The effect of these miRNA on invasion, soft agar colony formation, and apoptosis is currently tested *in vitro* and their effect on tumorigenicity is investigated *in vivo*.

P.009. BIM MEDIATES GEFITINIB-INDUCED APOPTOSIS IN GLIOMA CELL LINES EXPRESSING WILD-TYPE EGFR

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BACKGROUND: Tyrosine kinase inhibitors (TKIs), as gefitinib, are currently been used for the treatment of human tumors, including malignant glioma as a second-line treatment. Previous studies in lung cancer have observed that Bim, a pro-apoptotic protein from the Bcl2 family, is involved in the apoptotic effect of TKIs. They also propose that either inhibition in PI3K/Akt pathway or MEK/Erk pathway causes an increase in Bim levels. In this study, we analyze the apoptotic effects of gefitinib treatment and Bim expression in glioma cell lines. **MATERIAL AND METHODS:** Seven glioma cell lines (U118, SW1088, A172, SW1783, GOS3, SF767, and T98G) were treated for 48 hours with 10 μM of gefitinib or with solvent DMSO alone in a serum-free medium with 100 ng/mL of EGF. Apoptosis was assayed by flow cytometric analysis by Annexin V-FITC staining. Protein expression of Bim, p-Akt, Akt, p-Erk, Erk, and tubuline were performed by Western blot (WB) using total protein lysates from cell cultures. For WB, before collecting, cells were treated for 15 minutes with 50 ng/mL of EGF to activate the EGFR pathway. Detection was performed with IRDye680/800CW-conjugated secondary antibodies and quantification of proteins bands was carried out with Odyssey (Licor Bioscience) software. Bim gene copy number (BCL2L11) was analyzed by Multiple Ligation-dependent Probe Amplification Sequencing analysis of exons 18–21 of EGFR were done to study mutations in cell lines. **RESULTS:** None of the cell lines showed EGFR mutation in exons 18–21 and none of them showed deletion or gain of BCL2L11. Two of the 7 cell lines (SF767, U118) suffered apoptosis after treatment with gefitinib. These cell lines showed a decrease in Akt and Erk phosphorylation as well as an increase in Bim expression after treatment. Among the 5 cell lines that did not suffered apoptosis, 2 of them (GOS3 and SW1088) showed a reduction in p-Akt and an increase in Bim expression after gefitinib treatment. A decreased level of p-Erk in the other 3 cell lines

(T98, A172, and SW1783) after treatment was not enclosed with a BIM increase. **CONCLUSION:** Bim mediates gefitinib-induced apoptosis in glioma cell lines, independently of mutational status of EGFR, as opposed to results obtained in lung cancer cells. Bim expression increases when p-Akt levels decrease, confirming PI3K-AKT regulation of Bim. Moreover, an activation of Bim itself cannot induce apoptosis in glioma cell lines, suggesting that alternative mechanisms would be necessary to induce apoptosis.

P.010. ROLE OF KITENIN IN MIGRATION AND INVASION OF U251MG HUMAN MALIGNANT GLIOMA CELLS

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OBJECTIVE: Wnts have important roles in multiple cellular processes during development, including cell differentiation, migration, polarity, and proliferation. KITENIN is a major molecule in the Wnt/planar cell polarity (PCP) pathway. The objective of this study was to determine whether and in which way KITENIN modulates the migration and invasion of a glioblastoma cell line. **MATERIAL AND METHODS:** In U251MG, one of the human glioblastoma cell lines, the expression of KITENIN was determined using Western blot. The difference in the migration and invasion abilities between the human glioblastoma cell line empty vector (U251MG-E) and KITENIN transfectants (U251-KIT) was evaluated using simple scratch test and matrigel invasion assays. Downstream signaling changes involving JNK, c-Jun, c-Src, and CAS were determined by Western blot. The dual luciferase assay was used for detection of AP-1 transcription factor activity. **RESULTS:** Following transfection with KITENIN, the U251-KIT cell line exhibited decreased migration and invasion compared with the U251MG-E cell line. The U251-KIT cell line downregulated phosphorylated JNK and c-Jun, c-Src, and CAS. The dual luciferase assay showed that AP-1 transcription factor activity was also downregulated. **CONCLUSION:** KITENIN inhibits migration and invasion of the U251MG human glioblastoma cell line by WNT/PCP JNK signaling cascades and through downregulation of the c-Src- and CAS-signaling pathways.

P.011. WARBURG EFFECT INFLUENCES MIGRATION OF HIGH-GRADE GLIOMA IN VITRO THROUGH ENHANCED TGF- β 2 ACTIVATION BY THROMBOSPONDIN-1

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INTRODUCTION: Lactate dehydrogenase-type A (LDH-A) is a key metabolic enzyme catalyzing pyruvate into lactate and is excessively expressed in several human tumors. Transforming growth factor- β 2 (TGF- β 2) is a key regulator of invasion in high-grade gliomas, partially remodeling the extracellular matrix (ECM) and inducing proteinases. Thrombospondin1 (THBS-1) is an extracellular protein important for activation and processing of TGF- β 2. A microarray of LDH-A knocked-down glioma cell RNA showed downregulation of THBS-1 and TGF- β 2. In this study, we tested the hypothesis that LDH-A influences TGF- β 2 activation by upregulation of THBS-1 leading to an enhanced migration of high-grade glioma in vitro. **METHODS:** We performed LDH-A knock-down by transient transfection of glioma cells with small interfering RNA directed against LDH-A (siLDH-A). Expression levels of TGF- β 2 and THBS-1 in siLDH-A-transfected cells were investigated using microarrays, RT-PCR, Western blot, and ELISA. Migration of transfected cells was investigated by Boyden Chamber and scratch assays. **RESULTS:** siLDH-A suppresses TGF- β 2 in high-grade glioma and decreases the expression of THBS-1 on the RNA and protein level. THBS-1 leads to an increased level of activated TGF- β 2 in supernatants of siLDH-A-treated cells. In migration assays, siLDH-A leads to a decreased migration of high-grade glioma cells. **DISCUSSION:** We demonstrate, for the first time, that knockdown of LDH-A can decrease the RNA and protein level of TSP-1 and consecutively the processing of TGF- β 2. Additionally, knockdown of LDH-A decreases the RNA level of TGF- β 2. Both results may contribute to an enhanced level of TGF- β 2 and increased migration, given that LDH-A is expressed. An increased expression of LDH-A has been found in aerobic glycolysis, a mechanism well known from several human cancers. Recent paper showed that LDH-A is able to bind RNA. Thus, we suppose LDH-A could influence the level of TGF- β 2 RNA by RNA stabilization. Together with our recent results that show that TGF- β enhances migration in high-grade gliomas, we demonstrate a new panel of interactions between lactate metabolism and TGF- β 2 that might be crucial for glioma migration and possibly invasion.

P.012. INCIDENCE OF LOSS OF HETEROZYGOSITY IN CHROMOSOMAL REGION 14q32.31 WHICH CONTAINS THE LARGE 7 + 46 BIPARTITE MICRORNA CLUSTER, AND ITS RELATIONSHIP TO OTHER MOLECULAR MARKERS IN 95 GLIOMAS

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BACKGROUND: We demonstrated that the large 7 + 46 bipartite Dlk1-Dio3 microRNA cluster on chromosome 14q32.31 is uniformly downregulated in gliomas, embryonic stem cells, and neural progenitor cells. It might suggest that this cluster probably represents the largest tumor-suppressor microRNA cluster. Because the microRNAs from this large microRNA cluster are expressed only from the maternally inherited allele, deletion of the active allele may result in complete silencing of these microRNAs. There is strong evidence that this chromosomal region is frequently deleted or genetically altered in both haematopoietic and systemic solid tumors. In a preliminary small scale survey, we found a low rate of loss of heterozygosity (LOH) in this chromosomal region of only 5% in oligodendrogliomas and 35% in glioblastomas. The aim of this study was to verify the incidence of LOH in chromosome 14q32.31 and whether it might carry a potentially meaningful explanation for the previously observed downregulation of 7 + 46 bipartite microRNA cluster in gliomas. In addition, we examine whether there is any relationship between 14q32.31 LOH and other known prognostic markers, namely 1p, 19q, and 10q, and the methylation status of the promoters of MGMT and PTEN genes. **METHODS:** A microsatellite analysis was performed on DNA that was extracted from paraffin-embedded samples of 95 gliomas. LOH of 14q32.31 is evaluated by using D14S65 at 14q32.2 and D14S252 at 14q32.31 markers. The analysis includes 39 oligodendroglial tumors (54% WHO grade II) and 55 astrocytic tumors (91% WHO grades III and IV). The final data analysis and the correlation between 14q32.31 LOH and other markers will be presented at the conference.

P.013. RADIO-CHEMOTHERAPY RESISTANCE OF HUMAN GLIOMA: A ROLE FOR AKT INHIBITION?

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Standard treatment of glioblastoma multiforme (GBM) consists of surgery, radiotherapy, and temozolomide (TMZ). Clinical data show that GBMs with wild-type methyl-guanine methyl-transferase (MGMT) gene, which express the MGMT DNA repair protein, are resistant to TMZ. The PI3Kinase-Akt "survival" pathway, which is activated in the majority of GBMs, is a main determinant of radioresistance. Activation of the pathway is mediated by the tumor suppressor gene PTEN and by EGFR. The HIV protease inhibitor nelfinavir (NFV) has been identified as downregulator of this pathway via inhibition of its key protein Akt. Our studies focus on the interaction between irradiation, TMZ, and NFV in human established and long-term primary glioma cell lines with known genetic profile with regard to PTEN, EGFR, and MGMT. Cell lines were tested on expression of the MGMT protein and on key proteins of the PI3Kinase-Akt pathway. Cell survival was determined by clonogenic assay. A clear correlation was found between the sensitivity of glioma cells to TMZ, both with expression of the MGMT repair protein and with MGMT gene promoter methylation. Enhancement of the radiation response by TMZ was noticed in 3 of 5 MGMT promoter methylated, TMZ-sensitive cell lines. Treatment of D384 cells (methylated MGMT; wtPTEN) with NFV alone for 24 hours decreased cell proliferation and was cytotoxic at doses exceeding 30 μ M. Pretreatment with 20 μ M NFV for 24 hours enhanced the radiation response. The data indicate that targeted interference with disturbed molecular pathways in GBM is a promising approach to circumvent resistance to current therapies.

P.014. EGR-2-MEDIATED ACTIVATION OF BAK EXPRESSION IS INHIBITED BY THE NUCLEAR LOCALIZATION OF UPAR IN GLIOMA CELL LINES

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Glioma progression is a highly complex process that involves the deregulation of proteins and genes that are responsible for tumor invasion, angiogenesis, circulation of tumor cells in blood vessels, colonization at secondary organ sites, and the tumor's evasion of the host's defense systems. The uPA/uPAR system has been postulated to play a central role in the mediation

of these processes. We have demonstrated previously that the simultaneous downregulation of uPAR and uPA causes the activation of the extrinsic apoptotic pathway involving the mitochondrial $\Delta\psi$ collapse. From Western blot analysis of nuclear extracts from glioma cells (SNB19, U87) and xenografts (4910, 5310), we observed that uPAR is localized in the nucleus of these cells. Further analysis by immunoprecipitation, immunohistochemistry and transcription factor array revealed that uPAR was strongly associated with EGR-2 among other nuclear molecules. As determined by Western blot analysis of cytoplasmic extracts, RNAi-mediated downregulation of uPAR caused the activation of BAK expression and release of cytochrome c into the cytoplasm in SNB19, U87, and 4910 cells, and to a lesser extent, in 5310 cells. Downregulation of both uPAR and BAK retarded mitochondrial $\Delta\psi$ collapse as observed from Mito-PT staining and Western blot analysis of cytochrome c release when compared with cells downregulated for uPAR alone. To determine whether uPAR binding to EGR-2 involved genetic material, we performed CHIP analysis by immunoprecipitating uPAR and PCR amplification of EGR-2-binding sequences. From the CHIP analysis, we observed that uPAR and EGR-2 form a complex with DNA in SNB19, U87, and 4910 cells, but form weak complexes in 5310 cells. Taken together, our results indicate the involvement of uPAR as a regulatory element with potential therapeutic implications.

P.015. ABERRANT HYPERMETHYLATION OF NON-PROMOTER ZYGOTE ARREST 1 (ZAR1) IN HUMAN BRAIN TUMORS

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Zygote arrest 1 (ZAR1) is a novel maternal-effect gene that plays crucial roles during the oocyte-to-embryo transition. Comprehensive methylation analysis of tumor-specific differentially methylated regions in human malignant melanoma has recently led to the identification of nonpromoter hypermethylation of the ZAR1 gene that had never been previously linked to aberrant methylation. Strikingly, ZAR1 hypermethylation was frequently observed in melanomas but was absent in benign nevi, and ZAR1 expression was found to be upregulated in methylated tumors. We searched for nonpromoter ZAR1 hypermethylation in 90 primary human brain tumor samples, normal brain tissue from 1 autopsy case, and 7 glioma cell lines, employing Sequenom MassARRAY, in which bisulfite-treated fragments are quantitatively detected using time-of-flight mass spectroscopy. We also evaluated the ZAR1 transcript expression levels by quantitative real-time reverse transcription-PCR in 7 glioma cell lines. Hypermethylation of ZAR1 was frequently found in diffuse astrocytomas (7 of 7; 100%), anaplastic astrocytomas (16 of 17; 94%), glioblastomas (27 of 29; 93%), oligodendrogliomas (3 of 3; 100%), anaplastic oligodendrogliomas (3 of 3; 100%), and pituitary adenomas (9 of 10; 90%), but not at all in 3 pilocytic astrocytomas. Other tumor types showed infrequent ZAR1 hypermethylation: 1 (17%) of 6 of vestibular schwannomas and 4 (33%) of 12 meningeothelial meningiomas. The normal brain tissue revealed no evidence of ZAR1 methylation. Among the 7 glioma cell lines, all cell lines displayed aberrant hypermethylation of ZAR1, while detectable ZAR1 transcript was not found in any of the cell lines. Our data indicate that nonpromoter hypermethylation of ZAR1 is extremely frequent in diffuse gliomas and pituitary adenomas, although methylation-related aberrant ZAR1 expression is far less likely to be related to glioma tumorigenesis.

EPIDEMIOLOGY

P.016*. "ON-CALL" REFERRAL PATTERNS OF PATIENTS WITH BRAIN TUMORS IN TWO NEUROSURGICAL CENTERS: USING DATA TO ORGANIZE SERVICES

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BACKGROUND: In the UK, brain tumors account for up to 10% of all emergency referrals to a neurosurgical center and up to 6% of all out-of-hours emergency referrals. A better understanding of the patterns of referral could lead to an improvement in the organization and provision of services. **METHODS:** Data on all patients referred with suspected diagnosis of brain tumor to the "on-call" neurosurgical team in 2 units in the north east of England were collected over a 1-year period. Data included demographics, neurological status, and time of referral. These were analyzed using JMP 8.0.2. Categorical data were tabulated and a two-tailed χ^2 test was used to test for any association with a significance level of 0.05. **RESULTS:** A total of 451 referrals were

analyzed (mean age of patients was 60 years). Fifteen percent of referrals were received between 1700 and 0800 hours. Fifty percent of all "on-call" referrals were received between 1100 and 1700 hours with a peak at 1400 hours. Twenty-five percent of all referrals were received on a Friday. Up to 30% of brain tumor patients were referred from within the same hospital trust and increased significantly if the medical departments (which refer 60% of patients) were present on site ($P < .05$). Up to 27% of patients had focal neurology at the time of referral and 70% of the patients had a Glasgow Coma Scale score of 14–15. Eighteen percent of all the emergency tumor referrals were admitted to the neurosurgical unit and only 3 patients were operated upon out-of-hours. **CONCLUSIONS:** The majority of referrals of patients with suspected brain tumors are received during normal working hours. Most are from medical departments in the catchment area and most patients have good neurological status. Streamlining referral pathways to the neuro-oncology multidisciplinary team during working hours will improve services for patients and reduce the workload of the on-call neurosurgeons.

P.017. WHO GRADE II GLIOMA DISTRIBUTION ON THE FRENCH TERRITORY: PRELIMINARY DETAILED RESULTS CONCERNING 6 REGIONS (ALSACE, BOURGOGNE, CHAMPAGNE/ARDENNES, FRANCHE, CONTÉ, LANGUEDOC ROUSSILLON, AND LORRAINE)

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Diffuse infiltrating WHO grade II gliomas are relatively rare tumors. They account for 10%–15% of all gliomas with an incidence rate of 1 in 100,000 person-years, approximately. Epidemiological data are very rare even exceptional. The aim of the present study was to estimate, with this preliminary work, the French national geographic distribution of all the histological cases of WHO grade II gliomas during a 4-year period. **METHODS:** The French neurosurgeons, neuropathologists, and neurooncologists, in collaboration with epidemiologists and biostatisticians, have established the French Brain Tumor DataBase (FBTDB) which is the biggest database concerning primary central nervous system tumors (PCNST) in Europe. This database continues to record all PCNST in France for which histological diagnosis is available. One of the major aims of this structure is to allow the realization of epidemiological studies. The methodology used for the geographic distribution analysis was simple: (i) identification of all the histological cases of glioma diagnosed between January 1, 2006 and December 31, 2009; (ii) for each identified WHO grade II glioma cases, collection of the personal address (postal code) of the patient at the moment of the surgical procedure; and (iii) description of the geographic distribution of all cases in the French territory with the search of spatial clusters. **RESULTS:** We analyze at present intraregional distribution of cases in 6 French regions (Alsace, Bourgogne, Champagne/Ardennes, Franche-Comté, Languedoc Roussillon, and Lorraine) corresponding to a population of 11 million of inhabitants. Preliminary results seem to show an important intraregional heterogeneity. The analysis at a national level is in progress. **CONCLUSION:** This original study aims to describe the geographic distribution of the WHO grade II gliomas at the level of the French territory. We will present the preliminary results concerning a population of approximately 11 millions of inhabitants.

P.018. INCREASED SURVIVAL IN GLIOBLASTOMA, A POPULATION-BASED STUDY BY THE AUSTRIAN BRAIN TUMOR REGISTRY

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BACKGROUND: Historically, median survival times of glioblastoma (GBM) patients ranged from 6 to 9 months. Thirty-six months after diagnosis, only 2%–5% of the patients were alive (long-term survivors). In 2005, the

EORTC-NCIC NCT00006353 randomized phase III trial (Stupp et al., NEJM) demonstrated significant improvements in survival yielding 16.0% long-term survivors. The present study aims to analyze to what extent these improvements within the setting of clinical trials translated to the population level. **PATIENTS AND METHODS:** A population-based survey on primary GBM cases, newly diagnosed in 2005, was conducted by the Austrian Brain Tumour Registry. The patient cohort was followed-up until December 12, 2008. GBM cases in children and adolescents aged <18 years were excluded. Survival analyses were performed according to Kaplan–Meier with two-sided log-rank tests. **RESULTS:** A total of 375 adult primary GBM cases were identified, referring to an age-standardized incidence rate of 4.3 in 100,000 person-years (95% CI 3.7–4.9) in males and 2.6 in 100,000 person-years (2.2–3.0) in females (WHO world standard population). Median age at diagnosis was 63.8 years, range: 18.8–87.0 years. Overall survival (OS) of the total cohort was 39.2% (34.3%–44.1%) at 1 year, 20.0% (16.1%–24.2%) at 2 years, and 15.5% (12.0%–19.3%) at 3 years. Significant differences in OS ($P < .0001$) were encountered between age groups 18–69 ($n = 257$, 68.5%) and 70+ ($n = 118$, 31.5%). Whereas in the age-group 18–69, the OS was 47.9% (41.6%–53.8%) at 1 year, 26.1% (20.9%–31.6%) at 2 years, and 19.8% (15.2%–24.9%) at 3 years, the respective rates of the age-group 70+ were 20.3% (13.6%–28.0%), 6.8% (3.2%–12.3%), and 5.9% (2.6%–11.2%). **CONCLUSIONS:** We show, for the first time, at the population level that GBM survival has increased when compared with historic data. The increase in survival is most prominent in the age group 18–69, whereas it is still poor in patients aged 70+. This favorable development at the population level seems to be because of innovations in diagnosis and therapy of brain tumors in the past decades, including the recent introduction of combined postoperative radiochemotherapy as new therapy standard in GBM.

P.019. NEOPLASTIC DIAGNOSIS TIMING PROFILE IN VON HIPPEL–LINDAU’S DISEASE: A PERSONAL SERIES EVALUATION

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INTRODUCTION: Von Hippel–Lindau’s (VHL) disease is a rare autosomal-dominant genetic disease, with a prevalence close to 1 in 40,000 inhabitants. The affected patients present nervous system and retinal hemangioblastomas (HGB) associated among others with paragangliomas/pheocromocytomas (PGL), endolymphatic sac tumors (ELST), and renal cell carcinoma (RCC). The object of this study is to evaluate the presenting timing profile of these tumors in a series of affected patients. **MATERIALS AND METHODS:** Age and sequence of imaging confirmed diagnosis of intracranial, spinal cord, and retinal HGBs, PGLs, ELSTs, RCCs, evaluated in a series of 54 affected patients with a total amount of 245 diagnosed tumors, from 30 families studied and followed in a neurological familial neoplastic syndrome referral center, based in a neurosurgical unit. The patient’s clinical data are compared within the obtained clinical groups. **RESULTS:** One hundred thirteen intracranial, 33 spinal, and 44 retinal HGBs were diagnosed. Retinal HGB diagnosis was more precocious, with initial diagnosis at 8 years of age, and a median at 28. Intracranial HGBs are first diagnosed at 8 with a median at 34 years of age. Spinal HGBs have a later diagnosis, beginning at 9 years of age, with a median at 37. PGLs diagnosis begun at age 11, with a median diagnosis age of 33. ELSTs begun at 23 years, with a median age of 37. RCC was the latest performed diagnosis, starting at 20 years with a median at 39 years. Three disease molecule–confirmed carrier patients have not developed tumors yet. Five patients have died as a result of their HGB, at age 30–60 years old, and 2 more from RCC, some later. No relation has been observed between age of presentation and other clinical or molecular characteristics. **CONCLUSIONS:** In von Hippel–Lindau’s disease, the neoplastic occurrence begins at early age. Tumors are diagnosed in 20% of affected patients before age 19. A precocious diagnosis does not predict a more aggressive clinical course in relation to other clinical signs. On the other hand, the clinical temporal profile is not predictable with the molecular diagnosis. Deaths before 60 in these patients are commonly related to hemangioblastoma. A molecular diagnosis should be provided to all first-degree relatives of affected patients at an early age, so that clinical and imaging studies can be performed regularly following protocols, in order to obtain an early diagnosis and adequate management of these neoplasms.

QUALITY OF LIFE

P.020*. SUPPORTING PATIENTS WITH A DIAGNOSIS OF MALIGNANT GLIOMA: THE ROLE OF THE NEURO-ONCOLOGY NURSE SPECIALIST

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INTRODUCTION: In the UK, Guidance published by the National Institute for Clinical Excellence recommends that a “key worker” should be allocated to all patients with a diagnosis of intracranial tumor. It is a common practice that a neuro-oncology specialist nurse (NOSN) takes on this role and acts as a point of contact for patients, their carers, and all healthcare professionals involved in the patients’ management. Often the NOSN is a single-handed practitioner and data regarding their workload and involvement in patient care are scarce. **OBJECTIVE:** To assess the involvement of the NOSN in the management of patients with a diagnosis of high-grade malignant glioma. **METHOD:** Retrospective review of NOSN records relating to the management of all patients in our unit with a diagnosis of high-grade intracranial glioma (WHO Grades III and IV) during the period July 01, 2007 and June 30, 2009. NOSN involvement at key stages of the patient pathway together with liaison with patients, carers, and health professionals were assessed. **RESULTS:** A total of 123 eligible patients were identified (70 Males: 43 Females, mean age 59 years). Seventy-four percent patients had a diagnosis of glioblastoma. NOSN involvement at the time of initial consultation occurred in only 3% cases but rose to involvement in 93% cases during treatment. The NOSN had 705 contacts with patients and carers and dealt with 826 diverse issues, including clinic scheduling, symptom management, medication/steroids, psychological support, and referral to other agencies. Subsequently, the NOSN had 941 contacts with other health professionals (17 different agencies) to take forward patient care. **CONCLUSION:** The NOSN plays a key role in the management of patients with malignant glioma. This study indicates the high workload of the NOSN and their pivotal role in the management of this complex patient group.

P.021*. HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH BRAIN TUMORS TREATED WITH TWO DIFFERENT TYPE OF FRACTIONATION

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BACKGROUND: The aim of the study was to compare the quality-of-life (QOL) in patients with brain tumors treated with two different type of fractionation. **MATERIALS AND METHODS:** We measured the QOL in 78 patients with different type of brain tumors treated with postoperative radiotherapy with or without chemotherapy. The QOL was appreciated by using the QOL-C30 and QOL-BN20 questionnaires at the beginning and at the end of radiotherapy. There have been 27 women and 51 men with a median age of 53.5 years. The neurological index was 0 for 18 patients, 1 for 36 patients, 2 for 14, and 3 for 10 patients. They have been treated with conventional fractionation 1.8–2 Gy/fraction per day with a total dose of 54–60 Gy (53 patients) and with DT = 30–45 Gy with 3 Gy/fraction per day (25 patients). Conformal radiotherapy (3D) was applied in 60 patients. **RESULTS:** The acute toxicity at the end of radiotherapy was appreciated by using RTOG scale. This was 0 for 19, 23% of patients, 1 for 47, 44%, 2 for 32.05%, and 3 for 1.28% of patients. The health-related QOL coefficient was slightly better for all parameters at the end of radiotherapy, except nausea and vomiting (correlation coefficient $r = .34$). The correlation coefficient (r) was better for global health status (.93), physical functioning (.97), emotional functioning (.96), and cognitive function (.94). Motor dysfunction (.75), seizures (.78), and communication (.67) were altered at the end compared with the beginning of radiotherapy. The correlation between the type of fractionation (modified vs conventional) and QOL was analyzed by Roc curves and show a significant difference for nausea and vomiting ($P < .001$). The global health-related QOL at the end of radiotherapy was similar for the 2 types of fractionation. **CONCLUSIONS:** Assessment of QOL is possible in patients with brain tumors despite the neurological status. In our study, the QOL endpoints based on QOL-C30 and QOL-BN20 questionnaires show no difference between modified vs conventional radiotherapy. Hypofractionation could be a good alternative to treat patients with poor neurological status.

P.022*. PRELIMINARY VALIDATION OF THE EORTC CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY QUALITY OF LIFE QUESTIONNAIRE (QLQ-CIPN20) SPANISH VERSION IN A SERIES OF MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB

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INTRODUCTION: Bortezomib-induced peripheral neuropathy (BIPN) presents in up to one-third of multiple myeloma (MM) patients treated with bortezomib (BTZ). The EORTC core quality-of-life questionnaire, the QLQ-C30, has demonstrated to be reliable and valid when used with MM patients. The EORTC has developed the QLQ-CIPN20 questionnaire module to assess patients' symptoms and functional limitations related to chemotherapy-induced peripheral neuropathy (PN). QLQ-CIPN20 consists of 20 items grouped into three scales assessing sensory, motor, and autonomic symptoms and functioning. The aim of the study was to determine the usefulness of the Spanish version of the QLQ-CIPN20 in a series of MM patients treated with BTZ. **MATERIAL AND METHODS:** A sample of 18 patients participating in a study evaluating the risk factors for developing BIPN (J. Peripher. Nerv Syst 2010;15:17-25) were asked to complete the QLQ-C30 and the QLQ-CIPN20 at baseline and during treatment. PN was graded according to the Total Neuropathy Score, both clinical (TNSc) and reduced (TNSr) version. QLQ-CIPN20 and TNS changes were compared at baseline between patients with and without PN, and at last visit between patients with and without BIPN. **RESULTS:** Prior to BTZ therapy, 6 patients had PN (5 had received vincristine previously). At baseline, patients with PN reported significantly more sensory ($P = .01$) and motor ($P = .05$) problems on the QLQ-CIPN20 than those without PN. Five of 18 patients developed BIPN. Patients with BIPN reported significantly more sensory problems than those without BIPN ($P = .002$) scale. No significant differences were observed on the final QLQ-CIPN20 in BIPN patients with or without prior PN at baseline. TNSc and TNSr baseline scores were significantly different between patients with and without PN ($P = .001$). Patients who developed BIPN showed differences in TNSc ($P = .004$) and TNSr ($P = .048$) in comparison with patients without BIPN. In the whole series, TNS was significantly correlated with QLQ-CIPN20 sensory (TNSc: $r = .52$, $P < .001$; TNSr: $r = .57$, $P < .001$), motor (TNSc: $r = .37$, $P = .001$; TNSr: $r = .36$, $P = .002$) and autonomic (TNSc and TNSr $r = .59$, $P < .001$) scales. **CONCLUSION:** Our results confirm the usefulness of this questionnaire in detecting the impact related with sensory impairment in BIPN patients. Besides, QLQ-CIPN20 scales exhibit a significant correlation with TNS.

P.023*. COGNITION AND QUALITY OF LIFE IN PATIENTS WITH BRAIN TUMORS

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Damage to the brain areas involved in cognitive functions can have a crucial effect on the quality-of-life (QoL) of patients. QoL is an important topic that is increasingly relevant considering that gliomas typically affect young individuals potentially capable of returning to work. The objectives of this study were to develop a neuropsychological battery to investigate cognitive functions in patients with primary brain tumors, to identify any permanent cognitive deficit (in particular attention, language, and memory) and to study how patients after surgery perceive their functional status and social well-being. Two hundred patients with high- and low-grade gliomas in the right and left hemisphere were evaluated. We have developed an extensive neuropsychological battery that allows a careful evaluation of patients and a selection of the patients suitable for awake surgery, while detecting the stimuli to use intraoperatively. Cognitive function (memory, language, attention, visuo-spatial orientation, and intelligence) was evaluated by means of this battery and a short interview was run to test their well-being perception. Patients were evaluated before surgery, immediately after surgery, and 3 months after surgery. This neuropsychological evaluation often revealed deficits in language competences or in memory abilities that hardly emerged from a simple clinical evaluation. Patients immediately after surgery reported fatigue and sometimes altered mood. A comparison with the preoperative test showed poorer verbal fluency, attention, verbal or visual learning, or psychomotor speed. Cognitive function did not differ between the evaluation before surgery and follow-up and a high proportion of patients had a good recovery of cognitive function and were able to carry out everyday activities.

P.024*. THE LATE TOXICITY OF ADULT MEDULLOBLASTOMA TREATMENTS: THE EXPERIENCE OF 4 FRENCH CENTERS

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OBJECTIVES: Adult medulloblastoma is a rare tumor. Conventional treatment for the standard risk group (complete surgery or residual tumor lower than 1.5 cm², absence of malignant cells in the cerebrospinal fluid, absence of metastasis, absence of MYC amplification and exclusion of large cells medulloblastoma) is classically based on a 54/36 Gy cranio-spinal radiotherapy (54 Gy on the posterior fossa and 36 Gy on the neuraxis). Chemotherapy is proposed in complement for the high-risk group. This treatment is associated with an acute toxicity that decreases gradually when patient goes away from the treatment period. The French intergroup experience pleads also in favor of a late and progressive neurotoxicity for long survivors associated with a clear degradation of the quality of life. The purpose of this work was to estimate precisely this late toxicity for the surviving patients. **METHODS:** Four French neurooncology centers were associated for this work (Lille, Montpellier, Nancy, and Paris Salpêtrière). In each centre, it was proposed to all the patients alive more than a year after the completion of the treatment and without tumoral progression (i) an analysis of the social status, (ii) an analysis of the quality-of-life status (HR EORTC QLQ 30 + Brain module), and (iii) a neuropsychological assessment. **RESULTS:** Thirty-four patients fulfilled the inclusion criteria. At this day, we collected social data in 19 cases, quality-of-life data in 14 cases, and a neuropsychological assessment in 19 cases. The work is still in progress and we will have supplementary data at our disposal for the meeting. The preliminary analysis shows that (i) only approximately 40% of the patients resumed a professional activity, (ii) the quality of life is altered, and (iii) despite the heterogeneity of the assessments, neuropsychological modifications seem mainly to concern attention and memory processes. **CONCLUSION:** As for the pediatric population, medulloblastoma adult survivors seem to present a late toxicity of the treatment. It justifies a discussion about the adaptation of the treatment modalities at least for the standard risk patients.

P.025*. CHARACTERISTICS OF SPONTANEOUS SPEECH IN PATIENTS WITH LOW-GRADE GLIOMAS IN ELOQUENT AREAS BEFORE SURGERY

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INTRODUCTION: Although it is already known that language deficits could occur in patients with LGGs, no previous studies are reported with respect to a detailed analysis of spontaneous speech. It must be noted that spontaneous speech is the most natural form of linguistic behavior. Patients with preoperative language disturbances are at-risk for more persistent aphasic disturbances; therefore, more insight into this subject is profitable. This study was conducted to investigate the spontaneous speech preoperatively in patients with LGGs in eloquent areas. **METHOD:** Thirty-four patients (22 males, 12 females) were included, and 21 healthy controls (8 males, 13 females) matched for age and education. Spontaneous speech from LGGs patients was collected for analysis and recorded in an interview setting before awake craniotomy. Three different topics were discussed with a minimal intervention of the interviewer; medical status, work, and hobbies. In the control group, most recent doctor's visit, work, and hobbies were discussed. Within a sample of 300 words, speech from patients and controls is analyzed with regard to the following variables; lexical diversity (type token ratio), mean length of utterance (mlu), repetitions, self-corrections, and incomplete sentences. **RESULTS:** Statistical analyses revealed a significant difference ($P < .01$) between the patient group and the controls in lexical diversity, repetitions, self-corrections, and incomplete sentences. In the patient group, repetitions occurred most frequently, followed by self-corrections, and incomplete sentences. **Discussion:** The results of this study suggest that a word finding deficit is the background of the distorted spontaneous speech of LGG patients. The availability of different words is restricted.

Repetitions could be a sign of time-gaining before the next content word. Self-corrections point to an earlier erroneously selected word. Sentences might be incomplete because of a lack of meaningful words. However, a syntactic component might be involved too. Our next step is to perform a fine-grained analysis of the spontaneous speech of LGG patients on the main linguistic levels: semantics, phonology, and syntax. Our goal is to select the sensitive parameters for improvement and deterioration of linguistic behavior of brain tumor patients pre- and postoperation. A spontaneous speech analysis might be a more sensitive tool to detect language problems than structured language tasks, such as naming, all linguistic levels are involved.

P.026. CARING FOR A PATIENT WITH A MALIGNANT GLIOMA: AN ANALYSIS OF CARE-GIVER BURDEN

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BACKGROUND: The progressive physical and cognitive impairment experienced by patients with a malignant glioma (MG) places particular importance on the role of a patient's primary caregiver. Providing this care for a patient with MG can be particularly burdensome, impacting social, physical, and emotional quality of life (QOL). Given the importance of the caregiver in the course of treatment, the purpose of this study was to quantify and understand the nature of the caregiver experience. **METHODS:** Patients with MG within 6 months of initial diagnosis or relapse were eligible for this study if they had an involved caregiver. The Caregiver Quality of Life Index-Cancer (CQOLC) was given to caregivers at baseline as part of a series of validated instruments to assess involvement and impact on them. The CQOLC measures social, physical, and emotional strain on a caregiver through 35 items on 5-point Likert scales (0 = not at all to 4 = very much). The CQOLC has a maximum score of 140 points, with a higher score reflecting better QOL. **RESULTS:** Completed CQOLC questionnaires were collected from 22 caregivers to date. Of the 35 items, the 3 most strongly reported were increased levels of stress, distress over seeing their loved one deteriorate, and an increased fear of their loved one dying. The caregivers surveyed in this study scored an average of 80.6 on the 140-point scale. This is significantly lower ($P = .01$) than the average for caregivers across all cancers (93.3). We also found a significant difference between caregivers of patients with newly diagnosed vs recurrent MG. Caregivers of newly diagnosed patients were significantly more likely to feel sadness ($P = .055$) and feel that their life is imposed upon ($P = .02$), while caregivers of patients with recurrent MG were significantly more satisfied with their sex lives ($P = .03$). **CONCLUSIONS:** Results demonstrate the heavy burden placed on caregivers of patients with MG, particularly for those caring for newly diagnosed patients. This burden is greater than that experienced by caregivers of patient's with other cancers; this may be related to the neurologic compromise of patients with MG. Caregivers play a crucial role in assisting MG patients; these findings demonstrate the negative impact on caregivers and the importance of the physician awareness so psychosocial interventions might be instituted.

P.027. HOW DOES TUMOR RESECTION AFFECT COGNITION? HIGH-GRADE GLIOMA VS MENINGIOMA PATIENTS

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INTRODUCTION: Many patients with intracranial tumors suffer from cognitive deficits. Because of differences in localization and growth speed, high-grade glioma (HGG) more readily damages healthy brain tissue compared with meningioma (MG). Surgical resection may diminish the pressure on brain tissue, but it may otherwise harm neuronal tissue. **PURPOSE:** To compare the effects of tumor resection on cognition in patients with HGG and with MG. **PATIENTS AND METHODS:** Seventy-five patients (41 HGG, 34 MG) were tested preceding surgery. Testing was repeated following surgery, before subsequent therapy was instituted (median interval: 5 (HGG) vs 8 (MG) weeks). Tumor size and site, use of anti-epileptics (AED), and the extent of resection were recorded. Validated neuropsychological tests for 8 domains were applied: general cognitive functioning (GCF), memory, working memory (WM), fluency, speed, perception, construction, and attention. **RESULTS:** Compared with normative data, preoperatively up to 50% of HGG patients and up to 28% of MG patients suffered from cognitive

deficits. Mean preoperative test scores were lower in the HGG group than in the MG group, with significant differences in GCF, memory and speed. In the HGG group, patients with large tumors tended to perform worse in fluency. Tumors located in the dominant hemisphere were related to significantly lower memory and WM scores. For MG patients, tumor size and site did not correlate with cognition. For both groups, no significant influence of AED on cognition was observed. Fifty-two patients (30 HGG, 22 MG) were tested post-surgery. Reasons for drop-out included refusal, post-surgical stroke, and progressive tumor growth. For HGG patients, mean postoperative test scores—apart from perception—improved compared with presurgical levels. The improvement was significant for construction and speed. Changes in performance after surgery were not related to the extent of resection. For MG patients, mean postoperative test scores declined for perception (significantly), WM, and speed, while the other domains showed a nonsignificant increment compared with presurgery. All MG patients underwent a radical resection. **DISCUSSION:** HGG patients have more cognitive deficits than MG patients. Surgery leads to an improvement of cognitive functioning in HGG patients, while this effect is less clear in MG patients. This might be because of a shorter test interval in HGG, or because more severe cognitive deficits in HGG patients may more easily improve than the subtle deficits associated with MG.

P.028. A NEW ORIENTAL MEDICAL APPROACH TO ELIMINATE BRAIN EDEMA COMPLICATED WITH MALIGNANT BRAIN TUMORS: EFFICACY OF GOREISAN (AN AQUAPORIN INHIBITER)

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OBJECTIVES: Glyceol, steroids, and isosorbide, which are covered by Japanese health insurance system, are widely used as medical decompression agents to eliminate brain edema complicated with malignant brain tumors and to relieve headache and several focal neurological deficits. Their side effects, however, sometimes prevent them from long-term use. For reducing brain edema, the authors have used the traditional oriental medical prescriptions for promoting diuresis and eliminating dampness, such as goreisan. Goreisan constitutes of 5 types of herbs—Polyporus 3 g, Rhizoma Alismatis 4 g, Rhizoma Atractylodis 3 g, Poria 3 g, and Ramulus Cinnamomi 1.5 g, and it is well known as an aquaporin inhibitor to suppress pathologically emerged aquaporin 4 which increases in various pathological conditions such as malignant brain tumor, trauma, cerebrovascular disease, and so on. **METHODS:** Between October 2006 and February 2010, goreisan were prescribed to 63 cases (52 patients: males 29, females 23, ages range between 24 and 83 years, mean 55.4) with malignant brain tumors (primary tumor 16 patients and metastatic tumor 36 patients). Headaches were complained in 23 cases, and focal neurological deficits were complained in 44 cases. The efficacy was evaluated with improvement rate of symptoms and neurological deficits: excellent (improvement rate >50% or higher), good (improvement rate <50% or can significantly reduce the dose of glyceol and steroids), no effect, and deterioration. **RESULTS:** Excellent 18 (28.5%), good 30 (47.6%), and no effect 15 (23.8%). No significant complications were recognized. **CONCLUSION:** Goreisan can be used as a substitute for glycerol, isosorbide, and steroids to reduce mild brain edema.

P.029. STRENGTH OF SKELETAL MUSCLE IN GLIOBLASTOMA PATIENTS: AN ONGOING PILOT STUDY

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Glioblastoma (GBM) leads to a decrease in muscular strength as a result of neuromuscular dysfunction caused by GBM itself, and of corticoid treatment which is needed to decrease intracranial pressure. Aim of this pilot observation was to test feasibility of strength testing in GBM patients. **METHODS:** Strength testing was so far performed in 2 patients (m:f = 4:1 (patient 3), 54 ± 16a, BMI = 28 ± 4 kg/m²) at baseline and follow-up after 5 (± 2) months. One patient (Patient 5) dropped out because of death before follow-up; Patient 4 started with a training program after receiving the GBM diagnosis, the other patients reported no muscular training activity. Handgrip strength was measured by using a Jamar hand-dynamometer. Isokinetic testing of both thighs (isokinetic knee extension and flexion strength) was performed by using a Biodex 3 dynamometer.

RESULTS: None of the patients showed side effects during or after the strength examinations. Handgrip strength of right hand/left hand measured for Patients 1–5: 16–62/10–44 (range) lb. Handgrip strength of (dominant) right hand increased in Patients 1, 2, and 4 (+9%– +10%), and decreased in patient 3 (–37%). Handgrip strength of left hand decreased in Patients 1–3 (–20% to –70%) (range), and increased in Patient 4 (+17%). Peak torques/weight (PT) of right knee extensors were in Patients 1–5: 91–290 (range) Nm/kg; PT of left knee extensors were 128–311 (range) Nm/kg. PT of right knee flexors were in Patients 1–5: 32–182 Nm/kg; PT of left knee flexors were 28–187 Nm/kg. At follow-up, isokinetic strength of knee extension and knee flexion decreased in 3 patients: extension of right knee (Patient 1–3: –3% to –39%). For Patient 4, the value increased by 3%. Extension of left knee decreased in all 4 patients (Patient 1–4: –5% to –51%). Flexion strength of both knees decreased in 3 patients (right knee: Patient 1–3: –16% to –59%); left knee: –22% to –32%). In Patient 4, isokinetic strength increased (+21%). **CONCLUSION:** Testing of muscular strength seems feasible in GBM patients. The results of this pilot observation indicate that strength of thigh muscles appears more than handgrip to decrease during the survival time. However, Patient 4 was able to increase his muscular strength through training.

P.030. REHABILITATION OF PATIENTS WITH MOTOR DISORDERS AFTER SURGICAL TREATMENT OF LOW-GRADE GLIOMAS

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BACKGROUND: The increase of quality of life in patients with low-grade gliomas (LGGs). Motional defects are a basic factor that reduces quality of life of patients. **AIM AND METHODS:** We refer brain gliomas to infiltratively growing tumors, when zones of brain, which are functionality important for speech and motion and median structures, are involved in tumor process. After tumor removal, the motor defects can be appeared or increased its severity. Image-guided surgery and laser thermodestruction allow to perform safe tumor resection within growth border. Thirty-seven patients LGG with motional defects in early postoperative period received recovery treatment. Treatment course includes pharmacotherapy (prozerin, vascular preparations), physiotherapeutic methods (electro-miostimulation, lasertherapy), massage, medical gymnastic, and psychotherapy that depends on neurological disorders. The programs of individual recovery treatment depended on the volume of tumor resection, preoperative neurological disorders, and associated diseases. It allowed to improve the results of treatment of patients with LGG and promoted their social adaptation, provides high quality of life. **RESULTS:** All the patients had early renewal of the broken functions: multiplying the volume of active motions, improvement of walking and degree of domain domestic skills, positive psychotherapy effect. **CONCLUSION:** This study shows that early differentiation complex rehabilitation treatment effectively corrects neurological abnormalities and provides high quality of life of patients with LGG.

P.031. THE NEURO-ONCOLOGY SPECIALIST NURSE: COORDINATING THE CARE OF PATIENTS WITH INTRACRANIAL TUMOR

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INTRODUCTION: In 2006, the National Institute for Clinical Excellence (NICE) published guidelines in the UK for the management of adult patients who are affected by brain tumors. The guidance advises that all patients diagnosed with an intracranial tumor should be allocated a “Key Worker” to coordinate their care. In most neuro-oncology units in the UK, this role is undertaken by the neuro-oncology specialist nurse (NOSN) and the majority of nurses are single-handed practitioners. **OBJECTIVE:** To identify the involvement of the NOSN in the management of patients with brain tumor. **METHODS:** Retrospective casenote review of NOSN involvement in the management of newly diagnosed patients with high grade gliomas (HGGT), low-grade gliomas (LGGT), meningiomas and pituitary tumors presenting to a single neurosurgeon in the period July 1, 2008–June 30, 2009. **RESULTS:** The records of 140 adult patients were reviewed (59 M: 81 F). The most common tumor types were HGGT (37%) and meningioma (31%). The frequency of NOSN involvement in patient management was: HGGT 87%; LGGT 69%; meningioma 51%; pituitary tumor 48%. Patient and carer contact with the NOSN was greatest in the HGGT group with an average of 13 contacts. As a consequence, these contacts the

NOSN liaised with 10 other health professionals on average. Patient and carer contact was lowest in the meningioma and pituitary tumor group. **CONCLUSION:** NICE guidance recommends that all adult patients with brain tumor should have NOSN involvement in their care. This study suggests that we are nearing compliance in patients with compliance HGGT but there is still unmet need in patients in other tumor groups. There is a need to increase the number of NOSNs.

P.032. COGNITIVE REHABILITATION IN NEURO-ONCOLOGICAL PATIENTS

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INTRODUCTION: Still now, despite therapeutic achievements of last decades in oncology, neuro-oncological patients are characterized by poor prognosis and low quality of life (QoL) during disease progression. Among neurological deficits, cognitive decline is one of the most common symptoms, with a severe impact on patients' QoL. As pharmacologic interventions have not proven effective yet in the treatment of cognitive deficits, cognitive rehabilitation could represent an alternative approach. Literature research (Medline) evidenced 5 studies about this topic. Although all studies reported some successes, these are difficult to interpret because of limitations in the methods used. Additionally, both type and time of interventions, study population, and outcome measures were different, making studies not comparable. **MATERIALS AND METHODS:** Fifty-five patients (mean age 51 ± 14.4 years, range 25–79 years) affected by primary brain tumors referred to Palliative Home-Care Unit for Brain Tumor Patients, Regina Elena National Cancer Institute (Rome), from June to December 2009 were evaluated. All patients had already undergone surgery and chemotherapy and/or radiotherapy. All patients underwent a comprehensive neuropsychological examination assessing memory, language, attention, executive functions, and visuo-constructional skills. On the basis of literature review, we identified basic criteria for assessment of patients requiring cognitive rehabilitation program: (i) mild-to-moderate cognitive deficits (MMSE ≥ 18); (ii) life expectancy ≥ 6 months; (iii) KPS ≥ 70 ; (iv) age ≥ 18 years. Also we defined a standardized program consisting in: 1-hour-weekly individual sessions, lasting 10 weeks, carried out with a computer program. At now we enrolled 8 patients who are performing rehabilitation training. **CONCLUSIONS:** Limited data about cognitive rehabilitation in neuro-oncological patients has left many questions about the suitability of interventions in this population unanswered. Optimal timing, type, and intensity of interventions are unknown. Similarly, the role of factors such as tumor grade, prognosis, fatigue, disease duration, severity of cognitive impairment must still be clarified to establish eligibility criteria for cognitive intervention.

IMMUNOLOGY AND IMMUNOTHERAPY

P.033*. INTENSE HUMAN CYTOMEGALOVIRUS (HCMV) IMMUNE RESPONSE IN GLIOBLASTOMA PATIENTS: A NOVEL PROGNOSTIC FACTOR FOR SURVIVAL

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BACKGROUND: Glioblastoma is a lethal malignant brain tumor with overall survival rates of <9.8% at 5 years. HCMV is a ubiquitous human herpesvirus found in nearly all humans worldwide with a persistent infection occurring in over 70% of adults. HCMV has been implicated in the development of several human malignancies owing to oncomodulatory effects of HCMV infection. It has been recently recognized that there exists an association between HCMV and malignant gliomas. Expression of HCMV nucleic acids and proteins has been described in >90% of gliomas *in vivo*. To study the prognostic value of anti-HCMV immune response in glioblastoma we prospectively assessed the levels of serum HCMV IgM and IgG in newly diagnosed glioblastoma patients and correlated the results with the clinical course. **MATERIALS AND METHODS:** Serum from 24 glioblastoma patients treated with standard chemo-radiotherapy in our institution between November 2008 and October 2009 were analyzed. Any HCMV IgM over 0.5 U/mL was considered diagnostic for acute HCMV infection. HCMV IgG >16 U/mL was regarded as positive for latent infection. Intense HCMV IgG immune response was defined as HCMV IgG >100 U/mL. All clinical and pathological data were recorded in a database

system using SPSS 13.0 statistics package. Response and progression-free survival time were defined, respectively, as objective response according to the 2D Macdonald criteria. Survival curves were generated using the Kaplan–Meier method and univariate analyses for survival differences were tested using two-sided log-rank tests. Cox's proportional hazards regression model was used for multivariate analysis. RESULTS: After a median follow-up of 11.4 months, 13 patients (54%) have died. HCMV IgG was positive for latent infection in 9 patients (37%), 5 of whom had intense HCMV IgG immune response (20%). None of the patients had an acute HCMV infection. In univariate analysis, HCMV IgG >100UI/mL demonstrated a strong significant association with a longer overall survival ($P = .020$). Positive HCMV IgG was found to be marginally associated with survival ($P = .07$). In multivariate analysis, the only prognostic factors that retained statistical significance were complete tumor resection and age ≥ 65 years. CONCLUSIONS: Intense HCMV IgG immune response is significantly associated with longer overall survival in our series. Further larger studies are required to validate HCMV IgG as prognostic factor for survival in glioblastoma patients.

P.034*. MODULATING THE IL-1 SIGNALING DURING GLIOMA ONCOLYTIC VIROTHERAPY

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There are no effective treatments for glioblastoma (GBM), and patients with this cancer have a life expectancy of 15 months from diagnosis. This poor prognosis is because of the difficulty in delivering appropriately targeted drugs to the brain and infiltrating margins of GBM. Thus, the capacity of oncolytic viruses (OVs) to generate progeny on-site that can spread throughout the tumor represents an ideal strategy for GBM treatment. However, the host innate immune response reduces OV replication *in vivo* and limits their therapeutic potential. It is therefore crucial to understand the mechanisms regulating antiviral immunity to improve the OV efficacy. We have previously shown that intratumoral injection of OVs induces infiltration of phagocytes (macrophages and microglia) that inhibit viral replication and persistence. Pretreatment of animals with cyclophosphamide (CPA) depletes macrophages from the cancer and enhances OV spread and animal survival. However, the efficacy of the CPA + OV combined treatment is still poor. Indeed, more recent data indicated that macrophages are rapidly restored after CPA activity, leading to viral clearance, tumor re-growth, and animal death. Continuous systemic administration of CPA is toxic to the animals; therefore, it is important to find alternative means to prolong the immunosuppressive action of CPA in a more selective and less toxic fashion. We hypothesized that combination of CPA + OV armed with inhibitors of inflammation will prolong the immunosuppressive effects of CPA selectively in tumor tissue, thus resulting in enhancement of OV treatment while minimizing systemic toxicity. To test this hypothesis we have first identified the molecules that trigger OV-induced inflammation by analyzing tumor gene expression changes early after OV delivery. Our data showed 12 genes changing in expression; they were all induced by OV and belonged to the interleukin (IL)-1b signaling. Interestingly, IL-1b is an inflammatory cytokine with strong tumorigenic properties and the antagonist for IL-1b receptor (IL-1RA) is being tested for cancer treatment. We have then observed that IL-1b is induced in glioma cells by a combination of stromal factors and OV infection and inhibits viral replication *in vitro*. Systemic pretreatment of animals with IL-1RA prevents OV-induced intratumoral macrophage infiltration and increases OV spread. IL-1RA is a protein and does not cross the blood–brain barrier; thus, when delivered systemically, it did not inhibit activation of microglia in response to intratumoral OV. We expect that CPA + OV armed with IL1RA will result in a broad suppression of phagocytic cells and synergistic enhancement of oncolytic virotherapy. Altogether, we have identified the intratumoral signaling initiating OV-induced inflammation and these data can be used in a new strategy of virotherapy for GBM that presents strong potential for a synergistic treatment outcome.

P.035*. T-CELL BASED IDENTIFICATION OF TISSUE ANTIGENS BY AUTOMATED TWO-DIMENSIONAL PROTEIN FRACTIONATION

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BACKGROUND: Here, we describe a new method to comprehensively identify candidate tissue antigens that spontaneously cause T-cell responses

in disease situations. **MATERIALS AND METHODS:** We used the new automated two-dimensional chromatography system *PF2D* to fractionate the proteome of tumor tissues and tested protein fractions for recognition by pre-existing tumor-specific CD4+ T-helper cells and cytotoxic T-cells. **RESULTS:** Applying this method to the ovalbumin (OVA) specific, TCRtg OT-I mouse model demonstrates efficient separation, processing, and cross-presentation to CD8+ T-cells by dendritic cells of OVA expressed by the OVA-transfected mouse lymphoma RMA-OVA. Applying this method to human tumor tissues, we identified in patients with head and neck cancer MUC-1 and EGFR as tumor-associated antigens selectively recognized by patients' T-cells. Finally, we detected on an exemplary patient with a malignant brain tumor CD4 and CD8 T-cell responses against 2 novel antigens, transthyretin and calgranulin B/S100A9, which were expressed on tumor and endothelial cells. Immunogenicity of these antigens could be confirmed in 4 out of 10 other brain tumor patients. **CONCLUSIONS:** This fast and cheap method appears suitable to identify candidate T-cell antigens in various disease situations, such as autoimmune and malignant diseases without restriction to their expression by a certain cell type or HLA allele.

P.036. HUMAN GLIOBLASTOMA CELLS DERIVED FROM NEUROSPHERES ARE MORE SENSITIVE TO NK, LECTIN-DEPENDENT, ANTIBODY-DEPENDENT, IL-2-ACTIVATED NK CELL LYSIS AND ANTI-TUMOR T-CELL CYTOTOXICITY COMPARED WITH CELLS FROM ADHERENT CULTURES DERIVED FROM IDENTICAL GBM PATIENTS

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Glioblastoma multiforme (GBM) is a brain tumor with a very poor prognosis as a result of inevitable recurrence. During the past few years, a contingent of cells within tumors, so-called stem-like tumor cells (STC), has been characterized in GBM. These cells have similar properties to neural stem cells and can, with a limited number of cells injected *in vivo* in animals, recapitulate an entire initial tumor. STC are also resistant to current radio- and chemo-therapeutic treatments *in vitro*. Therefore, STC are considered to play a key role in GBM recurrence observed in patients. These cells may be appealing targets for new therapeutic approaches such as immunotherapy. In this study, cells obtained from GBM specimen were grown in DMEM 10% FSC (adherent culture) and in serum-free medium supplemented with EGF and basic FGF (neurospheres culture). Phenotypic analyses confirm expression of stem cell markers such as CD133, nestin, and A2B5 on cells from neurospheres but not on cells from adherent cultures using cell lines derived from 5 different GBM patients. Expression of HLA class I molecules is observed in cells from both neurospheres and adherent cultures. Regarding tumor antigen expression, IL13R α 2 antigen is only observed on adherent cells. In contrast, EGFRvIII is expressed at a higher level on cells from neurospheres compared with cells from adherent cultures. Cell lines are then tested for their sensitivity to cell cytotoxicity mediated by NK and anti-tumor T cells. Human GBM cells grown as neurospheres are more sensitive to NK and CTL lysis compared with the same cells grown as adherent layers. Indeed, in contrast to their corresponding cells derived from adherent cultures, cells from neurospheres are sensitive to cell cytotoxicity mediated by resting NK cells or activated NK cells (with lectins, antibodies, and IL-2). In addition, Melan-A–pulsed cells from neurospheres pulsed are sensitive to Melan-A–specific T cell lines, used as effectors, compared with cells derived from adherent cultures. In total, this study demonstrates that STC are suitable targets for immunotherapy using NK or specific T cells as effectors.

P.037. STUDIES OF NATURAL KILLER (NK) CELLS AGAINST GLIOMA INITIATING CELLS IN VITRO

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BACKGROUND AND OBJECTIVE: There is increasing evidence sustained the hypothesis that human gliomas originated from glioma-initiating cells or stem cells (GIC/GSC). And usually these cells could not be eradicated by conventional surgery, chemotherapy, and radiotherapy because of their “stem-like” properties. The cytotoxicity of activating natural killer (NK) cells against GIC *in vitro* was investigated. **METHODS:** The CD133+ glioma

cells were isolated from resected human glioblastoma specimens or glioma cell line and were used as GIC. The NK cells were separated from peripheral blood mononuclear cells of glioma patients or healthy donors by Miltenyi magnetic beads, and were activated with IL2/PHA. Their in vitro cytotoxicity against GIC was assessed by lactate dehydrogenase (LDH) assay, with K562 cells as positive control target cells. RESULTS: The allogeneic NK cells could be cultured and activated with GIC-cultured medium, the increased cytolytic activity against GIC was shown with the higher E:T ratio. At the same E:T ratio, the activated NK cells showed remarkable higher cytolytic activity against GIC than that of resting (freshly isolated) NK cells ($P < .01$). CONCLUSIONS: The allogeneic NK cells could be activated in the GIC culture system and showed killing effect against GIC. It was suggested that activated NK cells might be used in the clinic for GIC eradication.

IN VITRO/IN VIVO MODELS

P.038*. ESTABLISHMENT OF TEMOZOLOMIDE-REFRACTORY GLIOMA CELLS

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PURPOSE: Temozolomide (TMZ) is an effective drug for the treatment of malignant gliomas; however, relapse of the tumor with the drug resistance development may be one of the major problems to be solved. Here, we established TMZ refractory glioma cell lines in vitro and analyzed their molecular characteristics including MGMT status. **METHODS:** To establish the TMZ-refractory glioma cell lines, human (U87, U251, U373, T98G, A172) and mouse glioma cell line (GL26) were repeatedly treated with TMZ at lower concentration (25 μ M) and gradually at higher concentration (800 μ M) every 4 days for about 2 months. MGMT expression of the cells was analyzed by Western blot. **RESULTS:** TMZ resistance developed in all the TMZ-sensitive cell lines without MGMT expression (U87, U251, U373, A172, and GL26) except in TMZ-resistant T98G cells with MGMT expression and mutant type p53. The degree of resistance to TMZ appeared about 2–3-fold higher than that of normal glioma cell lines. No difference was found in MGMT expression level between TMZ refractory cells and normal cells. The drug resistance and MGMT expression were not affected by the cell passage number. **CONCLUSION:** The newly established cell lines would be useful in the development of in vitro and in vivo TMZ-resistance experimental model.

P.039*. HIGH-RESOLUTION NMR SPECTROSCOPY OF BRAIN-DERIVED STEM CELLS

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NMR spectroscopy of living organisms provides a noninvasive and comprehensive insight into cellular composition and metabolism. In addition, identifying cell-specific NMR spectroscopic signals and patterns may lead to in vivo detection and tracking of different cell types including stem cells. We established high-resolution ¹H-NMR spectroscopy of several cultured brain-derived stem/progenitor cell lines like Nestin-positive fetal murine neural progenitors (NPCs), DCX-positive adult murine neuroblasts, and CD133+ human glioblastoma-derived cancer stem cells (CSCs), all cultured under serum-free conditions. Measurements are performed at Bruker Avance 600 MHz (14.4 T) and 800 MHz (18.8 T) spectrometers equipped with TCI cryo probes. Employing a metabolomic approach including spectroscopic filtering techniques, sophisticated quantification methods, and correlation analysis of spectroscopic and biological data, we investigate both metabolites and NMR-visible macromolecules (ie, so-called mobile lipids and mobile proteins, regarding their stem cell specificity and their response to targeted modulation of proliferation and differentiation, for example, by means of transforming growth factor β [TGF β]). Subsequently, high-resolution NMR spectroscopy of cultured brain-derived stem cells may constitute the key link between the fundamentals of stem cell identity and metabolism on the one hand, and on the other hand the possibility of monitoring neurogenesis, neurodegenerative diseases, and tumors regarding both diagnosis and therapy noninvasively in vivo in humans.

PREDICTIVE BIOMOLECULAR MARKERS

P.040*. SCREENING CHEMOTHERAPY AGENTS USING SURGICALLY OBTAINED BRAIN TUMOR SPECIMENS

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BACKGROUND: Surgical brain tumor specimens can be used to obtain valuable information regarding sensitivity to drug therapies. Data collected on surgical specimens using an in vitro growth and invasion assay were correlated with patient response to chemotherapy. **METHODS:** Surgically obtained glioma tumor specimens were cultured in a 3-dimensional collagen gel matrix and observed for growth and invasion. Chemotherapy drug effects on mean invasion and growth were expressed as a ratio relative to control conditions. Temozolomide (TMZ) sensitivity was correlated with methyl-guanine-methyl transferase (MGMT) methylation status. Length of patient survival was compared between TMZ-treated patients whose screening results had predicted a positive response and those predicted to have a negative response. **RESULTS:** Tumors from individual patients differed in terms of response profiles, especially when response was defined as at least an 80% reduction in tumor invasion distance. Similarly, different drugs varied in their ability to inhibit tumor growth and invasion, with only 9 of 31 (29%) tumors responding to TMZ, compared with 100%, 94%, and 90% response rates for paclitaxel, cis-platinum, and vincristine, respectively. Length of survival in TMZ-treated patients who screened positive for a TMZ response averaged 301 days, vs just 98 days in their TMZ-negative counterparts. **CONCLUSIONS:** In this study, we report a novel assay system that generates individual patient drug profiles, and an ability to predict patient survival in TMZ-treated patients. Further research, including a formal prospective clinical trial, may be warranted.

P.041*. STROMAL-DERIVED FACTOR 1A (SDF-1A), A HOMING FACTOR FOR MESENCHYMAL PROGENITOR CELLS, IS ELEVATED IN TUMOR TISSUE AND PLASMA OF GLIOMA PATIENTS

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Malignant gliomas are a fatal disease without sufficient possibilities for early diagnosis and a lack of chemical markers to detect remission or relapse. The recruitment of progenitor cells like mesenchymal stem cells (MSC) is a main feature of gliomas. SDF-1, a chemokine produced in glioma cell lines, enhances migration in MSC and was associated with cell survival and apoptosis in gliomas. Therefore, this study was performed to evaluate (i) whether SDF-1 and its receptors are expressed in human malignant gliomas in situ and (ii) if SDF-1 might potentially play a role in recruiting MSCs into human glioma. In glioblastoma tissue, immunohistochemistry revealed that SDF-1 and its receptor CXCR4 are expressed in regions of angiogenesis and necrosis, qPCR showed that SDF-1 is elevated and public expression data indicate that CXCR4 is upregulated. The latter data also illustrate that SDF-1 could be up- or downregulated in glioma compared with normal brain in a transcript-specific manner. In plasma, SDF-1 is elevated in glioma patients. The level is reduced by both dexamethasone intake and surgery. Dexamethasone also decreased SDF-1 production in the cells in vitro. Undirected migration of hMSC was not enhanced by addition of SDF-1. However, SDF-1 stimulates directed invasion of hMSC in a dose-dependent manner. Taken together, we show that SDF-1 is a potent chemoattractant of progenitor cells like hMSCs and its expression is elevated in glioma tissue, resulting in elevated SDF-1 levels in the patient's plasma samples with concomitant decrease after tumor resection. The fact that elevated SDF-1 plasma levels are significantly decreased after tumor surgery could be a first hint that SDF-1 might act as tumor marker for malignant gliomas to detect disease progression or remission, respectively.

P.042*. CYTOPLASMIC SUBLOCALIZATION OF THE STEM CELL-ASSOCIATED PROTEIN ASPM IS AN INDEPENDENT PROGNOSTIC FACTOR IN ASTROCYTIC GLIOMAS

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OBJECTIVE: Recently, tumor initiation, tumor recurrence, and therapy resistance in astrocytic gliomas have been attributed to the existence of brain tumor stem-like cells. ASPM (abnormal spindle-like, microcephaly associated), a stem-cell associated protein, is a key regulator of the symmetric division of neural stem cells that controls spindle orientation during cell division and therefore localizes to the cytoplasmic centromeres during interphase (cASPM) and around the nucleus during mitosis (nASPM). In this study, we correlated its expression in a tissue microarray (TMA) comprising samples of 334 gliomas to WHO grade, the proliferation marker Ki-67, and patient outcome. **METHODS:** Formalin-fixed, paraffin-embedded tissue samples of 283 primary astrocytic gliomas WHO II–IV and 51 recurrences were used for TMA design. Detection of primary antibodies directed against ASPM, Ki-67, and the appropriate secondary antibodies was carried out with Vectastain ELITE ABC Kit. Staining was graded semiquantitatively from 0 to 5 according to the percentage of positive cells covering the whole tissue spot. Cytoplasmic and perinuclear staining was analyzed independently. The relationship between ASPM expression, WHO grade, and Ki-67 was quantified by Spearman's rank correlation rho. To exclude potential prognostic confounders of survival, WHO grade, age at diagnosis, and extent of resection were included in a multivariate Cox proportional hazards regression. **RESULTS:** Perinuclear expression of ASPM was neither associated with WHO grade nor with patient outcome. Cytoplasmic expression of ASPM, however, resulted in a significant prolongation of overall survival (OS) both in gliomas of all WHO grades ($P = .021$) and in the subgroup of glioblastomas ($P = .026$) as well as time to malignant progression ($P = .026$) in gliomas WHO II–IV, independent of known prognostic confounders. Even though no significant correlation between WHO grade and cASPM expression was found, there was a trend towards a decreased expression both in case of malignant progression and recurrent glioblastoma. Importantly, a significant inverse correlation between cASPM and Ki-67 was found both in tumors of all WHO grades ($P < .0001$) and in glioblastomas ($P = .0002$). **CONCLUSION:** Our study indicates that overexpression of cytoplasmic ASPM in astrocytic gliomas WHO II–IV is a strong prognostic factor for a favorable patient outcome and is associated with a less aggressive phenotype in terms of proliferative capacity and tumor recurrence.

P.043*. EPO AND EPOR IN HUMAN GLIOBLASTOMA: FRIEND OR FOE?

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INTRODUCTION: Erythropoietin (Epo) is a well-known factor of erythropoiesis and is therefore used to treat anemia in neoplastic disease. In addition, Epo exerts neuroprotective effects via Epo-receptor (EpoR) on neuronal cells. This makes a prophylactic use against neurocognitive impairment caused by radiochemotherapy probable. Epo-/EpoR signaling, however, has also been recognized in various tumors such as glioblastomas. Several studies during the last years performed in vitro and in vivo reported conflicting results on the effect of Epo on malignant gliomas. We analyzed here the impact of Epo and EpoR expression on the prognosis of human glioblastomas in different treatment groups. **METHODS:** We established retrospectively a bank of human glioblastomas with complete documentation of clinical course and treatment. The expression of Epo ($n = 64$) and EpoR ($n = 66$) was assessed by immunohistochemistry and analyzed with semi-quantitative scores. The results were assessed separately for short- or long-term survival in the treatment groups by univariate and multivariate analysis with respect to age, gender, chemotherapy, and extent of resection. **RESULTS:** High expression levels of EpoR were correlated with a median survival advantage of 8 months ($P < .01$) in patients under 60 years of age. High levels of both Epo and EpoR were associated with a significant prolongation of 9 months median survival when compared with low levels of both molecules. In patients treated with radiochemotherapy adjuvant to operation, a trend to 6-month longer median survival was observed in association with high levels of EpoR expression that just failed significance ($P = .06$). By multivariate analysis, a positive correlation of Epo ($P = .03$) and EpoR ($P = .02$) with longer patient survival proved to be significant. **DISCUSSION:** In accordance with some of the previous studies, we found evidence for a longer patient survival associated with higher expression

levels of EpoR in human glioblastomas. A therapeutic use of EPO for anemia in glioblastoma patients seems therefore to be safe with respect to tumor growth. A prophylactic use (ie, for neuroprotection, however) cannot be recommended in light of the functional studies described in the literature.

P.044. METHYLATION-SENSITIVE HIGH-RESOLUTION MELTING ANALYSIS: QUANTITATIVE ASSESSMENT OF MGMT PROMOTER METHYLATION IN HIGH-GRADE GLIOMAS

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The methylation status of the MGMT (O⁶-methylguanine-DNA methyltransferase) gene has been shown to be a predictive marker in high-grade gliomas treated with temozolomide. Methylation-specific PCR (MSP) is widely used for the detection of the MGMT methylation. Despite its widespread use, MSP has several disadvantages. False positives can arise if primers are badly designed or used at too low a temperature. Moreover, MSP is very sensitive, but is not quantitative. Here, we show that high-resolution melting analysis (HRM) can detect MGMT methylation with high sensitivity and estimate quantitatively the extent of methylation in tumors. We used genomic DNA derived from 72 high-grade glioma samples and universal methylated/unmethylated DNA standards. After bisulfite treatment, PCR was carried out in the presence of dye to fluoresce when intercalated with double-stranded DNA. Methylated and unmethylated DNA acquires different sequences resulting in PCR products with markedly different melting profiles. By comparing the melting profiles of unknown samples with the profiles of methylated and unmethylated template ratio, we were able to estimate quantitatively the methylation levels of samples. It took us only about 90 minutes to get the data from PCR. MGMT methylation could be detected at levels as low as 1%. Methylation level measured by this assay was inversely correlated to the MGMT mRNA expression level quantified by real-time RT-PCR. High-grade gliomas with MGMT methylation <40% showed significantly short progression-free survival. Methylation-sensitive HRM is the rapid and useful method for predicting the effect of Temozolomide in high-grade glioma therapy.

P.045. THE PROGNOSTIC/PREDICTIVE ROLE OF IDH1 GENE MUTATIONS IN PATIENTS TREATED FOR RECURRENT GLIOMA

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BACKGROUND: Somatic mutations in the isocitrate dehydrogenase 1 (IDH1) gene have been frequently found in low-grade glioma (WHO grade II–III), less frequently in secondary glioblastoma (sGB), are rare in de novo glioblastoma (dnGB), and associated with a significantly younger age and a better survival from primary diagnosis. The aim of this study was to investigate the correlation between IDH1 gene mutation status and clinical outcome in patients with recurrent glioma enrolled in phase II trials with the EGFR-targeted monoclonal antibody cetuximab and the VEGFR inhibiting small molecule sunitinib. **METHODS:** Somatic DNA was extracted from formalin-fixed and paraffin-embedded tumor tissues of 52 patients with recurrent glioma, 36 of which were treated with cetuximab, and 16 who were treated with sunitinib in the context of two prospective phase II clinical trials. Nested PCR and denaturing gradient gel electrophoresis (DGGE) were performed to detect IDH1 mutations; codon 132 of IDH1 was sequenced in case of an abnormal DGGE pattern. **RESULTS:** IDH1 mutations (G395A in 15 cases and C394T in 1 case) were found in 8 of 14 (57%) grade II–III glioma, 4 of 7 (57%) sGB, and 4 of 40 (10%) dnGB ($P < .05$), and were associated with a younger age ($P < .05$). Patients with IDH1 mutations had a longer progression-free survival (PFS) and overall survival (OS) from initial diagnosis ($P < .05$ for both). IDH1 mutation status was not significantly correlated with TTP or OS from the time of recruitment in the sunitinib and cetuximab studies. A trend ($P = .07$) was observed for IDH1

wild-type patients to have a superior survival in the cetuximab-treated cohort but not in the sunitinib cohort. **CONCLUSIONS:** We confirm in this study, population that mutation of the IDH1-gene is correlated with the WHO differentiation grade and survival from initial diagnosis. IDH1 mutation status does, however, not correlate with survival from the time of recruitment in these two studies on recurrent glioma. A negative correlation with OS may be present in patients treated with the anti-EGFR-targeted mAb cetuximab. Further study is currently ongoing in one-third cohort of patients treated with bevacizumab at the time of recurrence. Updated results will be presented at the meeting.

P.046. IDH1 AND IDH2 MUTATIONS AND THEIR CORRELATIONS IN GLIOMAS

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INTRODUCTION: Somatic mutations of isocitrate dehydrogenase enzyme isoforms 1 (IDH1) and 2 (IDH2) have been recently described in a high percentage of astrocytomas and oligodendrogliomas. The two isoforms catalyze the conversion of isocitrate to α -ketoglutarate with reduction of NADP+. Mutations are preferentially located in exon 4 of both IDH1 and IDH2 genes, affecting arginine at codon 132 (R132) of IDH1 gene and the codon 172 (R172) of IDH2 gene. **MATERIALS AND METHODS:** Search for mutations in IDH1 and IDH2 genes was investigated in a series of 120 grade IV (glioblastomas), 32 grade III (10 astrocytomas and 22 oligodendrogliomas), and 44 grade I-II gliomas (10 pilocytic astrocytomas, 10 diffuse astrocytomas, and 24 oligodendrogliomas). Analysis was performed on formalin-fixed and paraffin-embedded surgical samples by direct sequencing. **RESULTS:** Distribution of IDH1 mutations was inversely correlated with histological grade, occurring in 50% of diffuse astrocytomas grade II, 10% of pilocytic astrocytomas, 75% of oligodendrogliomas grade II, 36% of oligodendrogliomas grade III, and 2% of tumors grade IV. The majority of mutations were in IDH1 gene: R132H accounting for 91% of cases, R132Q for 6% and R132C for 3%. Those of IDH2 gene (R172K) were limited to 1 glioblastoma and to 1 oligodendroglioma. Associations of IDH1 mutations were tested for the following molecular markers: MGMT hypermethylation, LOH of 1p/19q, EGFR amplification, and TP53 mutations. IDH1 mutations showed a trend towards a positive association with MGMT hypermethylation and LOH of 1p/19q, whereas they were mutually exclusive with EGFR amplification ($P = .0346$). **CONCLUSIONS:** IDH1 and IDH2 mutations may represent an early genetic event in gliomagenesis, preceding the differentiation of precursors, and may be considered prognostic. It is conceivable that they are preserved in secondary glioblastomas. The latter must be rather rare in our collection where none of the glioblastomas was diagnosed at a second surgery after a previous astrocytoma.

P.047. SERUM S-100B PROTEIN IS A PREDICTOR OF SURVIVAL IN RECURRENT GLIOMA

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BACKGROUND: S-100B protein is raised in serum after cerebral damage and disruption of the blood-brain barrier. In a pilot study, high serum levels S-100B in glioma patients were associated with shorter survival. Our aim was to evaluate the value of S-100B in serum as a prognostic marker in terms of survival or response to chemotherapy. **PATIENTS AND METHODS:** Serial samples of 22 patients with recurrent glioma were obtained before, during, and after chemotherapy. Serum S-100B was measured and a Kaplan-Meier curve was drawn for high and low serum concentrations (cut off value of 0.1 $\mu\text{g/L}$). **RESULTS:** Recurrent glioma patients with a high serum concentration S-100B at baseline had a significantly shorter survival compared with patients with a low concentration ($P = .000$). No trends were detectable in serial measurements. No correlation was found between S-100B concentration and age, gender, tumor pathology, or response to chemotherapy. **CONCLUSION:** In patients with recurrent glioma, serum concentration S-100B is a strong predictor for survival.

P.048. TEMOZOLAMIDE AND RADIOTHERAPY IN NEWLY DIAGNOSED GLIOBLASTOMA PATIENTS: MGMT PROMOTOR METHYLATION STATUS AND KI-67 AS BIOMARKERS FOR SURVIVAL AND RESPONSE TO TREATMENT

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AIMS: This phase II study aims at investigating the correlation between O⁶-methylguanine DNA-methyl transferase (MGMT) promoter methylation together with Ki-67-labeling index, and response, time to progression (TTP), and overall survival (OS) in newly diagnosed patients with glioblastoma (GBM) who are treated with temozolamide (TMZ) concomitant with and adjuvant to radiotherapy (RT). **PATIENTS AND METHODS:** From June 2005 to [Unsupported Character-#1616;] August 2008, 34 patients with newly diagnosed GBM received TMZ 75 mg/m² as radiosensitizer plus RT 2 Gy/treatment up to 60 Gy, followed by TMZ 175 mg/m² for 5 days every 4 weeks for 12 doses. Methylation-specific PCR assay and Ki-67 expression were performed on the tissue blocks. The patients were followed by MRI while MR spectroscopy (MRS) was performed to confirm progression and accordingly bevacizumab 10 mg/kg every 2 weeks was added to 7 patients till further progression was proved. **RESULTS:** Three patients were omitted because of lack of tissue specimens, else, 12 specimens (38.7%) had unmethylated MGMT, whereas 19 (61.3%) were methylated, the cut off value of Ki-67 in relation to survival was 17%, where 15 specimens were <17% (48.4%), and 16 were \geq 17% (51.6%). The overall disease control rate (ODC) was 74.2%, where 2 patients had complete response (CR), 14 had partial response (PR), and 7 had stable disease (SD), while 8 patients (25.8%) had progressive disease (PD). The ODC was significantly higher among methylated patients and in those with Ki-67 \geq 17% ($P = .0003$). The median overall TTP was 12 months and the median OS was 20 months, the methylated patients had a higher median TTP of 13 months (range 8–18 months, CI 95% of 9.36–12.9), and OS of 24 months (range 12–31 months, CI 95% of 16.1–21.32), while the unmethylated patients had a median TTP of 6.5 months and a median OS of 12 months which was highly significant ($P = .0001$).

Patients with Ki-67 <17% had a median TTP of 16 months and median OS of 24 months compared with 7 and 12.5 months, respectively, for the patients with Ki-67 \geq 17%. The multivariate analysis of both methylation status and Ki-67% showed a nonsignificant correlation to ODC, TTP, and OS. Significant correlation was found between the ODC, TTP, and OS with age <52 years ($P = .001$), tumor excision vs biopsy ($P = .0001$), and the number of TMZ doses received \geq 10 doses ($P = .002$). The commonest G3 and G4 toxicities were lymphopenia and neutropenia in 3 patients (9.67%), thrombocytopenia in 4 patients (12.9%), and 1 patient with G3 constipations (3%), all were medically manageable. **CONCLUSION:** This study showed that MGMT promoter methylation status and the Ki-67 status could serve as independent predictive and prognostic markers of response and survival, they also might identify a group of patients who could benefit from combining further therapeutic agents to the TMZ.

P.049. EVALUATION OF IMMATURE AND ABSOLUTE PLATELET COUNT CHANGES AS PREDICTORS OF SEVERE THROMBOCYTOPENIA IN MALIGNANT GLIOMA PATIENTS TREATED WITH TEMOZOLAMIDE

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BACKGROUND: Temozolamide (TMZ) is commonly used for therapy of malignant glioma and induces severe thrombocytopenia in a small fraction of patients. Currently, no biomarkers predicting TMZ-induced thrombocytopenia are available. In this study, we investigated whether changes in platelet count (PLT) or the immature platelet fraction (IPF) may serve as predictor of TMZ-induced thrombocytopenia in malignant glioma patients. The IPF has been described to reflect platelet turn-over and has been proposed as useful parameter for the differentiation and monitoring of several forms of thrombocytopenia including chemotherapy-induced myelosuppression. **METHODS:** We prospectively included 52 malignant glioma patients receiving TMZ-containing therapy regimens in this study. Platelet counts and IPF were determined at each clinical follow-up visit (weekly during concomitant to radiotherapy or at least monthly during adjuvant TMZ monotherapy) using the Sysmex XE-2100 system. **RESULTS:** The highest combination of sensitivity and specificity was observed for a PLT change per day of $-0.65 \times 1000/\mu\text{L}$. At this cutpoint, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for prediction of significant thrombocytopenia (<100,000/ μL) were 80%, 50%, 6.6%, and 98%, respectively. The

highest combination of sensitivity and specificity was observed for an IPF change of 0.014 percentage-points per day. At this cutpoint, the sensitivity, specificity, PPV, and NPV for prediction of significant thrombocytopenia were 60%, 67%, 7.6%, and 97%, respectively. CONCLUSIONS: Low sensitivity, specificity, and PPV indicate that the time course of PLT counts and IPF measured at routine clinical follow-up are not useful for prediction of thrombocytopenia in glioma patients treated with TMZ.

P.050. IDH1 MUTATION IS AN IMPORTANT FACTOR PREDICTING OUTCOME IN HIGH GRADE GLIOMAS

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INTRODUCTION: A recent genome-wide mutational analysis revealed somatic mutations of the isocitrate dehydrogenase 1 gene (IDH1) in gliomas. IDH1 serves as major source for cytosolic NADPH production necessary for the regeneration of reduced glutathione, which functions as the main antioxidant in mammalian cells. To determine the prevalence and the prognostic impact of IDH1 mutation in gliomas, we investigated a series of these tumors of different WHO grades. In addition, we examined the correlation of IDH1 mutations with MGMT promoter methylation status and overall survival. **METHODS:** We screened a total of 83 gliomas, including 34 glioblastomas (GB), 5 anaplastic astrocytomas (AA), 3 diffuse astrocytomas (DA), 1 pilocytic astrocytoma (PA), 7 anaplastic oligoastrocytoma (AOA), 3 oligoastrocytoma WHO grade II (OA), 13 anaplastic oligoastrocytoma (AOG), 12 oligodendroglioma WHO grade II (OG), 3 ependymoma (EP), and 1 anaplastic ependymoma (AEP) from the Virgen de la Salud Hospital (Toledo, Spain) by using high-resolution melting (HRM). IDH1 codon 132 sequencing was performed in 31 of these 83 gliomas. MGMT promoter methylation status was analyzed by a nested methylation-specific PCR assay. Log-rank tests and Kaplan–Meier survival curves were performed to analyze the relationship of IDH1 mutational status with overall survival of the patients. **RESULTS:** We found IDH1 mutations in a total of 36 out of 83 gliomas: 21% (7/34) GBs; 60% (3/5) AA; 67% (2/3) DA; 71% (5/7) AOA; 100% (3/3) OA; 61% (8/13) AOG; and 67% (8/12) OG. No mutation was present in any of PA, EP or AEP cases. Sequencing confirmed the results obtained by HRM in all 31 sequenced tumors. Two different mutations were found in codon 132: Arg132His in 19 cases and Arg132Gly in two other cases. Almost all IDH1 mutated gliomas presented MGMT promoter methylation (92%). The presence of IDH1 mutations was associated with a better outcome in high grade gliomas ($P < .01$). **CONCLUSION:** We confirm the very high frequency of IDH1 mutations in WHO grade II and III astrocytic and oligodendroglial gliomas while the low or absent frequency of mutation in primary GBs and ependymal tumors. In addition, in this study, IDH1 mutation is an important factor associated with favorable prognosis.

P.051. MUTATIONS IN IDH1 AND TP53 AS PROGNOSTIC BIOMARKERS IN BULGARIAN PATIENTS WITH GLIOMAS

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Brain tumors, in particular gliomas, are characterized by high mortality rate. However, gliomas with similar features often show different behavior. This raises the issue about molecular markers for prognosis prediction. Recently, somatic mutations in *IDH1*, which encodes cytoplasmic isocitrate dehydrogenase 1, were reported to occur at high frequency in glial tumors and association with overall survival was found. Genetic aberrations in *IDH1* were predominantly detected in tumors that harbor *TP53* mutations and mutations at codon 248 and 273 were associated with good prognosis. To determine prognostic value of *IDH1* and *TP53* mutations in Bulgarian patients, 30 glial tumors of various grades were selected and DNA was extracted from them. All samples were examined by direct sequencing for mutations in exon 4 of *IDH1* and exon 5–8 of *TP53* gene. Mutations in *IDH1* were found in 8 (27%) glial tumor. All genetic aberrations were located at position 395 and caused amino acid substitution R132H. Patients with *IDH1* mutations were younger (median age 35.5 vs 54 in non-

mutated cases; $P = .001$) and had better overall survival (median survival 32.1 vs 5 months in non-mutated cases; $P = .01$). Mutations in *TP53* were detected in 7 (23%) tumor samples. Patients with mutated *TP53* showed increase in overall survival (median survival 38.9 vs 5.4 months in non-mutated cases; $P = .007$). Genetic alterations in *TP53* were found in 4 (50%) tumors with mutated *IDH1* and 3 (13.6%) gliomas without *IDH1* mutation. Median survival of patients harboring mutations in both genes was 43.8 months, while median survival of those with mutation in one of the genes and nonmutated cases was 31.6 and 6 months, respectively. Our results indicate that together *IDH1* and *TP53* mutations identify gliomas with better survival and may be applied as prognostic biomarkers in Bulgarian patients with gliomas.

P.052. PROMOTOR HYPERMETHYLATION-MEDIATED DOWN-REGULATION OF RUNX3 GENE IN HUMAN BRAIN TUMORS

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Brain tumors are usually caused by a change in genetic structure. Methylation of promoter regions, with corresponding downregulation of gene expression, has been implicated as an alternative mechanism for tumor suppressor gene inactivation. Runt-related (Runx) proteins are tissue-restricted and cancer-related transcription factors that regulate cell proliferation and differentiation. RUNX3, the smallest protein of the Runx family, has been shown to play an important role in neuronal development, localized at 1p36 and its loss leads to tumorigenesis. We aimed to evaluate the methylation-mediated expression regulation of RUNX3 gene in brain tumors.

Cases (10 females, 11 males) were recruited into the study that have been diagnosed as brain tumors (3 meningiomas, 3 anaplastic astrocytomas, 3 diffuse astrocytoma, 12 GBM) and taken the decision of surgical operation. The mean age was 52.43 ± 15.33 . Explant cell cultures were performed. Total RNA was isolated. Real-time quantitative RT-PCR analyses of RUNX3 gene were actualized. Genomic DNA and the bisulphite modification were performed for DNA methylation analysis. Quantitative methylation-specific PCR was used and primer pairs were designed. There was no significant difference between methylated and unmethylated quantitative ratio of RUNX3 gene promoter region and gene expression relative ratio. Methylated and unmethylated ratio in anaplastic astrocytoma, diffuse astrocytoma, GBM, meningioma, and in all groups were: 1.44, 1.09, 1.51, 1.52 vs 1.43, respectively. One allele was found methylated necessarily. No methylation was detected in GBM and anaplastic astrocytoma groups of one each case. There was no unmethylated promoter in one of the GBM cases. There were significant differences between relative ratio of RUNX3 gene expression and methylated/unmethylated ratio rate, compared with all groups ($P = .001$) and compared with GBM groups ($P = .041$). This study overemphasized the RUNX3 gene importance in brain tumors, as a result of the existence at least one methylated allele.

P.053. BEVACIZUMAB-BASED THERAPY RECURRENT MALIGNANT GLIOMA: CORRELATION BETWEEN SERUM VEGF AND RESPONSE TO TREATMENT

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BACKGROUND: Significant therapeutic benefit has been observed for recurrent malignant glioma (MG) patients treated with bevacizumab (BV), a neutralizing monoclonal antibody against the vascular endothelial growth factor (VEGF). The relationship between VEGF levels and response to BV has never been addressed so far. **METHODS:** We conducted a prospective analysis of recurrent MG patients treated with BV alone or in combination with chemotherapy performing serial evaluations of serum and plasma VEGF (sVEGF/pVEGF) levels and procoagulant factors such as Tissue Factor (TF) and Thrombin/Antithrombin Complex (TAT) plasma levels (ELISA). Baseline, and post-treatment samples were collected at each administration of BV. **RESULTS:** Eighteen recurrent MGs (glioblastoma = 7, anaplastic astrocytoma = 7, oligodendroglioma = 4) who received BV at 10 mg/kg intravenously every 14 days, of whom 13 in association with chemotherapy were included in the study. Median age was 39 years (27–65), and median

Karnofsky performance status was 80 (60–90). The median number of previous chemotherapy lines was 2 (2–3) and all patients had received prior surgery and radiotherapy. Out of the 12 evaluable, 4 partial responses (33%), 3 stable disease (25%), and 5 disease progressions (42%) were observed. Eight patients showed an improvement in neurological signs and symptoms. Basal sVEGF lower than <224.25 pg/mL were observed in responding patients. Overall, serum and plasma VEGF, TF, and TAT levels decreased during BV-based therapy. Patients of older age (≥ 40 years) had higher sVEGF and pVEGF levels at baseline compared with the younger ones (<40 years). CONCLUSIONS: BV-based therapy showed activity in patients with heavily pretreated recurrent MGs. Low sVEGF levels at baseline might help predict response in recurrent MG patients treated with BV-based therapy.

P.054. HES6 AS A GLIOMA BIOMARKER WITH FUNCTIONAL SIGNIFICANCE FOR CANCER GROWTH

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Malignant gliomas are the most common type of primary brain tumors affecting 16,000 new patients every year in the United States. In this study, we undertook a systematic large-scale transcriptomic data mining study of 9,783 Affymetrix samples from the Genesapiens database (www.genesapiens.org) in order to identify the most glioma-specific biomarkers. We searched for genes that were highly expressed in 322 glioblastoma multiforme (GBM) samples and in 66 anaplastic astrocytomas when compared with 425 samples of the normal central nervous system as well as all other normal and cancerous tissues in the database. Transcription cofactor HES6 (Hairy and enhancer of split 6) emerged as one of the most glioma-specific genes. Since the role of HES6 in glioma pathogenesis is poorly understood, we chose to validate its expression by immunostaining and functional role by siRNA knockdown studies in glioma cell lines. HES6 protein levels were studied in a glioma tissue microarray material that consisted of 414 samples as well as in normal brain tissue controls. Positive HES6 immunoreactivity was present in 99% of available gliomas. Recurrent tumors of grade 2 astrocytomas and grade 2–3 oligodendrogliomas showed higher levels of HES6 immunoreactivity than the corresponding primary tumors. Endothelial cells within the tumors were also stained in 75% of gliomas. In functional studies, cell viability was reduced by 60% and caspase 3/7 activity elevated after HES6 silencing by RNA interference in A172 and LN405 cells. HES6 silencing also increased apoptosis 2-fold in 2 cell lines as measured by Apo-ONE Homogeneous Caspase-3/7 Assay. The biological role and consequences of HES6 silencing was explored with genome-wide analyses following RNAi, which indicated a key role for HES6 in, for example, p53, c-myc, and CREB1 transcriptional networks. Gene ontology analysis implicated genes involved in cellular movement, development, and RNA posttranscriptional modification. HES6 protein was localized to the PML bodies by immunostaining and co-localized with the creb-binding protein (CBP). In conclusion, these results pinpointed HES6 as a potential therapeutic target playing a critical role in sustaining glioma cell growth, survival and possibly invasion. HES6 may also be a useful biomarker for gliomas.

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NEW DRUG DELIVERY METHODS

P.055*. GSH-CONJUGATION IMPROVES EFFICACY OF DOXIL AGAINST INTRACRANIAL XENOGRAFTS

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High-grade glioma is a uniformly fatal disease with an unmet need for better therapy. A major reason for this poor outcome is the invasive nature of gliomas. Novel therapies that target invasive brain tumor cells should be invented, but a major impediment to the delivery of adequate amounts

of therapeutics is the blood–brain barrier (BBB), which is largely intact in regions where invasive cells reside. Glutathione (GSH)-conjugated PEGylated liposomes may be suitable vehicles for targeted delivery of small molecule cytotoxic drugs across the BBB. GSH is a natural anti-oxidant that is found at high levels in the brain and its active transporter is abundantly expressed at the BBB. Previous studies using microdialysis with an increasing % of GSH conjugated to liposomes carrying ribavirin have shown a % GSH-dependent increase of drug levels in brain interstitial fluid (up to 5-fold higher), and GSH-liposomes carrying endomorphin-1 were more effective in hot-plate tests when compared with unconjugated liposomes. We have now tested GSH-conjugated PEGylated liposomes containing doxorubicin (GSH-Doxil) for treatment of mice carrying intracranial U87 xenografts. In a first series, we compared 5% GSH-Doxil to conventional Doxil, free doxorubicin (Dx), and untreated controls. Mice were injected with 10×5 U87-luc cells and bioluminescence (BL) imaging was used for follow-up. After 11 days, mice were stratified into control or test groups ($n = 9$ / group). Mice received 3 consecutive weekly dosing of 5 mg/kg Dx-equivalents. The cohorts receiving Doxil and Dx showed a marginal growth delay relative to controls. The response with 5% GSH-Doxil was more promising but variable: two animals receiving 5% GSH-Doxil showed complete regression, which was not observed in any of the other cohorts, whereas other tumors in this cohort responded more similar to the other treatment groups. Since the treatment was well tolerated, we performed another more dose-intense series, administering biweekly 5 mg/kg Dx equivalents. Moreover, 5%GSH-Doxil and 3%GSH-Doxil were tested relative to Doxil and untreated controls. Treatment started at Day 14 after tumor cell injection, stratifying only animals whose tumor BL signals increased relative to Day 11. After Day 25, the animals experienced weight loss that precluded further dosing. In this series, the variation in tumor response was small. There was again one complete regression in the cohort of 5% GSH and not in any of the other cohorts. Moreover, the growth delay in the other tumors in this 5% GSH-Doxil cohort was significantly longer than in any of the other groups. This growth delay translated into a significantly increased median survival of 32.5 days relative to 27 days for untreated controls. The response in the 3% GSH and Doxil cohorts was marginally better relative to controls. Overall, these results warrant further pre-clinical and clinical investigation using 5% GSH-Doxil liposomes.

NEUROIMAGING OF BRAIN TUMORS

P.056*. ROUTINE FOLLOW-UP IMAGING AFTER TREATMENT FOR GLIOBLASTOMA: HOW USEFUL IS IT?

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INTRODUCTION: Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumor (MPBT). It is common practice in Neuro-Oncology centers to use cranial imaging in the posttreatment follow-up of patients diagnosed with GBM. However, there is little published guidance regarding the recommended timing and frequency of follow up imaging, or the efficacy of imaging in detecting asymptomatic tumor recurrence. OBJECTIVES: Our local Neuro-Oncology guidance recommends that patients diagnosed with GBM are CT scanned at 3 months (defined as 12 ± 2 weeks) post treatment and thereafter at 3 monthly intervals. This audit assessed compliance with local guidelines and performance in detecting asymptomatic recurrence. METHODS: Retrospective review of case notes. Data collected regarding post-treatment imaging modality, frequency, indication, and detection of symptomatic/asymptomatic tumor recurrence. RESULTS: One hundred sixty-two patients diagnosed with GBM were identified between 2004 and 2008. One hundred fifty-six cases were analyzed. 55 (35%) patients did not receive any follow-up imaging, explained in part by high patient mortality (median survival 35 weeks from time of diagnosis) and the proportion of patients receiving palliative care where follow up imaging was not indicated. Two hundred sixty-five scans were performed on 100 patients, 54% of which were within the 12 ± 2 week target. Thirty-two percent of scans were performed earlier than the 12 ± 2 week target. 36% were undertaken because of a clinical deterioration in the patient, the main reason for the reduced time interval between scans. Of 124 recurrences detected, only 11 were asymptomatic. CONCLUSIONS: Guidelines relating to the follow up imaging of MPBT are limited. The National Institute for Clinical Excellence (UK) and the European Society for Medical Oncology recommend the use of cranial imaging in MPBT follow up, stating 3–4 monthly scans is ‘common practice’. Neither specifies the optimal timing or frequency of post treatment scans. The National Comprehensive Cancer Network (USA) recommends post treatment scans every 2–3 months post MPBT treatment, as does the Cancer Council Australia. There is lack of consensus and evidence regarding the role of post treatment imaging in patients with MPBT. Further studies are required to evaluate clinical and cost effectiveness.

P.057*. PERI-ICTAL PSEUDO-PROGRESSION IN BRAIN TUMOR PATIENTS

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BACKGROUND: During the follow-up of brain tumor patients, the appearance of new contrast-enhancing lesions on MRI frequently signs disease progression. However, conditions such as early or delayed radiation necrosis can mimic tumor progression and are important to recognize to avoid futile therapeutic escalation. Transient peri-ictal MRI changes have been described in epileptic patients and some reports have suggested that these changes can also mimic disease progression in brain tumor patients. However, the clinical and MRI features of these patients have not been specifically studied yet. **METHODS:** The databases of four institutions were consulted to identify brain tumor patients who presented during the follow-up period transient MRI lesions wrongly suggesting tumor progression in a context of epileptic seizures. **RESULTS:** Seven patients were identified. Five patients suffered from high-grade gliomas, one from a medulloblastoma and one from a single brain metastasis. All patients had been initially treated with surgery and radiotherapy and all had achieved a complete remission. The peri-ictal pseudo-progression episode occurred after a mean delay of 13.3 ± 8.6 years after radiotherapy (range 3–25). All patients demonstrated new focal neurological signs in a context of frequent epileptic seizures. The MRI features were highly similar across patients and consisted of focal cortical and/or leptomeningeal enhancing lesions that could extend beyond the initial tumor localization. The clinical situation progressively improved after adjustment of antiepileptic drugs and transient oral corticotherapy. MRI was normalized at 3 months in 4 patients and at 6 months in the others. Three patients demonstrated two or more relapses with the same clinico-radiological pattern. At last follow-up, after a mean period of 3.7 ± 2.3 years (range 1–7) since the initial peri-ictal pseudo-progression episode, none of the patients had presented a tumor recurrence. **CONCLUSIONS:** In brain tumor patients, especially in long-term survivors treated with radiotherapy, the appearance of new cortical and/or leptomeningeal contrast enhancing lesions in a context of seizures should raise the suspicion of peri-ictal pseudo-progression. We make the hypothesis that this phenomenon is in relation with a post-irradiation cortical vasculopathy.

P.058*. CORRELATION OF VESTIBULAR SCHWANNOMA VOLUME WITH GROWTH AND AUDITORY FUNCTION IN A WAIT AND SCAN POLICY

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INTRODUCTION: A wait and scan policy (W&S) is often proposed in vestibular schwannomas (VS). In this policy, volume measurements have proven to be more reliable than two-dimensional measurements to establish tumor growth. In this study, we use a novel volumetric measuring tool to evaluate the correlation between VS volume and audiological function at diagnosis and during follow-up. In addition, risk factors (patient characteristics and symptoms, VS growth and morphology on magnetic resonance images (MR)) predicting hearing loss and VS growth were assessed. **MATERIALS AND METHODS:** MRI scans, corresponding audiograms (with results of pure tone audiogram (PTA) and speech discrimination score (SDS)) of 63 patients, were analyzed retrospectively. Of 36 patients, 2 or more MRI/audiogram combinations were available. Mean follow up was 21,6 months. Volume measurements were performed on contrast enhanced T1-weighted images (CE T1-WI). Morphology was evaluated by checking the presence of central nonenhancement, VS stage and side and signal intensity of the affected labyrinth. Clinical charts were analyzed for symptoms. **RESULTS:** Growth occurred irrespective of hearing status (PTA/SDS), patient age, gender, VS side, symptoms at presentation and morphology (VS stage, nonenhancement, labyrinthine signal intensity), although significant growth in the first year was predicting further growth during FU. Patients complaining of sensorineural hearing loss (SNHL) showed significant worse hearing on PTA and SDS and a trend towards more profound hearing deterioration over time was seen. Hypointensity of the affected labyrinth was a predictive factor of significant hearing loss over time compared with isointense labyrinths. Volume measurements did not correlate with audiological function and deterioration. **CONCLUSION:** Hearing loss was more profound, and hearing will deteriorate faster in patients presenting

with SNHL. Hypointensity of the affected labyrinth will result in a significant faster deterioration of PTA. Audiological deterioration occurs irrespective of VS growth and significant growth during the first year of FU predicts further growth during FU. No other factors predicting growth were identified, therefore sequential MRI remains the only objective FU method of conservatively followed vs Volume measurements are not a reliable measure predicting auditory function, in a W&S policy. These findings can aid the clinician dealing with VS patients in a W&S policy.

P.059*. MRI AND THALLIUM-201 SPECT IN THE PREDICTION OF OUTCOME IN GLIOMA THERAPY

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BACKGROUND: The prediction of outcome and the detection of tumor progression in glioma patients are of great clinical importance. In previous studies, we found ²⁰¹Tl SPECT to be superior to conventional CT and MRI in the prediction of outcome during chemotherapy for recurrent glioma. The present study aims to study the value of MRI and ²⁰¹Tl SPECT in the prediction of outcome in glioma patients treated with temozolomide, and to optimize timing of radiological follow-up during treatment. **METHODS:** We included patients treated with temozolomide chemoradiotherapy for newly diagnosed glioblastoma multiforme (GBM) (study A), and with temozolomide for recurrent glioma (study B). MRI and ²⁰¹Tl SPECT scans were obtained at regular intervals. The value of both imaging modalities in predicting overall survival (OS) and progression-free survival (PFS) were examined using Cox regression analyses. **RESULTS:** Altogether 138 MRI and 113 ²⁰¹Tl SPECT scans in 46 patients were performed. Both imaging modalities were strongly related to OS and PFS. In study A, the predictive capacity of MRI and ²⁰¹Tl SPECT increased during treatment. In study B, baseline measurements appeared strongly predictive of outcome. The addition of one modality to the other did not contribute to the prediction of outcome. **CONCLUSIONS:** Both MRI and ²⁰¹Tl SPECT are valuable in the prediction of outcome. It is adequate to restrict to one of both modalities in the radiological follow-up during treatment. Without clinical progression, we suggest to perform MRI only at the end of the standard treatment protocol in newly diagnosed GBM, and only at baseline in recurrent glioma.

P.060*. ANALYZING RESPONSE OF MALIGNANT GLIOMA TO BEVACIZUMAB USING HIGH-RESOLUTION MAGNETIC RESONANCE IMAGING AT 7 TESLA

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BACKGROUND: Glioblastoma is a highly angiogenic tumor. Therapy with the monoclonal anti-vascular endothelial growth factor (VEGF) antibody bevacizumab aims at inhibiting neo-angiogenesis and has shown promising results in phase II trials in recurrent glioblastoma. However, the effect of bevacizumab has not been adequately investigated in vivo so far. In this study, we analyze the effect of bevacizumab therapy on recurrent glioblastoma and the tumor vasculature using high-resolution magnetic resonance imaging (MRI) at 7 Tesla including susceptibility-weighted imaging (SWI). **METHODS:** We performed repeated 7-Tesla MRI investigations in 4 male and 2 female patients with recurrent glioblastoma receiving bevacizumab therapy. MRI investigations were performed at baseline and 2, 4, and 8 weeks after start of treatment. Each MRI measurement was performed within 48 hours before bevacizumab administration. A three-dimensional, fully first-order flow-compensated gradient-echo sequence with a TE of 15 ms was performed to acquire SWI data. T1-weighted data were acquired using an MPRAGE sequence with the following parameters: image-matrix = 320×320 ; resolution = $0.75 \times 0.72 \times 0.7$ mm; slices = 208; parallel imaging factor = 2, TR/TI/TE 3800/1700/3.55 ms, acquisition time = 10:29 minutes. Contrast agent was injected in 2 of 6 patients before T1-weighted measurement. **RESULTS:** Image quality was in general excellent, although in few investigations image quality was impaired by movement artifacts caused by neurological symptoms. In 3 of 6 patients we found marked and rapid decrease of brain edema after initiation of bevacizumab therapy. In 2 patients we observed an increase of SWI signals already at the first follow-up MR investigation 2 weeks after initiation of bevacizumab therapy. Both patients developed progressive neurological worsening and bevacizumab therapy had to be suspended because of tumor progression after 6 and 8 weeks, respectively. In 1 patient showing rapid decrease of contrast enhancement and sustained clinical improvement under bevacizumab

therapy, SWI signals remained unchanged at all 7-Tesla MRI investigations. CONCLUSIONS: High-resolution 7-Tesla MRI is feasible in patients with malignant glioma. Our data indicate that in a fraction of recurrent glioblastoma patients early growth of tumor vasculature occurs despite bevacizumab therapy ("primary bevacizumab resistance"). Advanced imaging modalities may improve assessment of response to bevacizumab therapy.

P.061*. VALUE OF THALLIUM SPECT (SPECT) TO EVALUATE RESPONSE TO BEVACIZUMAB TREATMENT IN HIGH-GRADE GLIOMA (HGG) PATIENTS

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OBJECTIVES: To analyze whether Thallium-SPECT is a good method to evaluate response to bevacizumab in recurrent HGG patients. **METHODS:** Seventeen patients with recurrent HGG who were considered for treatment with bevacizumab ± irinotecan (Bev ± Iri) were studied with a SPECT and MRI, before and after treatment, in order to evaluate response correlation. **PATIENT CHARACTERISTICS:** Twelve out of 17 patients could be evaluated with both techniques: 1 in 16 patients without previous positive SPECT, 4 patients progressed before imaging evaluation. Male/female ratio was 9/3. There were 10 GBMs out of 12. Previous surgery was biopsy in 5 cases, and partial or complete resection in 7. Regarding previous treatment, Stupp regimen was administered in 6 cases, Number previous chemotherapy lines ≥ 2 were 7/12. KPS $\geq 60\%$ was in 11/12 and Barthel I $\geq 60\%$ in 11/12. Evaluated treatment was as follows: Bev 2/12 or Bev and Iri 10/12. **RESULTS:** Response rate by MRI was as follows: P 41.7%, SD 8.3%, PR 41.7%, and CR: 8.3%. Response rate by SPECT was as follows: P 16.7%, SD 41.7%, PR 16.7%, CR 25%. Response by modified MacDonald criteria: P: 58.3%, SD: 8.3%, PR: 25%, CR: 8.3%. Coincidence between SPECT and MRI was 24.9% and SPECT with MacDonald's criteria was 16.6%. The solely patient with a CR by MRI and MacDonald's modified criteria had a normalization of a previous positive SPECT lasting for 103+ weeks while continuous maintenance Bev. **CONCLUSIONS:** In spite of reduced number of patients, it seems that Thallium SPECT is not a good imaging method to evaluate response of bevacizumab treatment.

P.062*. PRESERVATION OF PYRAMIDAL TRACT BY NAVIGATION-ASSISTED INTRAOPERATIVE MAPPING IN GLIOMA SURGERY

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OBJECTIVE: The preservation of pyramidal tract is essential and very important issue to maintain the patients' quality of life. Recent technologies such as tensor-image of MRI and neuronavigator are unreliable method for precise identification as a result of intraoperative brain shift. Electrical stimulation of the resected tumor cavity has been a gold standard; however, it sometimes results in postoperative neurological deterioration. We developed a novel method to identify and protect the motor fiber during tumor resection. **METHODS AND RESULTS:** NY Tract Finder II, a new electrode designed for navigation-assisted detection of motor tract in cerebral white matter, was used during the resection of glioma adjacent to pyramidal tract. The bipolar needles are insulated except those tips and marked off in millimeters. The electrode was inserted into the cerebral white matter with guidance by a neuronavigator with continuous electrical stimulations. The muscle-motor evoked potentials were recorded to alert surgeons to the existence of motor fibers. In the recent cases, tractography images were integrated into the neuronavigation system and compared with intraoperative neurophysiological data. This technique enabled the detection of the pyramidal tract adjacent to glioma. The depth of pyramidal tract from the wall of tumor resected cavity could be measured by the ruler on the needles. There were discrepancies between tractography data integrated into neuronavigator and actual neurophysiological localization of pyramidal tracts. Postoperative MRIs revealed that the tumors were resected close to the primary motor cortices and pyramidal tracts even with brain shifts. None of the patients presented postoperative neurological deterioration. **CONCLUSIONS:** This technique may be a feasible method to detect and spare the motor pathways even with brain shifts. The combination of 2 modalities is easy-to-use technique in the glioma surgery in eloquent brains.

P.063*. MAGNETIC RESONANCE IMAGING OF VIRAL SPREAD AND INTRATUMORAL INFLAMMATION DURING ONCOLYTIC VIROTHERAPY

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One of the major drawbacks in treating gliomas is the poor efficiency of drug delivery. Oncolytic viruses (OVs) that can selectively replicate in tumor cells and potentially can reach the infiltrating margins of the tumor, represent a promising tool to overcome this problem. However, the *in vivo* therapeutic efficacy of OVs is limited because of host factors. We have recently demonstrated that the capacity of OVs to spread *in vivo* through the tumor is inhibited by intratumoral infiltration of phagocytic microglia and peripheral macrophages. Combining OVs with immune suppressive drugs can therefore increase their spread and therapeutic efficacy. However, the lack of a noninvasive imaging technique to detect intratumoral OV spread and phagocytic infiltration constitutes an important limitation in evaluating the results of new therapeutic strategies. We have tested 2 noninvasive magnetic resonance imaging techniques. The first one uses a gadolinium-based contrast agent to detect myeloperoxidase (MPO) activity, an enzyme present in phagocytic cells. MRI images show increased MPO activity after OV delivery. This enzymatic activity is reduced when animals are pretreated with cyclophosphamide. The MRI data correlate with immunohistochemistry staining of phagocytic cells and *ex vivo* measured MPO mRNA levels and activity. We also show that this technique presents a unique spatial resolution whereby the inflammation process at the border and in the center of the tumor can be distinguished and provides us with information on tumor size and shape. The second technique allows imaging of the spatio-temporal distribution of replicating agents (ie, OVs), through the use of artificial peptides presenting magnetic contrast through chemical exchange saturation transfer (CEST). We have thus generated an OV armed with a CEST-reporter gene to be tested in brain tumor oncolytic virotherapy. Because these two technologies can be combined, together they will provide a powerful diagnostic tool to monitor efficacy of OVs and glioma response to virotherapy, thus leading to the design of efficient strategies of virotherapy that overcome the influence of host factors.

P.064. EVALUATION OF MODIFIED METHIONINE PET IMAGING TO DISTINGUISH RADIATION NECROSIS FROM RECURRENT MALIGNANT GLIOMA: THE NEW METHOD TO EVALUATE TISSUE PROLIFERATION

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¹¹C-Methionine (MET) is useful for evaluating radiation necrosis (RN) and glioma recurrence. However, it is sometimes difficult to distinguish these lesions because the accumulation of MET is affected not only by tissue proliferative activity but also by vascular factors, such as the vascular bed volume or the disruption of BBB. RN has mild accumulation of MET mainly affected by the vascular factors. To exclude the vascular factors, we made modified MET (mod-MET) PET images. On the basis of our studies of infarction, accumulation of ¹¹C-Choline (CHO) is thought to be mostly affected by the vascular factors. The vascular MET-SUV, which reflected only the vascular factors, could be obtained from the CHO-SUV using linear regression for MET-SUV and CHO-SUV of the choroid plexuses. The mod-MET PET, which is obtained by eliminating the vascular MET-SUV from the original MET-SUV, is thought to mostly reflect tissue proliferation. The differentiation between RN and recurrent glioma was studied by using MET, CHO, ¹⁸F-Fluorodeoxyglucose (FDG), and mod-MET PET. The PET images were obtained from histologically verified 16 RNs, 16 recurrent grade-3 gliomas (Gr.3) and 17 recurrent glioblastomas (Gr.4). All lesion/normal (L/N) ratios for Gr.4 were significantly higher than those for RN ($P < .005$), but there was significant difference between Gr.3 and RN only in the MET and mod-MET L/N ratios ($P < .05$). ROC analysis indicated that mod-MET PET was the most accuracy for differentiating between RN and tumor recurrence. The best cutoff value of mod-MET L/N was 4.75, providing a sensitivity of 78.8% and a specificity of 93.7%. Even for cases in which RN is barely distinguishable from recurrence on the original MET-PET, the mod-MET-PET made it easier to visually distinguish these lesions.

P.065. FUNCTIONAL DIFFUSION MAP: NEW IMAGING ASSESSMENT OF GLIOBLASTOMA PATIENTS TREATED BY BORON NEUTRON CAPTURE THERAPY

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INTRODUCTION: Assessment of therapeutic efficiency for glioblastoma (GB) patients is traditionally accomplished by measuring changes in tumor size on gadolinium-enhanced T1-weighted MRI at 10 weeks after treatment. One disadvantage of size measures is the duration for changes to occur, with 10 weeks necessary to assess the response. The functional diffusion map (fDM) which is a new imaging assessment of GB patients was reported by Hamstra *et al.* This fDM analysis is able to assess at 3 weeks after initiation of treatment earlier than the traditional imaging assessment. In this study, we evaluated GB patients treated by boron neutron capture therapy (BNCT) by using this fDM analysis. **MATERIALS AND METHODS:** During 2003–2007 period, 17 patients with GB treated by BNCT were retrospectively enrolled onto a study of intratreatment MRI at 2 and/or 7 and/or 14 days, and/or 10 weeks. We used *I-ResponseTM-1.0 fDM analysis* that is analysis software to be able to assess changes over time of apparent diffusion coefficient (ADC) values. **Results and Discussion:** The volume of tumor with decrease diffusion analyzed by fDM at 2 days was the strongest predictor of patients survival time since BNCT ($R^2 = .7433$). Our study showed that greater decreases in ADC value in response to BNCT over time were observed in the good prognosis patients compared with the poor prognosis patients. The decrease in ADC value in response to BNCT at an acute stage was caused by BNCT as a high-dose radiation therapy, unlike a conventional radiotherapy as a low-dose radiation therapy. Briefly, BNCT might cause tumor cells to swell in an acute stage by the high-dose radiation therapy and fDM analysis captured it as an imaging of fDM. **CONCLUSION:** The fDM analysis could provide an earlier imaging assessment of GB patients treated by BNCT. Early detection of treatment failure can also allow more intensive therapy in patients with the worst prognoses. This fDM analysis will have the potential to replace size measures. Therefore, we will assess many more the number of GB patients and perform further animal experiments.

P.066. A RARE CASE OF EXTRA-AXIAL MEDULLOBLASTOMA PRESENTING WITH MULTIPLE CRANIAL NERVE THICKENING

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CASE REPORT: A 26-year-old male presented with left-sided hearing loss. MRI showed a small, subtle enhancing lesion in the left internal auditory canal (IAC), suggestive of schwannoma. Steroid therapy was started, and the patient was referred and scheduled for surgery in our centre. Two months later, while on the waiting list, the patient noticed right-sided hearing loss. Repeated MRI demonstrated a new right-sided IAC lesion and increased left-sided IAC mass and subtle, bilateral thickening of cranial nerves III–X. Lack of enhancement was attributed to steroid effect. Within the next week, the patient developed progressive cranial nerve deficits. Neither extensive blood, repeated CSF examination, nor PET-CT directed towards inflammation or primary malignancy. Biopsy of the IAC-mass was proposed, but declined by the patient. Steroid therapy was continued with vasculitis as working diagnosis and symptoms improved. Twelve months after his first presentation, the patient presented with paretic foot musculature. Spinal MRI demonstrated small intradural, extramedullary lesions on C3 and Th11 and thickening of the cauda equina. In a multidisciplinary session, biopsy of the Th11-lesion was decided. However a few days later, patient deteriorated very quickly, and MRI showed posterior fossa masses and extensive supratentorial and intraspinal leptomeningeal deposits. The right frontal leptomeningeal lesion was biopsied. Microscopy showed small round blue cells, rosettes, and leptomeningeal proliferation. CD56 immunohistochemical staining confirmed neurogenic origin. Final diagnosis was desmoplastic/nodular medulloblastoma with leptomeningeal deposits. **DISCUSSION:** Medulloblastoma is the most common pediatric central nervous system malignancy, usually presenting as an intra-axial infratentorial mass. Adult cases are extremely rare (annual incidence 1 per 2–20 million). Leptomeningeal spread occurs in 33% of cases. Extra-axial presentation of medulloblastoma is extremely rare, with only 9 reported cases in literature, the majority presenting as cerebellopontine angle masses.

To our knowledge, medulloblastoma presenting as multiple cranial nerve involvement has not been described before.

P.067. UNUSUAL IMAGING FINDINGS IN A TEMPORAL LOBE HIGH-GRADE GLIOMA WITH INVOLVEMENT OF BRAINSTEM WHITE MATTER TRACTS

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INTRODUCTION: We present a multilinear brainstem lesion, in conjunction with a right temporal mass, which turned out to be an astrocytoma. To our knowledge, this kind of presentation has not been described so far. **CASE DESCRIPTION:** A 51-year-old male patient presented at the neurology department with dizziness, speech disturbances and a strange feeling at the left side of his face. Initial neurological examination was unremarkable, except for a dysarthria. One day after the MRI scan was performed, the patient was admitted to the emergency department because of a secondary generalized seizure. Neurological examination revealed a (post ictal) left sided paralysis and a Babinski-reflex at the left side. In the following days and weeks, there was progressive neurological deterioration with progressive dysarthria, swallowing disturbances, diplopia as a result of abducens paresis and a left-sided hemiparesis. Laboratory results of blood and CSF were unremarkable. No signs of infection were present. Lues, Neuroborreliosis, HIV, Epstein-Barr, Herpes encephalitis were ruled out. CSF showed no pleocytosis or signs of malignancy. MRI showed a T2 hyperintense mass in the right temporal lobe, without enhancement after administration of gadolinium; radiologically suggestive of a low-grade glioma. Furthermore, hyperintensity along the white matter tracts in brainstem, pons, cerebellar peduncle, and thalamus was present. This white matter hyperintensity exerted some mass effect and enhanced partially, namely in the right-sided brainstem. Repeated MRI scan 2 weeks later showed progression of the hyperintense tracts, now extending into the left pons en cerebellar peduncle. The patient underwent a navigation-guided biopsy of the right temporal lesion. Pathological examination showed a diffuse infiltrating neoplasm of astrocytic origin with high cellularity and increased proliferation index. No tumor necrosis or microvascular proliferation was seen. A high-grade (WHO grade III) anaplastic astrocytoma of the temporal lobe and brainstem was concluded. Patient deteriorated further and palliative treatment was offered. **CONCLUSION:** The presented case is a radiological illustration of a high grade glioma diffusely infiltrating the white matter tracts in brainstem, pons and thalamus, thereby visualizing the normal anatomical connections. This rare presentation should be recognized to prevent unnecessary delay in establishing the diagnosis.

P.068. CONTRAST ENHANCEMENT ON INTRAOPERATIVE MRI: IS IT TUMOR?

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We describe a case of a patient with a right frontal glioblastoma. The tumor consisted of a large lesion and a ventral satellite lesion. Contrast enhancement of both lesions did not overlap. Both lesions were resected using ultra low-field strength intraoperative MRI (0.15 Tesla). The relation between contrast enhancement on intraoperative MRI and histological findings has not yet been evaluated systematically. This case report discusses intraoperative and histological findings, emphasizing several pitfalls involved in contrast dose and timing of contrast administration.

GLIOBLASTOMA MULTIFORME AND ANAPLASTIC GLIOMAS

P.069*. PROGNOSTIC IMPACT OF STEM CELL MARKER CD133 IN 61 GLIOBLASTOMA PATIENTS TREATED WITH CONCOMITANT RADIOCHEMOTHERAPY: A PROSPECTIVE STUDY

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Cancer stem cells (CSC) are thought to represent the population of tumorigenic cells responsible for tumor development. The CD133

antigen has been described as a putative stem cell marker in malignant brain tumor that could identify such a tumorigenic population in a subset of glioblastoma multiforme (GBM). To date, the correlation between CD133 expression in primary GBM and patients' prognosis is not clearly established. To address this question we investigated the relationship between the quantitative expression of CD133 stem cell antigen mRNA using real-time RT-QPCR and patient outcome in a prospective cohort of 61 primary GBM patients treated with radiotherapy combined with concomitant and adjuvant therapy with temozolomide. On multivariate survival analysis, CD133 stem cell antigen expression was a significant ($P = .007$) prognostic factor for adverse overall-survival independent of extent of resection ($P = .012$), patient age ($P = .037$), and MGMT status ($P = .002$). Furthermore, according to the conjoined expression of CD 133 and MGMT status, the patients were categorized into three groups. Among these three groups, patients with methylated tumors and low expression level of CD 133 (group I) had the best prognosis, patients with unmethylated tumors and high expression level of CD 133 (group III) had the poorest prognosis and others (group II) had an intermediate outcome. These findings constitute conclusive evidence that the measurement of the mRNA expression of CD133 stem cell antigen carried by some GBM cancer stem cells actually impact the survival of GBM patients, lending support to the current brain tumor stem cell hypothesis.

P.070*. PATTERN OF RECURRENCE AND PSEUDO-PROGRESSION IN GLIOBLASTOMA PATIENTS TREATED WITH POSTOPERATIVE RADIOTHERAPY WITH CONCURRENT TEMOZOLOMIDE

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BACKGROUND: Combined postoperative therapy with temozolomide (TMZ) and radiation has become standard first-line treatment in glioblastoma multiforme (GBM). Pseudo-progression is often observed since the introduction of this concurrent regimen. The objective of this study is to compare the incidence of pseudo-progression, pattern of recurrence, and overall survival (OS) in patients treated before and after introduction of concurrent TMZ in this indication. **METHODS:** A retrospective review was conducted of all pathologically proven GBM cases treated postoperatively in our center between 2004 and 2008. OS was calculated from the time of diagnosis. Pseudo-progression was examined by two different criteria. The first strict criteria define pseudo-progression as $\geq 25\%$ increase in tumor size or the occurrence of a new contrast-enhancing lesion, with, in absence of new active treatment, either subsequent spontaneous regression to baseline or smaller, or pathologically proven tumornecrosis by second resection. The second more liberal criteria also included cases with stable disease for at least 6 months after first progression. Recurrence was judged unusual occurring contralaterally or extracerebrally. Tumor status was assessed before and after surgery, 1 month after completion of radiotherapy, and every 3 months thereafter. **RESULTS:** A total of 136 patients were analyzed, 80 males and 56 females, median age 62 years (17–81), median Karnofsky performance index at diagnosis 70 (20–90). In total, 123 cases were primary GBMs, 13 were secondary, 13 patients had multifocal disease, 69 patients underwent macroscopically complete resection, 40 partial resection, 24 biopsy. 3 had no primary surgery, but were diagnosed by biopsy later. Seventy-three patients were intent to treat with concurrent chemo-radiation followed by 6 cycles adjuvant TMZ, of which 37 completed the full treatment as planned (50%). Median OS in the combined group was 16 months compared with 8 months in the other group ($P = .00003$). Pseudo-progression was observed in 10 cases in the combined group (16%) vs none in the other group using the strict criteria ($P = .003$). Applying more liberal criteria, pseudo-progression was found in 17 cases (27%) in the combined group vs one in the other group ($P = .0003$). The median time to pseudo-progression was 4 weeks after radiation. Only pseudo-progression assessed by the liberal criteria is associated with a significantly better OS. An unusual pattern of relapse was observed in 15 (21%) patients who were treated with the combination compared with 6 (10%) in the others ($P = .05$). **CONCLUSIONS:** The median OS in the group who received combined therapy was 16 months. Combined treatment is associated with higher incidence of unusual sites of relapse. Contralateral or extracerebral relapses were observed in more than twice as many patients. Pseudo-progression after combined treatment strongly depends on the criteria being used.

P.071*. CAN OS-6 REPLACE PFS-6 AS A PRIMARY ENDPOINT IN PHASE II STUDIES ON GLIOBLASTOMA PATIENTS GIVEN ANTIANGIOGENETIC DRUGS?

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BACKGROUND: In the last decade, progression-free survival at 6 months (PFS 6) has been considered the best endpoint in phase II trials on recurrent glioblastoma (GBM). However, since a "new standard" of care was established by the EORTC/NCIC phase III trial, no data have reported on the PFS-6 or overall survival (OS) obtained with second-line treatment following combined RT/TMZ. Moreover, antiangiogenic agents might alter data on response by repairing the blood-brain barrier and diminishing contrast enhancement, thus precluding the accurate assessment of any reduction in the tumor burden. The issue of a robust primary endpoint for phase II studies is therefore still open. The aim of the study was to evaluate outcome endpoints for second-line treatment at recurrence. **METHODS:** A retrospective analysis was made using a database on 653 GBM patients followed prospectively between May 2001 and August 2008. Eligibility criteria: age ≥ 18 years; PS 0–2; histological diagnosis of GBM; cytotoxic treatment at disease progression after RT/TMZ. The log-rank test was used to evaluate the significance of the prognostic variables, and the Cox model to ascertain any association between PFS and OS. **RESULTS:** A total of 150 patients (median age: 52 years, [24–76 years]) were enrolled. MGMT methylation status, evaluable in 110 patients, was present in 38% of cases. Median OS was 18.5 months. At disease progression, 40 patients (27%) received temozolomide, 92 patients (61%) received nitrosourea-based chemotherapy, and 18 patients (12%) received other treatments. At the time of recurrence, mPFS was 2.5 months (95% CI: 2.0–3.1), PFS-6 15% (95% CI: 9.5%–21.3%, mOS 7.6 months (95% CI: 6.9–8.3) and OS-6 64% (95% CI: 56.6–72.2%). In the Cox proportional hazard model, PFS with the second-line treatment was correlated with OS measured from the start of the second-line treatment ($P < .0001$). **CONCLUSIONS:** The findings made in the present study, the first in literature to evaluate outcome endpoints for a second-line treatment after RT/TMZ in GBM patients, confirm that a PFS-6 of 15% should be considered the cut-off for active cytotoxic drugs in phase II studies on recurrent GBM. For antiangiogenic compounds, OS 6 can be considered as a sound endpoint.

P.072*. A PHASE III RANDOMIZED CONTROLLED TRIAL OF SHORT-COURSE RADIOTHERAPY WITH OR WITHOUT CONCOMITANT AND ADJUVANT TEMOZOLOMIDE IN ELDERLY PATIENTS WITH GLIOBLASTOMA MULTIFORME

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INTRODUCTION: The EORTC (26981-22981)/NCIC CTG (CE.3) RCT in newly diagnosed GBM found sustained improvement in survival with the addition of concomitant and adjuvant temozolomide (TMZ) to radiotherapy (RT). Study patients were aged 19–71 (median 56) years, and a trend benefit analysis found less benefit with an increase in age. A recent RCT in elderly GBM patients found improved survival with RT compared with supportive care alone and another study detected equivalence of 40 Gy/15 vs a 60 Gy/30 RT regimen. Therefore, RT alone is considered standard for elderly patients and 40 Gy/15 is supported as an acceptable fractionation scheme. However, the question remains: does the addition of TMZ to RT confer a survival advantage in elderly patients for whom "short-course" radiotherapy is recommended? **Study Design:** In order to have 90% power to detect a 33% improvement in the primary outcome of overall survival (increased MST from 6 to 8 months) between arms, using a two-sided 5% alpha, a minimum of 520 deaths must be observed prior to final analysis. Assuming accrual of 150 patients per year, 560 patients will be accrued in 3.7 years with final analysis after an additional year of follow-up, yielding a study duration of 5 years. **Study Progress:** The trial is open in Canada (NCIC CTG), Europe (EORTC), Australia and New Zealand (TROG), and Japan. As of March 1 2010, 123 patients were randomized. Median

age is 73 (65–86) years with 78% over the age of 70. Seventy-five percent patients are ECOG PS 0 or 1 and 25% are ECOG PS 2; 69% had sub- or gross-total resection, 31% biopsy only. Discussion: The NCIC CTG CE.6 randomized study of RT alone vs RT and concurrent and adjuvant TMZ is an international cooperative effort addressing an important unmet need in the spectrum of care for newly diagnosed GBM.

P.073*. INFLUENCE OF HYPOXIA ON GLIOMA AND GLIOMA STEM-LIKE CELLS

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Glioblastoma multiforme (GBM) is the most common brain tumor but also the most aggressive one. Despite heavy treatment, life expectancy usually does not exceed 15 months. Therapy failure may be explained by GBM features such as high proliferation rate, invasiveness, and cellular heterogeneity. Similar to other malignant tumors, GBM appear to contain a subpopulation of cells that display stem cell properties and are able to survive in a hypoxic microenvironment. However, the mechanisms permitting stem-like cell survival under low oxygen conditions are poorly understood. Here we provide a detailed functional and molecular analysis of glioma stem-like cells grown under hypoxic conditions. Two stem cell-like cell lines NCH644 and NCH421k were compared with classical serum-dependent glioma cells (U87, U251, and U373) with regard to their behavior less than 1% and 0.1% O₂ culture conditions: proliferative potential, invasiveness, and clonogenicity were investigated. Stem cell-like cells showed marked differences in their response to hypoxic conditions as compared with non-stem-like glioma cells. Low oxygen levels dramatically inhibited glioma cell division whereas stem cell-like cell proliferation was only marginally affected by hypoxia. These cells appeared to survive even when oxygen level was as low as 0.1%. The optimal hypoxia conditions were chosen for further transcriptomic analysis based on cell survival data and protein expression profile of hypoxia inducible factor (HIF-1 α). The cellular response to hypoxia was studied at the transcriptomic level using whole-transcript expression analysis (GeneChip® Human Gene 1.0 ST, Affymetrix). Candidate genes for cell survival response to hypoxia were validated using quantitative PCR, protein-based approaches, and functional bioassays. Such genes may provide new molecular targets for GBM treatment by specifically targeting the glioma stem-like cell population.

P.074*. CIRCULATING ENDOTHELIAL CELLS DECREASE SIGNIFICANTLY IN RELAPSING HIGH-GRADE GLIOMA PATIENTS RESPONDING TO BEVACIZUMAB

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Angiogenesis, the recruitment of new blood vessels, is an essential component of tumor progression. High-grade gliomas (HGG) are highly vascularized tumors and bevacizumab, a humanized monoclonal antibody targeting the vascular endothelial growth factor (VEGF), is active in preclinical testing in vitro and in vivo. In the clinical setting, in combination with irinotecan, other chemotherapy agents or alone, it has shown significant activity in HGG patients. Therefore, the identification of patients who might benefit from antiangiogenic therapies is crucial for the optimization of treatment strategies. In other solid tumors, levels of circulating endothelial cells (CECs) and levels of circulating progenitors (CEPs), contributing to the formation of tumor vessels, correlate with the degree of tumor angiogenesis or the response to angiostatic therapy. Twenty-seven patients with recurrent HGG (22 glioblastomas (GBM) and 5 anaplastic astrocytomas (AA)) were treated at the Neurological Institute C. Besta, Milan, with irinotecan (340 or 125 mg/mq for patients on EIAEDs or not, respectively) and bevacizumab 10 mg/kg every 2 weeks. Median age was 53 years (range 15–66) and median Karnofsky Performance Status (KPS) was 70 (range 50–100). The median number of prior chemotherapy treatments was 2 (range 1–4). Median follow-up was 6 months. Number and viability of CECs and CEPs were measured on Day 0 and every 2 months by 6-color flow cytometry. CECs were enumerated as Syto + CD 45- CD31 + /P1h12+ cells, whereas CEPs as Syto + CD 45- CD31 + /CD133+ cells. CEC subpopulations expressing CD109 were also enumerated. No severe side-effect was observed during treatment. The first MRI, 2 months after treatment onset,

showed progressive disease in 6 subjects, partial response in 16, and stable disease in 4; 1 patient was lost to follow-up. Overall, 6M-PFS was 50% and 6M-OS was 64%. Median PFS and median OS were 6 and 10 months, respectively. For GBM patients, 6M-PFS was 37% and 6M-OS was 65%. At baseline the number of CECs was significantly higher in GBM patients than in AA (113.1 \pm 55.7 vs 61 \pm 31, $P = .04$). A significant reduction of CECs and viable CECs was observed only in GBM patients with a clinical and radiological response after 2 months of therapy (111.6 \pm 52.4 vs 70.9 \pm 55.3, $P = .05$ for CECs and 33.4 \pm 18.3 vs 16.2 \pm 16.4, $P = .03$ for CECs viable). No significant difference in CEPs number and viability among patients and during treatment was detected. The data suggest that investigations of CEC investigation levels could may contribute to a better understanding of clinical responses to bevacizumab action in HGG patients.

P.075*. THE EXPRESSION OF NG2 IDENTIFIES A TUMOR-COMPETENT POPULATION IN GLIOBLASTOMA WITH DISTINCT MOLECULAR SIGNATURE

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INTRODUCTION: We previously demonstrated that NG2 expressing (NG2+) cells in glioblastoma (GBM) exhibits robust proliferative and tumorigenic activity and share phenotypic and functional similarities with NG2 expressing glial progenitors. Here, we conducted comparative studies to address the difference of the molecular signature of GBM-NG2+ and GBM-NG2- cells. **METHODS:** GBM cell lines were derived from clinical samples according to our Cambridge Protocol. GBM-NG2+ cells were sorted using FACS. Comparative molecular studies were conducted using microarray, comparative genomic hybridization (CGH), and Western blot. **RESULTS:** Microarray data indicated that NG2+ cells overexpressed a group of genes previously recognized by Cancer Genome Atlas within the Mitosis and Cell-Cycle Module (MCM). Array data analysis showed overexpression of unique set of proliferative pathways and transcription factors (TFs) by GBM-NG2+ cells. Gene Ontology enrichment identified more than 200 gene categories that were enriched in the GBM-NG2+ cells. The top 10 categories were related to cell cycling, M phase, and DNA replication. Similarly, CGH demonstrated subtle structural differences between the molecular cytogenetic profile of GBM-NG2+ and GBM-NG2-. Finally, we demonstrated that MAPK and Akt pathways were significantly overactivated in GBM-NG2+ compared with GBM-NG2- cells. **CONCLUSION:** We previously showed the robust proliferative activity and tumorigenicity of GBM-NG2+ cells. Here, we provide evidence that our previous observations are linked to the distinct molecular signature of GBM-NG2+ cells. This signature includes notable structural chromosomal abnormalities, unique enrichment of TFs and MCM genes, and over activation of MAPK and Akt pathways.

P.076*. NEOADJUVANT TEMOZOLOMIDE FOR GRADE III AND IV ATROCYTOMA: A RANDOMIZED PHASE II STUDY

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BACKGROUND: A pilot study conducted with TMZ given neoadjuvant before radiotherapy (RT) for astrocytoma grade III and IV resulted in long survival times compared with historical controls. We therefore further investigated the value of neoadjuvant TMZ in a randomized phase II trial. During the enrolment period, concomitant radio-chemotherapy became standard treatment and was therefore incorporated in the later part of the trial. **MATERIAL AND METHODS:** Newly diagnosed patients with astrocytoma grade IV (glioblastoma, GBM) or grade III (anaplastic astrocytoma, AA) age \leq 60 years and performance status (PS) 0–2 were randomized to receive either 2–3 cycles of TMZ, 200 mg/m² Days 1–5 every 28 days, followed by RT 60 Gy in 30 fractions or RT only. The third TMZ cycle was administered to patients with nonprogressive disease after 2 cycles of TMZ. From June 2005, all patients received TMZ 75 mg/m² daily concomitant with RT. No adjuvant TMZ was given, but was recommended as first-line treatment at progression, unless patients progressed while on TMZ therapy. Primary endpoint

was overall survival and secondary endpoints were safety and quality of life. RESULTS: A total of 143 patients were enrolled in the trial. Of these 87 (61%) received TMZ concomitant with RT. GBM was diagnosed in 103 patients and AA in 40. Median age was 58 years (range 24–60) and 63% were male. PS was 0–1 for 93% of patients and 87% had undergone surgical resection. The treatment arms were well balanced. Of all patients, 71 (50%) were randomized to the neoadjuvant treatment. Of these, 67 (94%) received the first cycle of TMZ, 64 (90%) the second, and 58 (82%) also the third. At the time of data analysis, 98 patients (68.5%) were dead. Median survival time for all patients was calculated to 20.5 months. CONCLUSIONS: The role of TMZ in the treatment of GBM given concomitant with RT and adjuvant is well documented. We explored the value of TMZ given neoadjuvant for 2–3 cycles, before RT for GBM and AA in a phase II randomized trial. The final results will be presented at the upcoming EANO meeting.

P.077*. CANCER STEM CELLS IN GLIOBLASTOMA, WHAT ARE THEY?

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Glioblastoma multiforme (GBM) is one of the most heterogeneous tumors, both at the genetic and the cell morphology level. It has been proposed that only a subset of cancer cells display stem cell properties and are tumorigenic in vivo (cancer stem cells, CSCs). However, there is now growing evidence that expression of a putative stem cell marker, such as CD133, cannot define the only GBM subpopulation with tumor initiating capability. A number of studies have also shown that tumor initiation depends on the microenvironment and the animal model used, rather than being an intrinsic property of a subpopulation of tumor cells. In this project, we aim to characterize subpopulations of tumor cells with stem cell characteristics from GBM xenografts and determine whether these cells are a subpopulation of the tumor (bonafide CSCs) or represent a changing entity adapting to the signals from the microenvironment. To address this question we apply flow cytometry to identify and characterize small subpopulations of cells within a highly heterogeneous tumor population, according to cell surface and internal markers and according to their drug efflux properties (side population). We have set up an immunodeficient GFP expressing mouse xenograft model, which recapitulates the invasive and angiogenic features of human GBM. The use of a GFP mouse allows to distinguish between tumor and host cells, an important aspect since both populations could include cells with stem cell properties. The presence of several putative CSC markers as well as lineage-specific epitopes is examined within the tumor and host cell populations.

P.078. SMALL MOLECULE KINASE INHIBITORS IN GLIOBLASTOMA: A SYSTEMATIC REVIEW OF CLINICAL STUDIES

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The efficacy of small molecule kinase inhibitors has recently changed standard clinical practice for several solid cancers. Glioblastoma is a solid cancer that universally recurs and unrelentingly results in death despite maximal surgery and radiotherapy with concomitant and adjuvant temozolomide. Several clinical studies using kinase inhibitors in glioblastoma have been reported. The present study systematically reviews the efficacy, toxicity, and tissue analysis of small molecule kinase inhibitors in adult patients with glioblastoma as reported in published clinical studies and determines which kinases have been targeted by the inhibitors used in these studies. Publications were retrieved using a MEDLINE search and by screening of meeting abstracts. A total of 60 studies qualified for inclusion, 25 of which are original reports. A total of 2385 glioblastoma patients receiving kinase inhibitors could be evaluated. The study designs include 2 phase III and 37 phase II studies. Extracted data include radiological response, progression-free survival, overall survival, toxicity, and biomarker analysis. The main findings are (i) that efficacy of small molecule kinase inhibitors in clinical studies with glioblastoma patients does so far not warrant a change in standard clinical practice and (ii) that 6 main kinase targets for inhibitors have been evaluated in these studies (ie, EGFR, mTOR, KDR, FLT1, PKC β , and PDGFR).

P.079. NPAS3 IS A NOVEL LATE-STAGE ACTING PROGRESSION FACTOR IN GLIOMAS WITH TUMOR SUPPRESSIVE FUNCTIONS

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BACKGROUND: In our effort to better comprehend the genetics of gliomas, we explored new therapeutic targets. We previously cloned NPAS3, a transcription factor that maps to human chromosome 14. Our principal aim is to comprehend the disease associations of NPAS3, since we recently identified expression in human astrocytes. We investigated NPAS3 as a candidate for astrocytomas based on findings archived from the Cancer Genome Project demonstrating a loss of NPAS3 expression and with loss-of-function deletions of human chromosome 14 with NPAS3 in 30%–50% of astrocytomas. METHODS AND RESULTS: After undertaking extensive functional analyses, we now have novel evidence supporting NPAS3 as an astrocytoma tumor suppressor involved in late-stage tumor progression, based on: (i) Absent NPAS3 expression is predominant in high-grade astrocytomas (79–83%), in comparison with low-grade astrocytomas (29–35%). (ii) Loss of function mutations of NPAS3, which are associated with a loss of heterozygosity of the NPAS3 locus are identified in GBMs. (iii) Absent NPAS3 expression is predominant in >60% of malignant human glioma cell lines. (iv) An over-expressed NPAS3 in malignant glioma cell lines suppresses the transformation potential, while the converse reduced expression promotes an increase in transformation potential. (iv) A reduced NPAS3 expression (efficiency >90%) in concert with other gliomagenesis genes can transform a well-characterized TERT immortalized human astrocyte cell line and promote the growth of anaplastic astrocytomas. CONCLUSIONS: Our data provide compelling findings of NPAS3 as a novel gene involved in the cause of astrocytomas, with tumor suppressive and late-stage acting progression factor roles.

P.080. A NOVEL METHOD TO ENRICH FOR GLIOMA STEM CELLS FROM GLIOMA CELL LINES

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BACKGROUND: Glioma stem cells (GSCs) are inherently similar to stem cells except they can transform into tumors reminiscent of the pathological features of the originated tumor mass. GSCs serve as an excellent pre-clinical model to comprehend tumor re-growth and treatment resistance. Several approaches were previously described to purify GSCs, but seemingly appeared to be laborious, costly, and sometimes with poor yield. Our objective was to investigate alternative strategies to cost-effectively and efficiently enrich for GSCs. METHODS AND RESULTS: We grew 3 glioma cell lines in a modified serum-free media that promotes the growth of stem cells over a 10-day period and with ease of harvesting from the supernatant. The tumorspheres had cell line-specific morphologies. For instance, those from U87 and DB54MG were significantly larger with tightly associated spheres, in comparison with those from U251. The tumorspheres expressed stem cell markers and in fact were 80%–96% rich in CD133+ve cells. Upon growth in DMEM/10% FCS, tumorsphere differentiation occurred. In addition, the tumorspheres can transform in vitro and with the ability to grow into tumors having similar pathological hallmarks but faster growth in comparison with xenograft tumors derived from the growth of glioma cell lines. These findings were overall similar with passages 1, 10, and 30 GSCs examined. CONCLUSIONS: We have discovered an alternative strategy to enrich for glioma stem cells from glioma cell lines in a cost-effective, easy, and efficient manner. Current efforts are undertaken to utilize our protocol to enrich for glioma stem cells from surgical tissues.

P.081. A CHEMICAL GENETICS SCREEN IDENTIFIES NOVEL STEROID INHIBITOR DRUGS THAT INHIBIT THE GROWTH OF GLIOMA CELL LINES

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BACKGROUND: Gliomas are among the top 5 causes of cancer-related deaths, representing about ~60% of the cases in adults and ~30% in children. Despite current treatments (surgery, radiation, and chemotherapy), the

overall survival is still poor. Current promises exist with patients treated with adjuvant temozolomide; however, only 10%–15% typically have a positive response with combined surgery and radiation therapies leading to prolonged survival of up to 2 years. Since a wide range of steroid receptors are expressed in gliomas, our objective was to investigate whether novel classes of steroid inhibitor drugs can be used efficiently to inhibit glioma growth. To achieve this, we studied the effect of these drugs on the growth of glioma cell lines. **METHODS AND RESULTS:** We screened using a candidate chemical structure approach, a library of 400 steroid inhibitor drugs on 5 human glioma cell lines, and a normal human astrocyte cell line. We discovered 4 potent new drugs of the androsterone family that can induce significant death of glioma cell lines ($n = 5/5$) within a 24-hour period in contrary to normal human astrocytes. These drugs induced significant apoptosis resulting in an overall decreased viability and proliferation of the cells in a dose-dependent manner (5 and 10 μM). Furthermore, significant inhibition of transformation was noted. **CONCLUSIONS:** We have discovered a novel chemically distinct class of drugs that can significantly inhibit the growth of glioma cell lines. Current efforts are undertaken to study more of the mechanistic function of these drugs.

P.082. A CHEMICAL GENETICS SCREEN IDENTIFIES NOVEL STEROID INHIBITOR DRUGS THAT INHIBIT THE GROWTH OF GLIOMA STEM CELLS

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BACKGROUND: Glioma stem cells represent a fraction of cells within a tumor mass that are postulated to be responsible for tumor re-growth. Moreover, recent studies have associated glioma stem cells with impeccable chemoresistance mechanisms, leading to an overall poor survival and failure among patients treated by conventional adjuvant chemotherapy. Since a wide range of steroid receptors are expressed in gliomas, our objective was to investigate whether novel classes of steroid inhibitor drugs can be used efficiently to inhibit glioma growth. To achieve this, we studied the effect of these drugs on the growth of glioma stem cells. **METHODS AND RESULTS:** We screened using a candidate chemical structure approach, a library of 400 steroid inhibitor drugs on 5 human glioma stem cells established from surgeries ($n = 2$) and cell lines ($n = 3$), and a normal human neuroprogenitor cell line. We discovered 5 potent new steroid inhibitor drugs belonging to the methyl-piperazine family, which can induce significant death of glioma stem cells ($n = 5/5$) within a 24 hour period, and with some death of normal human neuro-progenitor cells. These drugs induced significant apoptosis, resulting in an overall decreased viability and proliferation of the cells in a dose-dependent manner (5 and 10 μM). Furthermore, significant inhibition of transformation was noted. **CONCLUSIONS:** We have discovered a novel chemically distinct class of drugs that can significantly inhibit the growth of glioma stem cells. Current efforts are undertaken to study more of the mechanistic function of these drugs.

P.083. BRAINSTEM GLIOMA IN ADULTS: A RETROSPECTIVE STUDY

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INTRODUCTION: Brainstem glioma are rare in adults. Literature on the subject is limited to small retrospective studies that described natural course, clinical-radiological presentation, and prognostic factors. Need for histopathological confirmation and therapeutic planning remains controversial. Surgery is first choice when tumor site permits it, even when only subtotal resection can be reached. Nevertheless, radiotherapy is very useful when tumor site is not easily accessible and for patients with poor clinical condition. Radiotherapy is better tolerable than surgery, has minor complications, and provides acceptable survival. Biopsy might be useful to differentiate with benign processes when MRI is not convincing and to define molecular genetics for future use of targeted agents. **MATERIAL AND METHODS:** The characteristics of 26 patients aged ≥ 16 years with brainstem glioma diagnosed in our center between 1987 and 2005 were reviewed. **RESULTS:** The median age at diagnosis is 35 years, with a median survival of 30.1 months (range 4–237.5 months). The main presenting symptoms were cranial neuropathy, ataxia, and/or hydrocephalus. Diagnosis is mostly based on MRI findings. Histological diagnosis was

available in only 8 of 26 patients. Contrast enhancement, central necrosis, or poorly delineated lesion on MRI correlate with poor prognosis (median survival of 23.1 vs 120.1 months). Three patients underwent a subtotal resection, but all 26 were irradiated with doses between 51 and 60 Gy. Three patients suffered from radiotherapy-linked complications consisting of necrosis ($n = 1$) and hearing disability ($n = 2$). Salvage chemotherapy was given in 5 patients at recurrence with a median survival of 15.7 months. The following patient features (characteristics) predict poorer prognosis: age above 40 years, hydrocephalus, WHO performance above 1 and high grade appearance on MRI. Duration of symptoms < 3 months was almost statistically significant to predict poorer prognosis. The more negative prognostic features present, the worse the survival. This was statistically significant. **CONCLUSIONS:** The need to perform a biopsy is a matter of dispute. In our series, contrast enhancement, central necrosis, and poorly margined lesions predicted shorter survival, indicating that radiological features can be used to diagnose high-grade glioma including biopsy is not necessary. All patients were irradiated with acceptable survival, only 3 suffered from complications. Radiotherapy alone is still an excellent therapeutic option. Age > 40 years, long tracts signs, hydrocephalus, and WHO score > 1 predicted for poorer prognosis. Shorter duration of symptoms was almost significant. This confirms the literature and can be used to decide which patients can benefit from more aggressive treatment. Chemotherapy should be preserved as rescue therapy at recurrence, seeing the median survival in our patient group.

P.084. GAMMA-KNIFE RADIOSURGERY FOR RECURRENT GLIOBLASTOMA RESISTANCE TO THE TEMOZOMOLIDE

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PURPOSE: The current standard of care for newly diagnosed glioblastoma is surgical resection to the extent feasible, followed by radiotherapy plus concomitant and adjuvant temozolomide. Ultimately, despite this current standard treatment, almost all patients with glioblastoma will have relapse. Gamma-knife (GK) stereotactic radiosurgery is a safe and less invasive treatment used as adjuvant therapy for patients with glioblastoma. Several studies have yielded conflicting results in the effectiveness of radiosurgery in glioblastoma. This article describes the results of our institutional experience with GK adjuvant therapy in the treatment of patients with recurrent glioblastoma resistance to the temozolomide. **METHODS:** Eighteen patients with newly diagnosed glioblastoma were treated with operation and concomitant temozolomide radiochemotherapy from 2006 to 2009. Six patients with recurrent glioblastoma were treated for 26 lesions with GK. Two patients were male and 4 were female. The median age at primary diagnosis of the tumor was 65.5 years (range: 53–81 years). All patients were received debulking surgery. Histology evaluations of all patients revealed glioblastoma. In all patients, radiotherapy was performed as first-line therapy, applied as fractionated external beam radiotherapy with concomitant temozolomide chemotherapy. The median interval between initial diagnosis and primary GK was 9.2 months (range: 6–11 months). The median target tumor size was 8.1 cm^3 (range: 0.65–38.8 cm^3). The median dose applied was 17.7 Gy (range: 15–20 Gy) prescribed to the 50% (range: 45%–80%) isodose line that encompassed the target volume. The median follow-up time was 22.5 months (range: 14–37 months). **RESULTS:** Median survival time of patients treated with GK was 19.0 months and without GK was 15.0 months. Treatment was well tolerated by all patients. No acute toxicities CTCAE Grade II occurred. Median survival time of patients treated with GK was 19.0 months and without GK was 15.0 months. **CONCLUSIONS:** GK radiosurgery is a relative safe and less invasive treatment and may play an important role in the treatment of recurrent glioblastoma resistance to the temozolomide.

P.085. DOES GENDER MATTER IN GLIOBLASTOMA?

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BACKGROUND: Clinical outcome of glioblastoma (GBM) patients who receive radiotherapy alone or plus chemotherapy is well established. However, little is known about patients who do not receive this treatment. In published studies, it is difficult to identify the percentage of patients who never receive oncological treatment after surgery and to determine the associated variables. **METHODS:** We reviewed all GBM patients operated in our hospital between January 2000 and December 2008. Patients' clinical data in our center are prospectively included in a database. We compare those who received oncological treatment and those who did not.

Variables analyzed were age, gender, clinical presentation, pre- and post-surgery KPS, size, location, extent of surgery, and surgical complications. RESULTS: A total of 216 patients with GBM were identified. Fifty-five (25%) did not receive any treatment after surgery. Univariate analysis showed that variables associated with the absence of oncological treatment were: gender, 33% women vs 20% of men were not treated, $P = .03$; age, median age 56 years (treatment) vs 64 years (no treatment), $P < .001$; initial KPS, 53% of patients with KPS ≤ 60 vs 18% of those with KPS > 60 were not treated, $P < .0001$; and post-surgery KPS, 68.3% of patients with KPS ≤ 60 vs 8% of those with KPS > 60 were not treated, $P < .0001$. In the multivariate analysis age (> 60 vs ≤ 60 , OR = 2.5 95% CI: 1.1–5.7, $P = .024$) and post-surgery KPS (KPS ≤ 60 vs > 60 , OR = 24.7, 96% CI: 11.0–55.5, $P < .0001$) were independent predictors of no treatment after surgery. We analyzed why there were more women in the non-treatment group. Women in the whole series were older than 60 years, $P = .01$, they had a worse KPS before, $P = .04$, and after surgery, $P = .02$, and had more biopsies, $P = .04$. In the multivariate analysis, gender was associated with a lower pre-surgery KPS (women vs men, OR = 2.7, 95% CI: 1.2–6.1, $P = .014$) and older age (> 60 vs ≤ 60 , OR = 2.0, 95% CI: 1.2–3.5, $P = .013$) at diagnosis. In the whole group, median survival time (MST) was 313 days for men ($n = 125$) vs 216 days for women ($n = 91$), log rank $P < .037$. However, in the treated group, we did not observe any difference in MST between men and women. CONCLUSIONS: One out of the 4 patients could not be treated after surgery. Associated factors were older age and lower KPS. These poor risk variables were more frequent in women, and they therefore had a lower survival in our series

P.086. RECURRENT SPINAL CORD GLIOBLASTOMA: SALVAGE THERAPY WITH BEVACIZUMAB

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BACKGROUND: Primary spinal cord tumors constitute 2%–4% of all primary CNS malignancies in adults of which less than 5% are glioblastoma. A retrospective evaluation to determine toxicity and response to bevacizumab in patients with recurrent spinal cord glioblastoma. PATIENTS AND METHODS: Six patients (4 males and 2 females; median age: 34 years) with recurrent spinal cord glioblastoma were treated with bevacizumab (10 mg/kg given once every 2 weeks wherein 2 treatments constituted a cycle of therapy). All patients had failed surgery and temozolomide-based chemoradiotherapy and post-radiotherapy temozolomide. Blood counts, chemistry panel, urine protein to creatinine ration, and neurologic examination were obtained bi-weekly. Contrast-enhanced spine MRI was performed after 1 cycle of therapy and thereafter following every 2 cycles of bevacizumab. RESULTS: Treatment-related complications included fatigue in 6 patients, constipation in 4, hypertension in 2, thrombophlebitis in 2, and infection without neutropenia in 2. There were 3 grade 3 toxicities (1 each fatigue, leukopenia, and thrombophlebitis). There were no treatment-related deaths. After one cycle of bevacizumab, 1 patient (17%) demonstrated progressive disease, 2 (34%) partial responses, and 3 (51%) stable disease. Overall median response or stable disease duration (disease free progression) was 7 months (range: 3–11 months). Overall median survival was 9 months (range: 5–13 months). CONCLUSIONS: Bevacizumab is well tolerated, has tolerable toxicity, and apparent activity in this small cohort of adults with recurrent spinal cord glioblastoma.

P.087. CONCURRENT 3-TIMES DAILY ULTRAFRACTIONATED RADIATION THERAPY AND TEMOZOLOMIDE FOR NEWLY INOPERABLE GLIOBLASTOMA: TEMOFAC, A PHASE II STUDY

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BACKGROUND: Ultrafractionation radiation therapy consists in irradiating cells or tumors several times daily, delivering low doses at which hyper-radiosensitivity occur. We have conducting a phase II clinical trial to determine the effect of a concurrent ultrafractionation regimen and temozolomide for

inoperable glioblastoma patients. METHODS: A phase II study has opened for accrual in February 2008. Patients over 18 years of age who are able to give informed consent and have histologically proven, newly inoperable diagnosed and supratentorial glioblastoma are eligible. Three doses of 0.75 Gy spaced by at least 4 hours are delivered daily, 5 days a week for 6 consecutive weeks (67.5 Gy), and concomitant chemotherapy consisted of temozolomide given 7 days per week during the ultrafractionated radiotherapy. After a 4-week break, chemotherapy is resumed up to 6 cycles of adjuvant temozolomide every 28 days. Tolerance and toxicity are the primary endpoints and survival and progression-free survival are the secondary endpoints. RESULTS: To date 31 patients have been enrolled in this study, 21 men and 10 women, median age 62, median Karnofsky performance status was 80. The concomitant ultrafractionated radiotherapy, temozolomide, has been well tolerated; no acute grade 3 and/or 4 CNS toxicity has been observed and 1 grade 4 hematological toxicity was reported during the combined therapy. Two patients progressed during the radiotherapy, and 2 patients died of pulmonary embolism. Median survival was not yet reached. CONCLUSIONS: Concurrent ultrafractionated radiation (temozolomide) is safe and well tolerated. At meeting, an analysis of survival will be given.

P.088. CONCURRENT RADIOTHERAPY–FOTEMUSTINE COMBINATION FOR NEWLY MALIGNANT GLIOMA PATIENTS: A PHASE II TRIAL

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PURPOSE: Fotemustine is a nitrosourea compound used for the treatment of malignant gliomas, especially in France. Recently, an EORTC-NCIC study has shown that a concomitant combination of radiotherapy plus temozolomide (an oral cytotoxic drug) improved survival in glioblastoma patients. We set out to test a concurrent combination of radiotherapy and fotemustine for newly malignant gliomas. METHODS: A prospective single-centre phase II study opened for accrual in September 2004. Patients over 18 years of age able to give informed consent and with histological proven, newly diagnosed supratentorial malignant gliomas were eligible. All patients were treated by a standard cranial irradiation (conformal irradiation, tumor bulk plus a margin of 2.5 cm) and concomitant daily administration of 10 mg/m² of fotemustine (5 days per week, 6 weeks, 1 hour 30 minutes before radiation therapy). Adjuvant chemotherapy, fotemustine, was administered at tumor progression as standard and classic regimen. RESULTS: Twenty-two patients were enrolled, 16 men and 6 women, median age 56 years (range: 32–74 years), median Karnofsky performance status 70 (range from 60 to 90). Histology included 16 glioblastomas, 3 anaplastic astrocytomas, 2 anaplastic oligodendrogliomas, and 1 mixed glioma. Eight patients underwent surgery (3 total resections) and 14 had a stereotactic biopsy. The concurrent radiotherapy–fotemustine combination was well tolerated: toxicity was mild and 3 hematologic toxicities grade 3–4 were observed. Median survival from initial diagnosis was 9.9 months, 2 patients are currently alive. Median survival was 11 months for surgery and 9 months for stereotactic biopsy. CONCLUSIONS: Concomitant radiotherapy–fotemustine combination is safe and well tolerated. Overall survival of over 10 months for the whole population compares favorably with other reports.

P.089. GENE EXPRESSION PROFILING PREDICTS RESPONSE TO TMZ IN GLIOBLASTOMAS IN VITRO

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Glioblastomas are highly lethal neoplasms that cannot be cured which currently available therapies. Temozolomide (TMZ) is a recently introduced alkylating agent that has yielded significant benefits and become a key agent in the treatment of high-grade gliomas including glioblastoma. However, its survival benefit remains unsatisfactory. Understanding the molecular basis of TMZ sensitivity/resistance is necessary for improving the treatment outcome by devising strategies that are able to circumvent primary drug resistance. We therefore combined the in vitro TMZ response with microarray gene expression data to identify genes that could potentially be used to predict the response of glioblastomas to

TMZ therapy. We first obtained the individual IC50 values for TMZ in 7 malignant glioma cell lines and then identified the genes whose expression correlated most highly with TMZ sensitivity employing a cDNA microarray. We present here a list of the most highly up- and downregulated genes which may be involved in conferring TMZ sensitivity/resistance in malignant gliomas including glioblastomas, although most of the genes have not been implicated as a causal factor in the TMZ response except MGMT. We also demonstrated and confirmed the MGMT methylation status, quantitative MGMT mRNA levels, and MGMT protein expression levels in TMZ resistant glioma cells in vitro. Our results are thus consistent with previous studies and suggest that a dominant mechanism conferring sensitivity/resistance to TMZ exists in malignant glioma cells. Although the present study dose has several limitations, our reported candidate genes could represent not only the potential molecular markers for TMZ sensitivity/resistance but also the chemotherapy targets. Furthermore, the present study could provide a foundation for alternative therapeutic strategies including novel combination treatments that incorporate additional reagents directed at overcoming resistance to TMZ.

P.090. TEMOZOLOMIDE DOSE DENSE AS SALVAGE TREATMENT FOR GLIOBLASTOMA

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BACKGROUND: There is no clear agreement for the choice of the best second-line regimen after failure of temozolomide standard schedule (TMZ-SS) in patients with recurrent glioblastoma (GBM). In patients with relapsed GBM, dose-dense regimens of TMZ (TMZ-DD) have been applied and the weekly alternating one showed low toxicity and good efficacy. **METHODS:** We have retrospectively analyzed clinical data of 14 patients treated with TMZ-DD 150 mg/m² 1 week on-1 week off after failure with TMZ-SS in patients with primary GBM. **RESULTS:** Between July 2007 and January 2010, 14 patients (9 men and 5 women; median age 56; range: 36-72) followed at our institution for primary GBM underwent TMZ-DD after the evidence of clinical and/or neuroradiological progression during TMZ-SS. All patients had a diagnosis of primary GBM: 11 were radically operated (78.5%) and 3 were submitted to partial exeresis (21.5%). MGMT status was as follows: unmethylated MGMT: 9 patients (64%) and methylated MGMT: 5 patients (36%). Eleven patients (78.5%) received concomitant chemo- and radiotherapy (RT) (Stupp regimen); 2 patients received radiotherapy (RT) only (14.3%): 1 for age and 1 for low PS (he received only 45 Gy palliative treatment). One patient (7.2%) were not submitted to RT for the extension of the disease (both frontal lobes). After chemo-RT, RT alone, or as primary treatment, all patients were all submitted to TMZ-SS: median number of cycles delivered was 4 (range: 2-12 cycles). At clinical and/or neuroradiological progression, all patients underwent TMZ-DD: 12 after the first progression (85.7%) and 2 patients (14.3%) for progression after second surgery. Six patients showed a disease control defined as the sum of objective response (1 patient with complete response) and stable disease (5 patients), with a median duration of response of 4.7 months (1-30 months); 3 patients (50%) were unmethylated and 3 patients were methylated (50%). One patient achieved the resectability after 3 months of TMZ-DD. Median progression free survival was 3.4 months. Median overall survival was 12.3 months (range: 9-39 months). No grade 3-4 toxicity (CTC 3.0) was recorded: 4 patients presented hematologic toxicity (G2) and 1 skin rash (G2). **CONCLUSIONS:** TMZ-DD is feasible and may be a good option after failure of TMZ-SS for its good safety profile. Its role as neoadjuvant treatment might be further investigate.

P.091. HYPOFRACTIONATED HIGH-DOSE IRRADIATION PLANNED BY METHIONINE PET FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

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PURPOSE: The ability of intensity modulated radiation therapy (IMRT) to deliver a high conformative dose of irradiation to the target has promoted

its application studies for patients with glioblastoma multiforme (GBM). Generally, the dose-escalation efforts have relied on the contrast-enhanced MRI, although contrast enhancement is not always an accurate measure of tumor extension. Metabolic imaging studies such as 11C-methionine PET (MET-PET) may improve the ability to identify the target volumes at highest risk of local failure. In this study, we evaluated the clinical significance of hypofractionated high-dose irradiation planned by MET-PET with MRI for the treatment of GBM. **MATERIALS AND METHODS:** Thirty-nine postoperative patients with GBM were treated by IMRT using the tomotherapy system with concurrent chemotherapy. The gross target volumes by MRI (GTV-MRI) were defined as the residual gross tumor or resection cavity, based on the contrast-enhancement MRI. GTV-MRI was expanded uniformly by 1.5 cm to form the MRI clinical target volumes (CTV-MRI). GTV-MET was considered to be that the area of intensive MET uptake, demonstrating a threshold of 1.7 for the standardized uptake value (SUV) of the tumor, compared with that of the normal brain. CTV-MET was considered to be that the area of moderate MET uptake, demonstrating a threshold of 1.3 for the SUV. GTV was finally defined as the area including the total volume of GTV-MRI and GTV-MET, and CTV was defined as the area including the total volume of CTV-MRI and CTV-MET. The GTV and CTV were expanded uniformly by 0.5 and 0.2 cm to generate planning target volumes, PTV-1 and PTV-2, respectively. IMRT was performed in 8 fractions, planning the dose for PTV-1 at 56 Gy and PTV-2 at 40 Gy with concurrent chemotherapy by temozolomide (TMZ) of 75 mg/m² daily. Adjuvant chemotherapy by TMZ of 150 mg/m² was repeated every 4 weeks. At a median follow-up of 13 months, the treatment outcomes and toxicity were evaluated. **RESULTS:** The median survival time was 18.5 months. The 1- and 2-year progression-free survival rates were 59% and 20%, respectively. The 1- and 2-year overall survival rates were 74% and 24%, respectively. The high incidence of CSF dissemination was demonstrated as the pattern of recurrence, while the incidence of local recurrence was relatively low. Five patients experienced acute grade 1 toxicities during the treatment. Nine patients experienced late toxicities, including radiation necrosis, cerebropathy, and intratumoral hemorrhage. **CONCLUSIONS:** Our regimen of IMRT with TMZ using MET-PET contributed to the control of residual infiltrating tumor, resulting in better survival of GBM patients. Survival times in this pilot study are encouraging, and relative favorable result might be from the decreasing of local failure, which suggested that original lesion could be well controlled by high-dose radiation planned by MET-PET. On the other hand, radiation-induced late toxicity was the next problem.

P.092. EFFICACY AND TOLERABILITY OF LEVETIRACETAM MONOTHERAPY IN PATIENTS WITH PRIMARY BRAIN TUMORS AND EPILEPSY

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OBJECTIVES: Epilepsy is a common symptom in patients with brain tumors, particularly gliomas. Enzyme-inducing or -inhibiting antiepileptic drugs (AEDs) are known to interact with antineoplastic drugs and corticosteroids, resulting in altered drug levels and potential inefficacy or toxicity. Levetiracetam does not have these interactions and may benefit these patients. We aimed to determine the efficacy and tolerability of levetiracetam monotherapy in glioma patients with epilepsy. **METHODS:** Forty glioma patients with epilepsy were recruited. All patients had undergone surgery and were on levetiracetam monotherapy at the time of inclusion. They were included within 6 weeks postoperatively. Treatment with levetiracetam was according to the routine daily care of patients with epilepsy. Clinical characteristics regarding patient, tumor, and epilepsy history were documented. Follow-up took place after 3 and 6 months. Seizure reduction (compared with preoperative baseline) and drug withdrawal as a result of adverse effects or inefficacy were defined as endpoints. **RESULTS:** Three patients were lost during follow-up: all 3 because tumor progression. After 6 months, 21 patients (57%) were seizure-free, whereas 6 patients (16%) reported a reduction in seizure frequency of >50% and 2 patients (5%) reported no changes in seizure frequency compared with their preoperative status. Seven patients (18%) had to switch to another AED because of lack of efficacy ($n = 4$) or adverse effects ($n = 3$). Efficacy was not related to any clinical characteristic. **CONCLUSIONS:** Although earlier studies indicate that add-on therapy with levetiracetam seems effective, there is hardly

any information available on levetiracetam monotherapy. Our results indicate that levetiracetam monotherapy is efficacious in reducing seizures and is well tolerated in the majority of glioma patients suffering from epilepsy.

P.093. AGGRESSIVE TREATMENT IS APPROPRIATE FOR ELDERLY (70 YEARS AND OLDER) PATIENTS WITH GLIOBLASTOMA MULTIFORME: A RETROSPECTIVE REVIEW OF 206 CASES

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PURPOSE/OBJECTIVE(S): Elderly patients have largely been excluded from large, randomized trials of therapy for glioblastoma multiforme (GBM). Small randomized trials have been done, evaluating the effect of individual treatment modalities. We reviewed our results of aggressive treatment with combined surgery, chemotherapy, and radiation in this group of patients. **PATIENTS AND METHODS:** From an IRB-approved institutional brain tumor database, we identified patients 70 years of age and older who were newly diagnosed with GBM from May 1979 through September 2007. Overall survival (OS) was the primary endpoint of this retrospective study. Univariate and multivariate analyses were performed, utilizing the log-rank test and Cox proportional hazards model to assess the difference in survival for patients with various characteristics and treatment. **RESULTS:** Two hundred and six patients 70 years of age and older were identified from the database. The median age was 75 years (range: 70–90 years). Patients had a wide variety of treatment modalities ranging from no treatment to a combination of surgery with chemoradiation with temozolomide. Median OS was 4.5 months. In univariate analysis, factors that significantly affected OS included Karnofsky performance status (KPS) (1.8 months for KPS \leq 50 vs 17.2 months for KPS = 90–100, $P < .001$); age at diagnosis (5.1 months for age 70–79 vs 3.1 months for age 80 or greater, $P < .001$); and the extent of disease with patients with bilateral disease ($P = .003$), multifocal disease ($P = .02$), and multicentric disease ($P = .002$) doing worse in all cases. Patients treated with radiation had longer OS of 6.7 vs 1.9 months for those not treated with radiation ($P < .001$) as did patients treated with chemotherapy who had an OS of 11.2 vs 3.5 months for those not treated with chemotherapy ($P < .001$). On multivariate analysis, higher KPS ($P = .006$), surgical resection ($P < .001$), radiation ($P < .001$), and chemotherapy ($P < .001$) were all found to be independently associated with improved OS. **CONCLUSIONS:** These data suggest that aggressive treatment with radiation, chemotherapy, and surgery improves OS in patients 70 years or older with newly diagnosed GBM.

P.094. TRABEDERSEN IN RECURRENT HIGH-GRADE GLIOMA: IMPACT ON TUMOR CONTROL AND SURVIVAL

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INTRODUCTION: TGF- β 2 regulates key mechanisms of cancerogenesis, namely immunosuppression, metastasis, angiogenesis, and proliferation. The antisense oligonucleotide trabedersen (AP 12009) is a TGF- β 2-specific inhibitor and is developed for the treatment of patients with highly malignant tumors such as recurrent or refractory high-grade glioma (HGG). **METHODS:** Clinical studies with trabedersen in HGG have included 3 phase I/II studies and 1 randomized, active-controlled, open-label, multinational dose-finding phase IIb study. These studies were performed in adult patients with recurrent or refractory HGG (AA, WHO grade III and GBM, WHO grade IV). Trabedersen was administered intratumorally by convection-enhanced delivery. **RESULTS:** In the phase IIb study, a total of 145 patients were randomized to either 1 of the 2 doses of trabedersen (10 or 80 μ M) or to chemotherapy (TMZ or PCV). One hundred and thirty-four patients (AA = 39; GBM = 95) received study medication during a treatment period of about 6 months. In the entire study population, the 10- μ M

trabedersen group had the highest tumor control rate (CR + PR + SD) at 14 months (22.5% for 10 μ M trabedersen vs 6.7% for chemotherapy). The highest efficacy was observed in AA patients treated with 10 μ M trabedersen. The proportion of patients showing a response (either CR, PR, or SD) in AA patients was 83% (10 of 12 patients) in the 10- μ M trabedersen group, 53% (8 of 15 patients) in the 80- μ M trabedersen group, and 58% (7 of 12 patients) in the chemotherapy group. In addition, the duration of response was highest in the 10- μ M trabedersen group with 29.1 months compared with the 80- μ M trabedersen (24.0 months) and the chemotherapy group (8.0 months). The median time to progression was 22.4 months for the 10- μ M trabedersen group and 13.0 months for the chemotherapy group. Furthermore, the 10- μ M trabedersen group showed a remarkable median survival benefit of 17.4 months over chemotherapy (39.1 vs 21.7 months). In addition, promising efficacy data were observed in GBM, especially in patients with age \leq 55 years and KPS $>$ 80%. Trabedersen generally had a good tolerability and safety profile. **CONCLUSIONS:** Trabedersen treatment has a clear clinical benefit in HGG. On the basis of the phase IIb results, the pivotal phase III study SAPPHERE in patients with recurrent/refractory AA was started. Patient recruitment is ongoing. Primary endpoint is 2-year survival; secondary endpoints include overall survival, tumor response, quality of life, and safety.

P.095. INCIDENCE AND SURVIVAL IN GLIOBLASTOMA PATIENTS WITH PSEUDO-PROGRESSION IN A SINGLE INSTITUTION

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We evaluate incidence and impact in survival and progression-free survival of pseudo-progression (PSP) in patients with glioblastoma (GBM). From December 2006 to September 2009, 61 primary GBM were treated in our institution with resection (54) or biopsy (7) followed by TMZ radiotherapy followed by TMZ (150–200 mg/m²/day for 5 days each 28) for 2 years or until disease progression or unacceptable toxicity. One month after chemoradiotherapy, MRI was performed and compared with the MRI done within 72 hours after surgery. The 12 and 24 months survival rate and PFS were analyzed by the Kaplan–Meier method, with the use of 2-sided log-rank test statistics. The median age was 60 years (range: 18–72), 43 were males. The median follow-up was 12 month (range: 2–37). The MRI 1 month after the end of radiotherapy showed progressive enhancement in 35 patients (57.3%) and stable disease or minor partial response in 26. All continued with 2 or 3 cycles of temozolomide. Of the 35 patients with progression in the post-radiotherapy, MRI 14 (22.9%) had PSP and 21 (34.4%) real early progression (REP). Fifteen of 21 patients (71%) with REP developed new clinical symptoms during or 1 month after radiotherapy but only 4 of the 14 patients (28%) with PSP had new symptoms during this period. PFS was 37% and 21% at 12 and 24 months, respectively; the 12 and 24 months survival rate was 59% and 43%, respectively. There was a statistical significant difference in PFS in patients with PSP ($P < .0013$) and a trend toward better overall survival for patients with PSP but it did not reach statistical significance $P = .08$. These data support the notion to continue TMZ in the case of progressive lesions immediately after TMZ radiotherapy. Further research is needed to establish reliable imaging parameters that distinguish between REP and PSP.

P.096. SALVAGE THERAPY AFTER FAILURE OF FIRST LINE TREATMENT FOR GLIOBLASTOMA MULTIFORME

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Concomitant chemo-radiotherapy is a mainstay of treatment for glioblastoma multiforme; however, all patients progress after the initial treatment. We analyzed treatment our patients received after the progression. Since the introduction of concomitant chemo-radiotherapy in the year 2004, we treated 226 patients until the end of 2008. Of those 94 had radiologically demonstrated disease progression and were considered for second-line therapy. Their median age was 53 years (22–78, SD 12.3 years) and after discussion at MDT 39 patients were offered best supportive care, 3 patients were re-challenged with temozolomide, 17 had surgical resection followed by systemic therapy (either BCNU or PCV), 24 received BCNU chemotherapy, and 11 received other systemic therapy (either dose dense temozolomide or bevacicicabumab and irinotecan). Overall median survival was 16.6 (SD 1) weeks, in patients receiving best-supportive care it was 7.1 (SD 3.3) weeks, 15.5 weeks in patients re-challenged with temozolomide, 26.5 (SD 7.9) weeks in patients which were operated and later received systemic therapy,

21 (SD 5.4) weeks in patients receiving BCNU, in patients receiving dose dense temozolomide or bevacizumab and irinotecan median survival was not reached after minimal observation time of 29 weeks with maximum observation time of 54 weeks. As the sample is small, only the absence of active treatment was significant in survival analysis, but not age and performance status. This may be because only those in reasonably good performance status were attending regular follow-ups. In summarize, active intervention seems too beneficial for patients with recurring glioblastoma still in good condition.

P.097. THE USEFULNESS OF MS-MLPA FOR DETECTION OF MGMT PROMOTER METHYLATION IN THE EVALUATION OF PSEUDO-PROGRESSION IN GLIOBLASTOMA PATIENTS

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We investigated the usefulness of the methylation-specific multiplex ligation probe amplification (MS-MLPA) designed for evaluating semi-quantitative *O*⁶-methylguanine DNA methyltransferase (MGMT) promoter methylation status to diagnose the pseudo-progression, which is a major diagnostic dilemma in modern treatment protocol involving concurrent chemoradiotherapy in malignant gliomas. Methylation ratio of promoters of MGMT and mismatch repair (MMR) genes and their copy number variation is analyzed with MS-MLPA on 24 samples of glioblastoma patients. The results were compared with those of methylation-specific polymerase chain reaction (MSP) and the protein expression of gene was confirmed by immunohistochemical (IHC) staining. Correlation between those molecular signatures and clinical outcome was analyzed. In case of radiological progression after chemoradiotherapy, the sensitivity and specificity of the MS-MLPA were 100% and 75% which was better than MSP in the decision of pseudo-progression using cut-off value of 0.2 for methylation ratio and 0.8 for copy number ratio. MS-MLPA result of MGMT gene was well correlated with those of MSP and IHC staining while there was insignificant correlation between MSP and IHC staining. MMR genes had little variability in promoter methylation and their protein had homogeneous tissue expression. We conclude that MS-MLPA is a useful method for the early detection of pseudo-progression in glioblastoma patients.

P.098. INTRAOPERATIVE TISSUE FLUORESCENCE USING 5-AMINOLEVOLINIC ACID (ALA) IS MORE SENSITIVE THAN CONTRAST-MRI OR AMINO ACID (FET)-PET GUIDED GLIOBLASTOMA (GBM) SURGERY

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OBJECTIVE: The ability of 5-ALA to visualize white matter infiltration zones of GBM compared with MRI contrast or [18F] fluoroethyltyrosin positron emission tomography (PET) was investigated. **METHODS:** Fluorescence tissue margins were mapped intraoperatively by neuronavigation and compared with pre- and postoperative MRI and FET-PET scans in 3 glioblastoma patients (2 temporal, 1 fronto-central tumor). **RESULTS:** In all patients, the intraoperatively detected 5 ALA fluorescence exceeded the MRI contrast tumor areas and FET-PET uptake, verified by intraoperative neuronavigation. Furthermore, all patients received complete resection of contrast affine tumor parts, which was verified by contrast MRI scans within 24 hours postoperatively. Although intraoperative fluorescence tissue was generously left in place, because it was estimated as tissue at risk for neurological deterioration, no contrast affine tissue could be detected by postoperative MRI. Additionally, postoperative FET-PET uptake was demonstrated only in one patient as a small residual spot. FET-PET did not show any uptake at the intraoperatively mapped large marginal areas of 5 ALA fluorescence, left in place in account of neurological preservation. **CONCLUSION:** Our findings demonstrate that 5 ALA fluorescence is more sensitive than FET-PET and MRI contrast uptake in detecting glioblastoma multiform white matter infiltration zones.

P.099. EVALUATION OF ADVANCED MR TECHNIQUES FOR DEVELOPMENT OF EARLY BIOMARKERS FOR TREATMENT EFFICACY IN MALIGNANT BRAIN TUMORS

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BACKGROUND: Glioblastoma multiforme (GBM) is the most common group of brain tumors in adults with a median survival of only 1 year. A bottleneck in treatment evaluation is that changes in radiologic tumor size are a late effect. Hallmarks of malignant gliomas including tumor vascularity, high cell density, and heterogeneity can indirectly be measured using advanced MRI techniques, such as dynamic contrast enhancement (DCE), MRI, diffusion MRI, and MR spectroscopy (MRS). **PATIENTS AND METHODS:** Fifteen patients with GBM were included in the study: 10 patients obtaining first-line therapy (radiotherapy 2 Gy/60 Gy) concomitant with temozolomide (RT/Tmz) and 5 patients obtaining second-line therapy: irinotecan 125 mg/m² concomitant with bevacizumab (Bvz) 10 mg/kg every 14 days. MRS, diffusion MRI, and DCE-MRI were performed at baseline, day 1, 2 weeks, and 6 weeks after treatment start. All measurements were performed with a 1.5-T Siemens Espree. Calculations of apparent diffusion coefficient (ADC) maps were based on a SE-EPI sequence (*b*-values: 0 and 1000). CSI-MRS was performed using an echo-time of 135 ms. DCE-MRI measurements utilized a pharmacokinetic model to construct parameter maps for V_e , K^{trans} , and V_p . Registrations between images acquired at different time points were performed using the Insight registration and segmentation toolkit (ITK) implemented in a Matlab® environment. **RESULTS AND DISCUSSION:** In general, following observations were made, but with pronounced inter-individual differences. MRS: In patients treated with RT/Tmz, there was a steady decrease of the Cho/NAA and the Cho/Chr ratio which persisted during the treatment period and was detected as early as 2 weeks after treatment start. The decline was more pronounced during the first 2 weeks than the rest of the 6-week period. **DIFFUSION MRI:** An increase in mean ADC values could be visualized at day 1, and this gradual increase persisted during the 6-week follow-up. **DCE-MRI:** In patients treated with Bvz/Tmz a clear decrease in K^{trans} and V_e could be seen after only 3 weeks of treatment with DCE-MRI, indicating a decrease of vascular permeability and leakage respectively. **CONCLUSIONS:** Important surrogate markers for angiogenesis, diffusion, and cell density tend to assume a more “normal” pattern after a very short treatment period in a series of patients. Studies with the objective to expand the number of patients and correlate these findings to patient outcome are ongoing at our department.

P.100. MULTI-PROFESSIONAL, PRE-TREATMENT ASSESSMENT CLINIC FOR PATIENTS WITH GLIOBLASTOMA RECEIVING CONCOMITANT CHEMORADIATION

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BACKGROUND: The investigation and management of patients with glioma is increasingly complex with the introduction of routine biomarker profiling, multimodality care, and complex protocols for clinical trials. Delays in starting nonsurgical treatments can be deleterious and should be minimized. The need, therefore, for patients rapidly to understand the issues and make complex decisions is paramount. We have introduced a multidisciplinary Pre-Treatment Assessment clinic (PTAC) into routine practice to improve the patient's illness related education, optimize therapeutic strategy and implementation, maximize symptom control, and facilitate trial entry. **METHODS:** Following surgery, patients with newly diagnosed primary brain tumors are assessed by a Consultant Oncologist and a Clinical Nurse Specialist (CNS) in a Neuro-Oncology outpatient clinic. During this consultation, the patient is informed of their diagnosis and proposals for further treatment are discussed. This consultation has been shown to be traumatic and ineffective in terms of information transfer and decision-making. The next contact between patient and specialist team was not normal until the start of radiotherapy planning, several weeks later. Patients now attend the new PTAC 1–2 weeks following their initial consultation. This innovative clinic is led by the same CNS as attended the initial consultation and a Specialist Therapy Radiographer. There is access to medical, psychological, and physiotherapy advice. A competency structure for the involved professionals has been developed and clinical supervision is provided by Neuro-Oncology Consultants. The PTAC addresses issues of patient and carer education, psychological adaptation, symptom control, the ongoing appropriateness of the therapeutic strategy, detailed

planning and preparation for treatment, and eligibility and entry for clinical trials and consent. Patients are offered as many appointments as they require. **DISCUSSION:** Patients with brain tumors are being offered increasing options for treatment; however, the trauma of the diagnosis and the complexity of the discipline call for much greater communication with and planning from the treatment team. We have implemented a novel PTAC run primarily by nonmedical staff as an efficient and effective mechanism to respond to these demands. We plan to audit measures of effectiveness and satisfaction during the change-over period to demonstrate its value.

P.101. MALIGNANT GLIOMA SURGERY IN ELOQUENT BRAIN AREAS

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OBJECTIVES: The surgical studies have demonstrated that the extent of anaplastic glioma resection is significantly correlated with patient median survival. Removal of brain tumors adjacent to eloquent areas remains a procedure with high-level postoperative neurological disorders as a result of wide tumor infiltration of functional cortex and subcortical pathways. Accurate preoperative and intraoperative identification of the eloquent cortex is an essential adjunct to successful surgical excision of gliomas involving motor and speech area. **METHODS:** A total of 36 patients (21 males, 15 females, mean age 48.3 years, range 21–70 years) who underwent resection of cortical or subcortical tumors located within or close to motor areas have been included in the series. The preoperative planning was done by integrating CT, MRI, fMRI, SPECT, and computed EEG studies. Brain tumors located in eloquent area in 21 patients (motor area in 12 cases, sensory area in 9 cases) and in close to eloquent area in 15 patients (motor area in 8 cases, sensory area in 7 cases). Tumor microsurgery resection was carried out using the StealthStation navigation system accompanied with intraoperative laser thermodestruction (808 nm, 18 W). **RESULTS:** Sixteen patients had glioblastoma multiforme and 20 had anaplastic astrocytoma. A gross total or nearly gross total tumor resection was accomplished in 19 patients, and subtotal resection was carried out in 17 patients. Neuronavigation technology helped to define the radiographic limits of the tumor, to perform preoperative planning and minimal access craniotomy. The intraoperative orientation by connection of anatomical and functional landmarks allowed performing image-guided tumor excision beyond functional zones with maximal extension of tumor resection volume. Laser thermodestruction of residual tumor parts was used after microsurgical tumor removal in regions around sensorimotor and speech areas. Laser thermodestruction increased the rate of complete citoreduction and performed an aimed coagulation without traumatization of eloquent cortex. **CONCLUSIONS:** The gross total resections of anaplastic gliomas could be performed in eloquent brain regions with an acceptable level of motor and speech impairment. The improved preoperative functional mapping, intraoperative neuronavigation techniques with laser thermodestruction allow maximal safe resection of tumor that predict higher levels of quality of life in patients with brain gliomas in eloquent area.

P.102. NEAR-CONTINUOUS TEMOZOLOMIDE AND LOW-DOSE WEEKLY CCNU: A NOVEL CHEMOTHERAPY REGIMEN WITH ACTIVITY IN MALIGNANT GLIOMAS RESISTANT TO DOSE-DENSE TEMOZOLOMIDE ALONE

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BACKGROUND: Alkylating chemotherapy with CCNU is active in recurrent malignant glioma, but may be antagonized by anti-alkylating MGMT. Here, we present feasibility and activity of a novel regimen aiming at depletion of MGMT with lower dose, near-continuous temozolomide followed by low-dose weekly CCNU to treat recurrent malignant gliomas resistant to dose-dense temozolomide. **METHODS:** Eleven consecutive patients with recurrent malignant gliomas (4 glioblastomas, 3 gliosarkomas, and 4 anaplastic gliomas) were treated: 6 males (55%), 5 females (45%); mean age at first diagnosis was 55.9 (19–76) years; median Karnofsky Performance Status 70%; 9 patients were treated for a second recurrence and 2 for first recurrence. All patients were pretreated with dose-dense temozolomide (day 1–21/28 or 1–5/7, initial dose 100 mg/m²). Nine of the 11 patients were switched without delay from dose-dense temozolomide monotherapy to combined near-continuous temozolomide (50–60 mg/m² day 1–5/7) plus weekly low-dose CCNU (40 mg fix dose at day 6/7). **RESULTS:** In total, 32 cycles of chemotherapy were applied. The combination was well tolerated in terms of nausea and fatigue. Blood counts decreased

continuously, enabling a gradual dose adaptation. Hematological WHO grade III + IV toxicity occurred in 5 of 11 patients (45%), 2 of them were symptomatic. One patient had a prolonged elevation of liver enzymes which improved partially after termination of levetiracetam. Best responses after ≥2 months were: 1 complete and 2 partial remissions (27%), 3 stable diseases (27%), 5 progressive diseases (46%). Median overall survival after start of chemotherapy was 4.5 months, progression-free survival at 6 months (PFS 6) was 18%, and overall survival from first diagnosis 23.5 months. **CONCLUSIONS:** In spite of adverse prognostic signs, the objective remissions indicate activity of combined near-continuous temozolomide and low-dose weekly CCNU after failure of dose-dense temozolomide alone. Hematotoxicity, though, has to be controlled vigorously. The results have to be controlled in a larger, prospective series.

P.103. RADIOTHERAPY PLUS CONCOMITANT AND ADJUVANT TEMOZOLOMIDE FOR GLIOBLASTOMA IN ELDERLY PATIENTS

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OBJECTIVES: The optimal treatment for elderly patient with glioblastoma remains controversial. The aim of this study was to verify the activity and the toxicity of temozolomide (TMZ) administration concurrent and adjuvant to radiotherapy in elderly patients with glioblastoma (GBM), and to explore correlation between clinical outcome and O(6)-methylguanine-DNA-methyltransferase (MGMT) promoter methylation status. **PATIENTS AND METHODS:** From April 2006, 25 consecutive newly diagnosed GBM patients aged 65 years or more (median age 72 years) and a KPS of >50 were treated with radiotherapy (total of 60 Gy for 18 patients and 40 Gy for 7 patients) plus continuous daily TMZ (75 mg/m²/day), followed by maintenance TMZ cycles (200 mg/m² once a day for 5 consecutive days ever 28 days) until complete response or unequivocal progression. **RESULTS:** The median OS was 13.7 months and median PFS was 8.3 months. The 6- and 12-month survival rates were 79% and 61%, respectively. The 6- and 12-month PFS rates were 54% and 40%, respectively. Four patients had grade III neutropenia and 1 patient had grade III thrombocytopenia and 11 patients had grade III/IV lymphocytopenia. Two patients had grade III infection resolved with medical therapy. Leukoencephalopathy was diagnosed in 2 patients who survived more than 12 months. This was associated with memory loss in 1 patient. The methylation status of the MGMT promoter was evaluated in 23 patient samples. The median OS was 25.8 months vs 9.0 months in patients with MGMT promoter methylated status and with unmethylated MGMT promoter status, respectively (*P* = .05). **CONCLUSIONS:** Radiotherapy plus concomitant and adjuvant TMZ was well tolerated with an acceptable rate of toxicity, and patients with a MGMT promoter methylated status had a better survival. However, further prospective trials are needed to confirm these results.

P.104. SAFETY ANALYSIS OF RANDOMIZED BELGIAN PHASE II TRIAL OF EXTENDED USE OF ADJUVANT TEMOZOLOMIDE IN NEWLY DIAGNOSED GLIOBLASTOMA PATIENTS

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PURPOSE: We conduct a Belgian randomized phase II trial to determine whether prolonged administration of temozolomide (TMZ) in newly diagnosed GBM patients increases their progression-free survival (PFS). We also assess the safety profile of this extended use of TMZ since no data are available yet. **METHODS:** Patients with newly diagnosed glioblastoma presenting with a response or stable disease after the standard treatment of 6 months adjuvant TMZ, were included. These patients were randomized between continued treatment with TMZ or observation, using residual tumor and MGMT status as stratification factors. Patients randomized in the observation arm were rechallenged with TMZ upon progression. We will report here the PFS at 6 months and the safety analysis from the interim analysis. **RESULTS:** The first 20 patients included were comparable between both arms in terms of the well-known prognostic factors such as median age at diagnosis 55 vs 56 years, Karnofsky score 84 vs 92%, residual tumor in 8 vs 9 patients, and steroids use for 5 vs 2 patients in prolonged TMZ arm and observation arm, respectively. The progression-free survival (PFS) at 6 months was 70% vs 57%, respectively. The median number of cycles was 6 in the prolonged TMZ arm. Three patients presented with a complete response after 6 months of added TMZ. The mean number of all

grade toxicity per patient was $3.5 (\pm 3.3)$. The toxicities of TMZ were, in the majority of cases, limited to grade 1–2; 4 patients had an asymptomatic grade 3 lymphopenia, 3 had grade 3 asymptomatic thrombopenia, and 1 patient had a grade 4 lymphopenia. But only 1 patient out of the 10 included in this arm had to stop TMZ because of hematological toxicity. In the observation arm, 5 patients were rechallenged and 3 cycles were given without any response. Patients presented with grade 1 toxicity and only 1 patient had a grade 2 lymphopenia. **CONCLUSION:** Hematotoxicities are more frequent in the prolonged adjuvant TMZ arm but only 1 patient had to stop treatment. This analysis shows a potential benefit of the experimental arm in terms of PFS at 6 months. Accrual is nearly completed and updated results on safety will be reported.

P.105. VAULTS AND THE MAJOR VAULT PROTEIN (MVP): IMPACT ON THE MALIGNANT PHENOTYPE OF HUMAN GLIOBLASTOMA MULTIFORME

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Vaults are highly conserved ribonucleoprotein particles containing a small untranslated RNA (vRNA). The 110-kDa major vault protein (MVP) has been implicated in the regulation of multiple cellular processes, including transport mechanisms, chemoresistance, and several signaling cascades/molecules (eg, MAPK and PI3K pathway). While in normal brain the expression of MVP is very low, MVP expression is consistently upregulated in gliomas including glioblastoma multiforme (GBM). Aim of this study was to investigate whether MVP/vaults have an impact on GBM cell growth and aggressiveness, including chemotherapy responsiveness and to clarify underlying molecular mechanisms. MVP and MVP–GFP fusion proteins were overexpressed by plasmids and adenoviral constructs. Endogenous MVP expression was repressed by shRNA. Protein expressions were detected by immunofluorescence and Western blot. Consequences of MVP modulation on cell proliferation, migration, and invasion capacities were analyzed by growth curves, scratch and transwell-chamber assay, respectively. Moreover, tumor formation in dependence of MVP expression was tested in SCID mice. Ectopic MVP expression in MVP-negative H7 glioma cells led to a significantly enhanced proliferative and migratory potential *in vitro*. Especially responsiveness to epidermal growth factor (EGF)-mediated growth stimulation was increased paralleled by significant upregulation of MAPK and PI3K pathway indicated by phosphorylation of ERK and AKT, respectively. Accordingly, several signal mediators of these pathways, including PTEN and pS6, were detected in immunoprecipitates from MVP-overexpressing cell clones. MVP transgenic cells were significantly resistant to cell death induced by serum-starvation but only marginally protected against the cytotoxic effects of diverse chemotherapeutics. Expression of a truncated MVP molecule harboring only the MVP coil-coiled domain and/or MVP downmodulation by shRNA in MVP-positive GBM cells induced programmed cell death as well as hypersensitivity to growth factor starvation. Tumor growth in SCID mice was significantly enhanced in all MVP overexpressing H7 subclones when compared with vector controls. Our data proof a significant contribution of vaults/MVP to the malignant phenotype of human GBM cells by supporting activation of oncogenic signaling pathways and growth/survival factor responsiveness.

P.106. A FIVE-YEAR FOLLOW-UP EXPERIENCE IN NEWLY DIAGNOSED GLIOBLASTOMA AND CONCOMITANT PROTOCOL: TOLERANCE, COMPLIANCE, EFFECTIVENESS, AND SECOND-LINE THERAPIES

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Since 2005 the Stupp protocol with concomitant regimen of chemoradiotherapy followed by monthly adjuvant cycles of temozolomide has become the standard first-line approach in newly diagnosed glioblastoma after surgery. In this study, we present a 5-year follow-up experience in newly diagnosed glioblastoma treated with the concomitant protocol at the Neurosurgery Units of Policlinico and Galeazzi Institutes. From January 2005 to December 2009, we enrolled 91 patients eligible to complete the concomitant phase. We excluded patients in poor general or neurological conditions who needed a rehabilitation period prior to be submitted to

radiotherapy. People over 70 years old were addressed to a modified protocol with a reduced dosage of radiotherapy, except for 3 patients in very excellent conditions who were submitted to standard protocol. There were 38 women and 53 men ranging from 18 to 75 years. All of them were submitted to gross total removal of the lesion (as assessed by postoperative gadolinium-enhanced CT or MRI scan) except for 6 deep-seated lesions, submitted to stereotactic biopsy. In 63 cases, MGMT status was analyzed. All the patients were able to finish the concomitant phase of the protocol. In 4 cases, a reduced dose of temozolomide was administered because of the onset of pias-trinopenia. In the adjuvant phase, we preferred to administer 12 monthly cycles of temozolomide instead of the standard 6 months regimen. In the majority of patients (90%), we adopted a modified schedule (150 mg/day 1–5 day, 75 mg/day 6–10 day). Four patients experienced a bronchopneumonia, 2 during the concomitant phase, and 2 during the adjuvant phase. At 6 months 100% of patients were alive, at 12 months 75% of patients, at 24 months 22%, and at 36 months 10%. Median survival was 15 months and median time tumor progression was 12 months. Twelve patients had second surgery at recurrence (in 9 of them Gliadel wafers were placed in the cavity), and 80% of the patients with recurrence received a second-line therapy: rechallenge of temozolomide (the 1-week on–one week off regimen in unmetastylated tumors), fotemustine, bevacizumab. We also analyzed the quality of life of the patients, the clinical (Karnofsky score) and neuropsychologic status (MMSE), the duration of hospital stay, and days spent in outpatients' visits. Finally, we concluded that a good tolerance of the concomitant regimen was observed in the majority of patients, determining an acceptable quality of life and, thus, an improved access to second-line therapies.

P.108. UPDATED RESULTS OF A PHASE II TRIAL OF BEVACIZUMAB AND IRINOTECAN IN RELAPSED HIGH-GRADE GLIOMA

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BACKGROUND: Relapsed glioblastoma multiforme (GBM) has a poor response to current chemotherapy and prognosis of patients with recurrent disease is dismal, with a median survival of 3–6 months. Numerical trials using bevacizumab, a humanized IgG1 monoclonal antibody to vascular endothelial growth factor (VEGF), with or without chemotherapy, have reported excellent response rates using 10 mg/kg or 15 mg/kg every 2 weeks, and allowed expedite FDA approval for its use as a second-line treatment in relapsed GBM. We performed a phase II trial of bevacizumab using 5 mg/kg only, with irinotecan (CPT 11) every 2 weeks as reported in the initial presentation by Stark Vance. In our interim analysis, we had demonstrated excellent response rates and similar results to others. This is an update of the final results. **PATIENTS AND METHODS:** This phase II trial accrued 30 patients with recurrent GBM who received bevacizumab at 5 mg/kg and CPT 11 at 125 mg/m² every 2 weeks, after failing radiation therapy and adjuvant TMZ. All patients on antiepileptic drugs (AEDs) had their regimen changed to nonenzyme-inducing antiepileptic drugs (NEIAEDs) prior to receiving CPT 11. Patients with KPS \geq 50% were allowed regardless of prior relapses. Patients were evaluated clinically and with contrast-enhanced MRI scan every 4 treatments of bevacizumab until progression. **RESULTS:** All 30 patients were evaluable. Responses were assessed radiographically according to the MacDonald criteria and comparing T2 or Flair weighed Sequences; 19 patients (63%) had a documented response (CR + PR), 6 patients (20%) had stable disease (SD) and 5 patients (19%) progressed (PD). The average number of bevacizumab treatments received was 5.6 (1–20). The 6-month progression-free survival was 33.4%; 6-month overall survival was 66.7%, median overall survival was 8.7 months (36.3 weeks); median progression-free survival was 5 months (22.8 weeks). Several complications were reported: 3 DVTs and 2 PEs requiring IVC filter placement, 2 intracranial hemorrhages and one nonneurosurgical complication. All patients had a clinical benefit and were taken off steroids rapidly after starting bevacizumab regardless of radiological response. Clinical and radiographic responses correlated well. Failures were mostly local progression in 12 cases, infiltrative nonenhancing (gliomatosis like) in 10 cases and multifocal including subependymal and leptomeningeal in 8 patients. **CONCLUSION:** Bevacizumab-based regimen for relapsed GBM demonstrates superior activity when compared with historical treatments. It is safe and improves overall quality of life in this patients' category. Our results were as attractive as previously reported series despite lower KPS on enrollment, and using lower doses of bevacizumab.

P.109. EARLY INITIATION OF RADIOTHERAPY PLUS CONCOMITANT AND ADJUVANT TEMOZOLOMIDE (TMZ) AND OVERALL SURVIVAL (OS) IN GLIOBLASTOMA (GBM) PATIENTS

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OBJECTIVE: The influence of early start of radiotherapy plus concomitant and adjuvant temozolomide on overall survival (OS) in GBM patients was investigated. **METHODS:** Forty-eight consecutive histologically verified glioblastoma patients (median age 62.5 years, from 25 to 82, 19 females and 29 males, WHO performance stratus 0–1) received surgery (15 biopsy, 18 partial, and 14 complete resections) and radiotherapy (start median 17.5 days, from 12 to 27 days after surgery) with concomitant and adjuvant TMZ (all patients completed 60 Gy in 2 Gy/day fractions, 5 day/week, 40 of 46 completed concomitant TMZ, 18 of 40 additionally 6 cycles of adjuvant TMZ). No significant wound complications leading to revision surgery occurred. **RESULTS:** Altogether, the 12 of 24 month OS was 54/20.2% with a median survival of 13.7 month. In younger patient (<65 years, median 57.5, 28 patients), the 12 of 24 month OS was 68.4/34.3% with 16.9-month median survival, in older patient (>65 years, median 73, 20 patients) the 12 of 24 month OS was 28.8/5.8%, with 7.7-month median survival (Log-rank, $P = .0005$). The OS comparing RT start <16days vs >16days was not significantly different. Extent of surgery influenced OS in the young age group (biopsy vs complete: $P = .06$), but not in patients >65 years ($P = .5$). **CONCLUSION:** As the 12 of 24-month OS in our patients <65 years (median 57 years) exceeds the OS of the EORTC study (61.1/26.5%) with comparable demographics except for therapy start (EORTC median 5 weeks), we speculate that early start of the RT/TMZ might be of additional benefit.

P.110. PHASE II STUDY OF IFOSFAMIDE, CARBOPLATIN, AND ETOPOSIDE IN PATIENTS WITH A FIRST RECURRENCE OF GLIOBLASTOMA MULTIFORME

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OBJECTIVE: The prognosis of recurrent glioblastoma multiforme (GBM) remains unsatisfactory. The authors conducted a Phase II study of ifosfamide, carboplatin, and etoposide (ICE) for a first recurrence of GBM to determine whether it prolonged a patient's good quality of life. **METHODS:** This trial was an open-label, single-center Phase II study. Forty-two patients with a first GBM relapse after surgery followed by standard radiotherapy (60 Gy) and first-line temozolomide- or nimustine-based chemotherapy were eligible to participate. The primary endpoint was progression-free survival at 6 months after the ICE treatment (PFS-6), and secondary endpoints were response rate, toxicity, and overall survival. Chemotherapy consisted of ifosfamide (1000 mg/m² on Days 1, 2, and 3), carboplatin (110 mg/m² on Day 1), etoposide (100 mg/m² on Days 1, 2, and 3), every 6 weeks. **RESULTS:** Progression-free survival at 6 months after ICE treatment was 35% (95% CI 22–50%). The median duration of PFS was 17 weeks (95% CI 10–24 weeks). The response rate was 25% (95% CI 9–34%). Adverse events were generally mild and consisted mainly of alopecia. **CONCLUSIONS:** This regimen was well tolerated and has some activity and could be one of the options for patients with recurrent GBM.

P.111. LONG-TERM SURVIVORS OF GLIOBLASTOMA

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There is no generally accepted definition of long-term GBM survivors (LTGBMS). Usually, most authors define long-term GBM survivor as a patient who survives at least 3 years after the histological diagnosis of glioblastoma. LTGBMS are uncommon and are reported to occur in 0.5%–16% of cases. In our ENOK (Ege University Neuro-Oncology Council) cases we have 12 of 372 GBM patients who survived more than 3 years (3.2%). The clinical and molecular factors that contribute to long-term survival are still unknown. Authors underline the association of glioblastoma long-term survival with prognostically favorable clinical factors, in particular young age and good initial performance score (KPS) as well as MGMT promotor hypermethylation.

P.112. HIGH-RESOLUTION GENOMIC PROFILING OF XENOGRAFTED HUMAN GLIOMAS TO DELINEATE NONANGIOGENIC AND HIGHLY ANGIOGENIC PHENOTYPES IN A CLINICALLY RELEVANT MODEL SYSTEM

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Glioblastoma multiforme (GBM) is the most common form of malignant brain cancer in adults. Patients with GBM have a uniformly poor prognosis, with a median survival of 1 year thus, advances on all scientific and clinical fronts are needed. We have developed a human glioblastoma xenograft model in nude rodents that is characterized by a highly infiltrative nonangiogenic phenotype. Upon serial transplantation, this phenotype will develop into a highly angiogenic tumor. Thus, we have developed an animal model where we are able to establish two characteristic tumor phenotypes that define human glioblastoma (ie, diffuse infiltration and high neovascularization). It is well established that the cancer genome is molded by the dual processes of somatic mutation and selection. In order to assess whether the observed phenotypic shift is because of clonal selection *in vivo*, we have performed high-resolution aCGH on primary tumors and xenografts derived thereof. We show that although the overall genomic pattern is highly conserved, additional genomic events trigger the switch from an invasive to a highly angiogenic phenotypic observed *in vivo*. We are currently further analyzing our findings by applying whole exome resequencing to the analyzed samples to delineate the mutational evolution of xenografted gliomas at single-nucleotide resolution and identify new mutational events linked to the phenotypic switch. This molecular dissection of the 2 hallmarks of GBM could lead to the identification of potential biomarkers and will facilitate the elucidation of the molecular pathways involved in the switch from invasive to angiogenic growth, thereby potentially opening new diagnostic and treatment avenues in the clinic.

P.113. THROMBOCYTOPENIA IN GLIOBLASTOMA PATIENTS TREATED WITH ANTI-EPILEPTIC DRUGS AND RADIO-CHEMOTHERAPY BASED ON STANDARD REGIMEN

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INTRODUCTION: Seizures in brain tumor patients are a common event. Tumoral epilepsy treatment guidelines based on clinical studies are scarce. Knowledge on hematological effects of antiepileptic drugs (AEDs) in this population is limited. Thrombocytopenia is a recognized side effect of valproate (VPA). Thrombocytopenia because of temozolomide (TMZ) is frequent; however, grade 3–4 toxicity has only been reported in 12% of patients. The aim of the study was to investigate the factors involved in thrombocytopenia in a cohort of glioblastoma (GBM) patients treated with standard protocol. **MATERIAL AND METHODS:** We reviewed 101 newly diagnosed GBM patients treated with Stupp schedule until July 2009, from 2 institution database. Clinical data, presence of seizures, use of AEDs, platelet count, and accumulated TMZ dose were analyzed at each cycle. AED treatment was categorized as follows: VPA (alone or combined with non-enzyme-inducing AEDs), LEV (levetiracetam), enzyme-inducing AEDs (alone or in-combination with other AEDs) and non-AEDs users. Thrombocytopenia was operationalized both as a continuous platelet count and as a dichotomic variable (cut-off 100 × 10⁹/L) aimed at detecting effects on clinical decision-making. A linear and a probit pooled cross-sectional regression analysis, respectively, were used to study the impact of covariates on thrombocytopenia. **RESULTS:** Thirty-five (35%) patients presented seizures at onset and it appeared during follow-up in 18 (27%). Five (9.4%) and 2 (3.8%) patients needed 2 and 3 AEDs to control seizures, respectively. Thrombocytopenia was observed in 37% of all GBM patients. Grade 3–4 thrombocytopenia was found in 11%. Decrease in platelet count was related with TMZ dose ($P < .001$), age ($P < .001$) and VPA ($P = .004$). Platelet count <100 × 10⁹/L was only associated with TMZ dose ($P = .001$). Age ($P = .87$) and VPA ($P = .12$) lost their influence. AEDs were not associated with time to progression (TTP), being RPA prognostic class the only variable with significant impact on TTP in Cox regression analysis. **CONCLUSION:** Accumulated dose of TMZ was the main determinant factor of thrombocytopenia. Although VPA and age were also factors associated with decreasing platelet count, thrombocytopenia modifying clinical management was only significantly related with TMZ. The lack

of VPA effect on critical thrombocytopenia for treatment decision-making could be related with the sample size of this study.

P.114. IDENTIFICATION OF CD133⁻/TELOMERASE^{LOW} PROGENITOR CELLS IN GLIOBLASTOMA-DERIVED CANCER STEM CELL LINES

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Glioblastoma multiforme (GBM) is paradigmatic for the investigation of cancer stem cells (CSC) in solid tumors. The CSC hypothesis implies that tumors are maintained by a rare subpopulation of CSC that gives rise to rapidly proliferating progenitor cells. Although the presence of progenitor cells is crucial for the CSC hypothesis, progenitor cells derived from GBM CSC are yet uncharacterized. Our data suggest that in the subgroup of CD133⁺ primary astrocytic GBM, CD133⁺/telomerase^{high} CSC give rise to non-tumorigenic, CD133⁻/telomerase^{low} progenitor cells. The progenitor cell population proliferates rapidly resulting in significant telomere shortening when compared with the CD133⁺ compartment comprising CSC. The average difference in telomere length as determined by a modified multi-color flow FISH (fluorescent in situ hybridization) was 380 bp corresponding to 4–8 cell divisions. Taken together, we demonstrate that CD133⁺ primary astrocytic GBM comprise a rapidly proliferating, CD133⁻/telomerase^{low} progenitor cell population in addition to CSC and terminally differentiated cells.

P.115. BEVACIZUMAB FOR THE TREATMENT OF PATIENTS WITH RECURRENT HIGH-GRADE GLIOMA, RESULTS FROM A DUAL-CENTER CLINICAL EXPERIENCE IN BELGIUM

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BACKGROUND: Vascular endothelial growth factor (VEGF) plays a key role in the neo-angiogenesis that characterizes high-grade gliomas (HGG). Bevacizumab (BEV), a humanized immunoglobulin G1 monoclonal antibody that inhibits VEGF, has demonstrated activity against recurrent HGG. **PATIENTS AND METHODS:** Patients with progressive HGG following prior standard care (including at least surgery, radiotherapy, and temozolomide) who received BEV (at a dose of 10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks) outside a clinical trial protocol were identified in 2 Belgian university hospitals. Tumor response and anti-edema effect were assessed by magnetic resonance imaging (MRI, including T1 ± Gd, T2, and FLAIR sequences); available results of amino-acid PET scan imaging were used to assess the metabolic response. **RESULTS:** Twenty patients with recurrent HGG were identified (11 M/ 9 F; median age 40 [range 28–70]); initial WHO grade: grade 2: 2 patients, grade 3: 4 patients, grade 4: 14 patients; baseline KPS: 90–80%: 3 patients, 70–60%: 17 patients). A median number of 3 prior systemic therapies had been administered. A total of 93 BEV administrations were performed (median 6, range 1–11). BEV-related toxicity consisted of CTCAE-grade 3 abdominal pain syndrome (1 patient), grade 3 skin ulceration (1 patient), grade 1 epistaxis (1 patient), grade 1 subungual bleeding (1 patient), and grade 1 hypertension (1 patient). There were no dose reductions. Fourteen patients stopped BEV because of tumor progression, 2 because of toxicity in the absence of a clinical response. At the time of this analysis, tumor response assessment by MRI is available for 14 patients; Response by T1 + Gd: 1 CR, 4 PR (BORR 36%); 11 of 14 patients (79%) had regression of edema on T2/FLAIR. A reduced uptake of amino-acid tracer on PET scan was documented in 3 of 4 patients at the time of MRI response. After a median follow-up of 8.5 months, 4 patients currently remain under treatment. Three out of 15 patients with sufficient follow-up remained progression-free after 6 months of BEV (with regression of all tumor related symptoms and the ability to maintain their personal and professional lives). **CONCLUSIONS:** In this analysis of the off-study use of BEV for recurrent HGG, activity and tolerability were comparable with what

has been reported from prospective phase II trials. A meaningful subgroup of patients in this series derived a durable treatment effect with evidence for a metabolic response in addition to an edema reducing effect.

P.116. FUNCTIONAL VALIDATION OF NOVEL MOLECULAR TARGETS FOR ANTI-ANGIOGENIC TREATMENT OF MALIGNANT GLIOMAS

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Glioblastoma multiforme (GBM) remains one of the most malignant tumors in mankind. Despite a multimodal treatment, prognosis and survival of patients remain poor. The highly angiogenic properties of GBM makes anti-angiogenic therapy an attractive addition to the current treatment protocol. Our group is currently active in identifying and characterizing novel biomarkers and therapeutic targets involved in the angiogenic switch observed in malignant gliomas. Making use of a clinically highly relevant xenograft model that recapitulates the invasive and angiogenic properties of GBM within consecutive generations of rats, we have previously performed a large-scale iTRAQ-based proteomics study comparing nonangiogenic to angiogenic GBM phenotypes. From more than a thousand quantifiable proteins identified in membrane fractions, about 50 proteins showed increased expression in angiogenic tumors. Known and novel candidate proteins have been identified. On the basis of their expression profile and established literature knowledge, we have selected a dozen of proteins for which the differential expression in angiogenic gliomas was validated by quantitative PCR. For functional analysis, we are currently targeting these genes using small interfering RNAs (siRNA) to specifically knockdown the proteins in various glioma cell lines, including glioma stem cell-like cells. The transfected cell lines are analyzed with regard to their proliferation rate, migration capacity, invasiveness, and clonogenic potential. Tube formation in siRNA-treated HUVEC endothelial cells is measured to evaluate the effect of the proteins on angiogenesis. Complementary data are obtained by overexpression studies of the selected proteins. We will report on the functional involvement of new candidate proteins in glioma angiogenesis, which may represent novel molecular targets for the development of anti-angiogenic therapy in the management of GBM.

P.117. BEVACIZUMAB AND FOTEMUSTINE AS SALVAGE THERAPY IN RECURRENT GLIOBLASTOMA: A PHASE II MULTICENTER ITALIAN STUDY

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BACKGROUND: Recent evidence indicates that antiangiogenic therapies have an important role in recurrent high-grade gliomas. Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), has been reported to be active with acceptable toxicity. We report the preliminary results of a multicenter phase II study on bevacizumab plus fotemustine in GBMs recurrent after standard treatment (surgery, radiation, and temozolomide). **PATIENTS AND METHODS:** Fifty-nine patients (38 males and 21 females) with a median age of 58 years (range 24–78), and a median KPS of 80 (range 60–100) were enrolled in a phase 2 protocol with bevacizumab at 10 mg/kg on day 1, 15 and fotemustine at 75 mg/m²/day on day 1, 8 (induction phase) and, after 3 weeks interval, bevacizumab at 10 mg/kg and fotemustine at 75 mg/m² every 3 weeks as a maintenance. Treatment was maintained until tumor progression or unacceptable toxicity. MRI was performed at baseline, after the induction phase and thereafter every 2 months. Response was defined as a reduction of enhancement on T1-weighted images associated with stability or reduction on FLAIR images (according to recently modified Macdonald's criteria). Primary endpoint was 6-month progression-free survival, whereas secondary endpoints were response rate, overall survival, and safety. **RESULTS:** The 6-month progression-free survival was 32% and median survival time was 6.7 months (range: 1.2–18+). The overall response rate was 43.4%, with 4.3% complete responses, 8.6% major partial responses, 30.5% partial responses, and 41% stabilization. Maximal responses were precocious, after the induction phase. Steroids were reduced or stopped in 61% of

patients. Forty percent of responders had unmethylated MGMT promoter. The most frequent side effects were: hematological grade 3–4 toxicity (21%); fatigue (46%); mild arterial hypertension (9%); and one grade III hypertension with reversible hypertensive encephalopathy; hemorrhagic events (12.5%) (2 lobar hemorrhages, 2 asymptomatic intratumoral bleedings, 1 esophageal bleeding); thrombotic events (9%) (one pulmonary embolism, 2 TVP, and 1 stroke); mild proteinuria (14%). CONCLUSIONS: Bevacizumab in combination with fitemustine was well tolerated and active in recurrent glioblastoma. The correlation between MGMT status, MRI perfusion, and response is ongoing.

P.118. RADIOTHERAPY AND CONCOMITANT AND ADJUVANT TEMOZOLOMIDE: TRANSLATION OF RANDOMIZED CONTROLLED CLINICAL TRIAL EVIDENCE INTO ROUTINE CLINICAL PRACTICE

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INTRODUCTION: Glioblastoma multiforme is the commonest primary malignant brain tumor in adults, and is usually rapidly fatal. Until 2004, standard treatment in the UK comprised of maximal surgical debulking followed by radiotherapy. The most important advance in recent years has been the addition of concomitant and adjuvant temozolomide, which has been shown to improve overall survival in this group of patients. In addition, data have demonstrated differing outcomes for patients treated with temozolomide depending on the methylation status of the promoter region of the gene for methyl guanine methyl transferase (MGMT) within tumor tissue. MGMT is a DNA repair enzyme that may repair sublethal damage because of alkylating agents. Methylation of the promoter region of the gene may decrease levels of the enzyme in tumor tissue, with a consequent increase in cytotoxicity. **METHODS:** Trial patients often represent a highly selected group. We undertook a retrospective review of all patients with glioblastoma multiforme treated with concomitant temozolomide at our center between June 2005, when temozolomide was licensed in the UK for this indication, and December 2007. **CONCLUSION:** We demonstrate close correlation between our outcomes at a minimum follow up of 2 years with those seen in the sentinel trial. In addition, patients were assessed for tumor methylation status and again we demonstrate close correlation with published data. This clinical audit confirms that the benefit of this regimen can be translated into routine clinical practice within the National Health Service.

P.119. TREATMENT WITH MULTIKINASE INHIBITOR SORAFENIB FOR RECURRENT CENTRAL NERVOUS SYSTEM TUMORS

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OBJECTIVES: Treatment for recurrent central nervous system (CNS) tumors is limited. The multikinase inhibitor sorafenib induces apoptosis and blocks cell proliferation in a variety of tumors and cell lines. The response rate and safety was evaluated in a pilot series. **PATIENTS AND METHODS:** Twenty patients (3 women and 17 men) with recurrent CNS tumors received sorafenib 200 mg twice daily. The median age was 48 years (range from 23 to 65 years). Seven patients presented with GBM, 6 with anaplastic astrocytomas, 2 with ependymomas, 3 had meningiomas, 1 hemangiopericytoma, and 1 hemangioblastoma. All patients had previously received more than two lines of systemic treatment. **RESULTS:** Two (of 7) patients with GBM had partial remission (PR), lasting for 3–5 months, stable disease (SD) was observed for 2–7 months in 4 cases. One patient showed clinical and radiological progress (PD) 5 weeks after treatment start. Regarding anaplastic astrocytomas, 4 (of 6) patients showed SD for a time period of 2–9 months. Partial response in 2 patients was lasting for 5 months. In ependymomas, 1 PR (9 months) and 1 SD (8 months) was observed. Patient with hemangiopericytoma has still an SD for more than 20 months. After 7 months of therapy, patient with hemangioblastoma showed a progressive disease. The best-achieved response within meningiomas was SD (1/3) for 6 months. Median time to progression for all patients was 3.5 months. Dose reduction was necessary in all patients mostly because of hypertension that was controlled with angiotensin-converting enzyme inhibitors or with antihypertensive combination therapy. More than 10 patients

had skin changes similar to hand-food syndrome grad II–IV. One gastrointestinal bleeding was reported, but no origin of hemorrhage was found. Some patients reported episodes of diarrhea which in one case led to treatment discontinuation. Deep venous thrombosis was not observed in our patient cohort. **CONCLUSION:** Multikinase inhibitor sorafenib showed responses in heavily pretreated patients with recurrent CNS tumors.

P.120. ACTIVATION OF P53 BY MDM2 ANTAGONISTS DOWN-REGULATES SURVIVIN AND INDUCES CELL-CYCLE ARREST AND APOPTOSIS IN HUMAN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults. Despite concerted efforts to improve current therapies and develop novel clinical approaches, patient survival remains poor, owing to the radio- and chemoresistance of the tumor. Recently, nutlins, small-molecular antagonists of MDM2, have been developed to inhibit the p53–MDM2 interaction and activate p53 signaling in cancer cells. We investigated whether nutlin-3a might be effective in inducing apoptosis in GBM. Glioma cell lines and primary cultured glioblastoma cells with known *TP53* status were treated with nutlin-3a, and inhibition of cell growth and apoptosis induction were evaluated. Upon treatment with MDM2 antagonists, p53-dependent G2-M cell cycle arrest and apoptosis were effectively induced in p53–wild-type glioma cell lines. Nutlin-3a induced down-regulation of Survivin mRNA and protein expression together with up-regulation of PUMA mRNA and p21 and PUMA protein induction. In wild-type *TP53* primary cultured glioblastoma cells, nutlin-3a induced p53-dependent suppression of Survivin, overexpression of PUMA and/or Noxa proteins and apoptosis. Primary cultured glioblastoma cells and glioblastoma cell lines with mutant p53 or functionally impaired p53 pathway as a result of a polymorphism R72P are resistant to nutlin-3a apoptosis induction. The results suggest that MDM2 inhibition induced p53-dependent cell cycle arrest and apoptosis in wild-type *TP53* glioblastoma cells. This effect seems to be at least partially because of Survivin down-regulation.

P.121. THE POTENTIAL ROLE OF SURGICAL RESECTION IN THE TREATMENT OF RECURRENT MALIGNANT GLIOMAS

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The founded relevance of surgical removal in the combined therapeutic strategy of malignant gliomas and the opportunity offered in the last few years by new unconventional treatments or second-line chemotherapeutic schedules have deeply modified the indications to surgical treatment of progressive and recurrent malignant gliomas. In the last 2 years, we have operated 22 recurrent malignant gliomas (12 glioblastoma, 7 anaplastic oligodendroglioma, and 3 anaplastic astrocytoma). At the moment of the initial diagnosis, all the patients have been submitted to first-line surgical treatment, radiotherapy, and chemotherapy with temozolomide, and/or fitemustine in a limited number of cases; all the patients presented documented localized tumor progression or recurrence at least 6 months after the previous surgery, and were evaluated suitable for second-line treatments. In 16 cases, it was possible to realize an extended resection (as radical as possible, or subtotal), and in 8 cases, during the same procedure, intratumoral CT wafers were positioned into the resection cavity. The main early complications of second surgical treatment have been local, as CSF fistula and wound dehiscence (4 cases), and were observed during the immediate post-operative period. Only in one case of temporal GBM, the second surgical resection was followed by an early progressive neurological deterioration, and the patient died 3 months after. The patients, followed in our department, with the relevant contribution of our home assistance service, have been submitted to second and/or third line chemotherapy; in 4 cases a highly focused stereotactic radiotherapy has also been performed. The present preliminary data tend to confirm the relevance of surgical treatment

and subsequent combined treatments for recurrent malignant gliomas, providing for a median extension of overall survival of at least 8.5 months (3–15+ months in the present series), with a significant improvement of neurological status and prompt resolution of intracranial hypertension. The results in terms of clinical benefit, progression-free survival, and overall survival will be presented, trying to design a patients' setting with more specific indication at second surgical removal.

P.122. MANAGEMENT OF GLIOBLASTOMA MULTIFORME RECURRENCES

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Glioblastoma multiforme (GBM) is the most frequent form of brain tumor and the most malignant astroglial neoplasm. GBM comprises 15%–20% of all primary intracranial tumors and ~60% of astrocytic neoplasms. The incidence is in the range of 3–4 new cases per 100,000 of population per year. It typically afflicts adults between 45 and 75 years of age, with a peak at 61.3. More than 80% of patients are older than 50. Despite progress in diagnostic procedures and treatment, prognosis in patients with GM is unfavorable and the survival time is limited. The crucial prognostic signs are the age and clinical condition of the patient. If not treated, the patient dies within several weeks after the diagnosis has been made. Surgery prolongs survival for 8–10 months. Subsequent radiotherapy extends lifetime for an additional 3 months. Chemotherapy originally did not play an overly significant role. Only the introduction of new alkylating chemotherapy Temodal for primary brain tumor brought a more significant change resulting in prolongation of both overall survival (OS) and performance-free survival (PFS). These problems with recurrent GM are evoked again. Our aims are to evaluate surgery indications, and also to achieve the planning of general treatment strategy. We would like to point our treatment strategy for recurrent GM out 15 patients group. The clinical and MRI follow-up of patients after first surgery (also during oncotherapy) will be carried out. Change of MRI often precedes change of clinical status. We assess as relapse of the tumor a growing mass more than 20%–30% of the neoplasm's volume (using MRI volumetric evaluation), or the origination of a new tumor. PET/CT is used in the case of doubts about the reliability of differentiating the tumor's relapse in the MRI image from other expansive, postcontrast enhancement processes (necrosis). We recommend for surgery the following patients: (a) Karnofsky Scale (KS) \geq 70% and performance status (PS) WHO \leq grade 2; (b) only local relapse, without multifocality; (c) possibility of cytoreduction minimally 70% of the size. Our purposes are (a) obtaining maximally receivable radical surgery; (b) avoiding postoperative morbidity; (c) securing a sufficient amount of tumor tissue for histological, immunohistological, and cytogenetic investigation. Selected patient's group benefit from recurrent GM surgery supplemented by adequate subsequent oncotherapy has a positive effect on performance-free status and overall survival. We endeavor to adjust our treatment strategy based on these above mentioned assignment of a suitable treatment process for every subgroup. Surgery impact is only limited without a following oncotherapy. Indication for surgery, repeated radiotherapy, and chemotherapy remains a challenging task. A close cooperation between each of these neuro-oncology team members is essential for the good results.

P.123. AVASTIN-CAMPTO (AC) IN HIGH GRADE GLIOMA: ARE STANDARD MCDONALD'S CRITERIA APPROPRIATE TO ASSESS EFFICACY?

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INTRODUCTION: Since the publication in 2007 of the of AC results in recurring high-grade glioma, this treatment has widely been used and enables some improved survival benefit. The MRI evaluation is a standard practice to follow the treated patients. We observed discrepancies between T1Gado enhanced (Gado enh), T2 sequences, and the neurological patient outcome. **OBJECTIVE:** To analyze the Gado enh and T2 sequences every 3 months compared each other and to the base line at the onset of the treatment and clinical outcome in recurrent high-grade or transformed glioma. **PATIENTS AND METHODS:** Recurrent glioblastoma (GBM) previously treated by radiotherapy (RT) and continuous temozolomide (TMZ) then monthly TMZ, and recurrent grade II–III glioma previously treated by RT and chemotherapy (CT). Avastin 10 mg/kg and Irinotecan 125 mg/m²

were administered every 2 weeks until progression. MRI, clinical examination, and corticotherapy changes were performed every 3 months. **RESULTS:** A total of 44 patients were included in the study. 54% grade IV, 18% grade III and 13% transformed grade II–III. Performance status was 0–1 in 54%, 2 in 36%, and 3–4 in 9%, median age 54 (24–78), Ki67 $<$ 15% in 64%. Initial treatment was RT-TMZ and monthly TMZ in 66%, RT and CT in 34%. The median number of cycles was 9 (1–43). The median time to progression was 4.6 months. On MRI axial Gado enh sequence, the best response was PR 22.5%, SD 55% whereas PD occurred in 22.5%; in T2 sequence, the best response was PR 12.5%, SD 72.5%, and PD 15%. The median survival was 18 months from diagnosis and 6.3 months from the onset of the treatment. The concordance evaluated by kappa coefficient between Gado enh and T2 sequences was low at 0.32 (0.06–0.57). At 6 cycles of AC, the neurological status correlated well with both Gado enh and T2 sequences. **DISCUSSION AND CONCLUSION:** despite McDonald's criteria remain the tool usually used in glial tumor evaluation, antiangiogenic drugs lead to extend MRI evaluation to T2, or FLAIR sequences, so as to not be mistaken by false favorable response observed in Gado enh sequence. Decreased T1 enhancement cannot be considered an accurate marker of tumor mass in AC treatment.

LOW-GRADE GLIOMAS

P.124*. PROX1 IS A PREDICTOR OF SURVIVAL FOR ASTROCYTIC GLIOMAS BUT NOT FOR OLIGODENDROGLIOMAS GRADE II

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The clinical course of grade II gliomas remains variable and their transformation into a more malignant phenotype is highly unpredictable. There have been attempts to identify biological markers that can facilitate prediction of prognosis in individual cases of low-grade astrocytomas, but so far without much success. PROX1 is a transcription factor that plays a critical role in the development of various organs and that has been ascribed both oncogenic and tumor suppressive functions in human cancers. In a recent study, we have shown that PROX1 may act as a diagnostic marker for high-grade astrocytic gliomas. The aim of this study was to address the prognostic value of PROX1 in a cohort of patients with grade II gliomas. A retrospective chart review of all adults with grade II gliomas operated between 1982 and 1999 at the Uppsala University Hospital, Sweden, was performed. Eligible paraffin-embedded tumor blocks were collected and a total of 128 samples were evaluated by immunohistochemistry for their PROX1 expression. The number of immunoreactive cells was used as a variable in multivariate survival analysis, together with established prognostic factors for this patient group. Patients with tumors showing high PROX1 expression had poor outcome, both in the univariate analysis ($P = .0151$) and multivariate analysis ($P = .0250$), compared with those with low PROX1 expression. When analyzing the separate histological subgroups, PROX1 expression was associated with shorter survival in astrocytomas and oligoastrocytomas but not in oligodendrogliomas. We conclude that PROX1 is a novel predictor of survival for grade II astrocytic gliomas.

P.125. PLEOMORPHIC GRANULAR CELL ASTROCYTOMA IN THE PINEAL GLAND

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BACKGROUND: Pleomorphic granular cell astrocytoma in the pineal region is exceedingly rare, and its clinicopathological features are distinctive. **CASE REPORT:** A 67-year-old woman was admitted with a staggering gait. Magnetic resonance imaging revealed a mass lesion at the pineal gland accompanied by obstructive hydrocephalus. Following surgery, pathological examinations demonstrated a pleomorphic granular cell astrocytoma. The patient has been free from recurrence for 24 months after surgery without adjuvant therapy. **PATHOLOGICAL FINDINGS:** The specimen exhibited nuclear and cytoplasmic pleomorphism. The nuclei varied in size, shape, and coarseness. Variability was also observed in the eosinophilic granular bodies, Rosenthal fibers, and spindle-shaped tumor cells. GFAP, S-100, and vimentin were immunohistochemically positive. Reticulin network was absent between the tumor cells, and granular cells with ballooned

cytoplasm showed positive staining for PAS. DISCUSSION: Pleomorphic granular cell astrocytoma is believed to be a form of astrocytoma originating from the pineal gland. Its clinicopathological features resemble those of pleomorphic xanthoastrocytoma. However, it can be differentiated from the latter by the absence of reticulin fibers, absence of basement membrane between adjacent cells, and presence of large numbers of mitochondria.

P.126. RADICAL SURGERY AFTER CHEMOTHERAPY FOR WHO GRADE II GLIOMAS: A STRATEGY PROTECTING NEUROCOGNITION AND QUALITY OF LIFE (ABOUT A SERIES OF 12 PATIENTS)

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OBJECTIVE: The aim of this study was to evaluate the impact on cognition and quality of life (QOL) as well as the results of a temozolomide (tmz)-based chemotherapy (CT) delivered before a radical surgery for World Health Organization (WHO) grade II gliomas. **PATIENTS AND METHODS:** We selected patients treated by tmz and then by surgery for a WHO grade II glioma, from the database of two French centers (Montpellier and Nancy). Usual oncological data were collected. Patients benefited, at the end of the sequence, of a cognitive and QOL assessment. Global efficiency, laterality, executive functions, attention, information processing speed, psychomotor functioning, working memory, verbal and visual memory, language, visuo-spatial ability were considered for the cognitive assessment. Used tests will be presented. For the QOL, EORTC QLQ C30 + BN 20 scales were chosen. Radiological responses were evaluated by tumor volumes before and after CT and surgery. The presence of a gadolinium enhancement was systematically analyzed. **RESULTS:** Twelve patients with a median age at diagnosis of 40.5 years (22–51) and at assessment of 48 years (26–55.5) were selected. The median follow-up was 64 months (31.5–120) and the median time between assessment and the last surgery was 20.5 months (3–70). Symptoms at diagnosis were partial seizures in 4 (33.5%) cases and were generalized seizures in 8 (66.5%). Tumor location was frontal in 6 cases (3 right and 3 left), fronto-temporal in 4 cases (1 right, 3 left), and left temporal in 2 cases. Tmz alone has been prescribed for 11 patients and tmz + fotemustine for 1 patient. Clinical and radiological evaluation after chemotherapy will be detailed. Seven patients had one functional surgery, 3 had two procedures, and 2 had three procedures. Pre and postoperating volume will be clarified. After the last surgical procedure, 10 (83%) patients had a WHO grade II oligodendroglioma (4 with some anaplastic foci), 1 patient has a grade II astrocytoma, and 1 patient has a grade II oligoastrocytoma. Molecular data (including 1p19q status) will be presented. Analysis of neuropsychological and QOL data is in progress. Definitive data will be discussed. Preliminary results are in favor of the conservation of a high quality of cognition and QOL. **CONCLUSIONS:** The sequence “chemotherapy and then functional surgery” seems to protect cognitive functions and QOL for patients with WHO Grade II glioma even with multiple surgical procedures. Definitive results will be presented during the meeting.

P.127. THE BRAIN TUMOR EXPERIENCE FROM THE RELATIVES' PERSPECTIVE

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INTRODUCTION: This research examined the experience of relatives of people suffering from a low-grade tumor using interpretative phenomenological accounts. There is a plethora of information about cancer in general, the carer experience, and on adjustment to difficult situations. There is also a wealth of information from the medical and oncology literature providing the factual context to the brain tumor experience. However, there has been limited work carried out on how the family experiences and adjusts to a brain tumor diagnosis. Initial explorations have tended to examine patients and carers, and

different types of brain tumors, as if they were universal phenomena. However, patients with brain tumors and their carers comprise a diverse group with a wide range of supportive care needs, depending on time since diagnosis and degree of malignancy. Therefore, the purpose of this study was to explore the specific experience of relatives of individuals recently diagnosed with a low-grade brain tumor. **METHOD:** A qualitative approach, based on interpretative phenomenological analysis (IPA), was adopted. Semistructured interviews were conducted with a purposive sample of 3 participants who were the spouse/partner of someone recently diagnosed with a low-grade brain tumor. Each interview was recorded and transcribed verbatim, and analyzed in accordance with IPA. **RESULTS:** Four superordinate themes emerged from the data including discovery, communication, emotional reactions, and contextual factors. Participants discussed the importance of discovering the tumor, both in terms of getting a diagnosis, and learning about the tumor. Disclosure and nondisclosure to others about the tumor diagnosis were also significant in the early illness experience. An important theme to emerge involved the participants describing what is difficult for them in the experience, and how they coped with this difficulty. The final theme placed the brain tumor experience within a wider context, where factors such as the relationship with the patient, relationship with professionals, and the hospital environment were described as significant. **CONCLUSIONS:** This research detailed the early tumor trajectory and the salient processes involved in this journey. A framework was proposed to help conceptualize the findings of the study and could be used to aid patients, families, and health professionals to reflect on, and better understand, parts of the early illness experience.

P.128. COMPARATIVE ANALYSIS OF IDH1 MUTATION, TP53 MUTATION, AND MGMT HYPERMETHYLATION IN ASTROCYTOMAS

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TP53: mutation, MGMT hypermethylation and, more recently, IDH1 mutations have been identified as precocious genetic aberrations in gliomas, but their mutual relationships during glioma formation have not been clarified. We performed a comparative genetic analysis in a selected group of 18 patients affected by low-grade astrocytomas and in these same patients at tumor recurrence, irrespective of anaplastic progression. The aim of the study was to investigate the natural history of disease, excluding the interference of the radio-chemotherapeutic agents. **MATERIALS AND METHODS:** We studied 18 patients affected by fibrillary astrocytoma (9), gemistocytic astrocytoma (3), protoplasmic astrocytoma (6). They did not undergo radiotherapy or chemotherapy after first surgery. After a median follow-up of 72 months, 15 of 18 patients recurred and the tumor showed a more malignant phenotype. Three patients underwent a third surgical intervention, all progressing to more malignant phenotypes. Specimens were investigated for MGMT hypermethylation using methylation-specific PCR after sodium bisulfite modification; LOH on chromosomes 1p, 9p, 10q, 17p, and 19q; IDH1 and TP53 mutations by sequencing analysis after PCR amplifications. **RESULTS:** Primary low-grade astrocytomas showed IDH1 mutation in 17 out of 18 cases, MGMT hypermethylation in 8 out of 18, and TP53 mutation in 8 out of 14. At recurrence, all MGMT hypermethylation and IDH1 and TP53 mutations in primary tumors were confirmed. Furthermore, all losses of heterozygosity observed in the first sample were present also at recurrence. While IDH1 mutations were already present in all primary tumors but one, the MGMT and TP53 status showed changes at recurrence: 3 primary MGMT unmethylated tumors becomes hypermethylated at recurrence and one of them, initially wild type for TP53, showed a TP53 mutation. A possible predisposing role of IDH1 toward accumulation of genetic aberrations was not investigated because of the small number of IDH1 wild-type patients at primary tumor. Finally, 5 patients out of 8 with MGMT hypermethylated and 8 of 10 with MGMT unmethylated at primary surgery acquired new losses of heterozygosity at recurrence. **CONCLUSIONS:** IDH1 mutation, MGMT hypermethylation, and TP53 mutations are precocious events in astrocytomas. Our results confirm that IDH1 mutation is the earliest genetic aberration in these tumors and that is widely represented in low-grade astrocytomas. Interestingly, only tumors with unmethylated MGMT changed their methylation status becoming methylated.

PEDIATRIC BRAIN TUMORS

P.129*. FEASIBILITY AND TOLERABILITY OF INTRAVENTRICULAR THERAPY WITH ALTERNATING ETOPOSIDE AND LIPOSOMAL CYTARABINE: EXPERIENCE IN 33 CHILDREN WITH MALIGNANT BRAIN TUMORS
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Intrathecal chemotherapy is a crucial element in the treatment of leptomeningeal disseminated brain tumors in children. We report on our experience with an intraventricular therapy with alternating etoposide and liposomal cytarabine concomitantly to conventional and/or antiangiogenic chemotherapy. Since May 1998, patients received intrathecal etoposide at a dose of 0.25–0.5 mg × 5 days every 2–6 weeks for a total of 306 courses (1–41 per patient) via an Ommaya reservoir. Intrathecal methotrexate and mafosfamide were additionally administered to 10 and 2 children, respectively. From October 2004 on, liposomal cytarabine was added and 33 patients aged 8 months to 25 years with various intensely pretreated disseminated brain tumors received liposomal cytarabine (25–50 mg) via an Ommaya reservoir or sporadically by lumbar puncture (210 doses, 1–20 per patient). Dexamethasone was used concomitantly for 3–5 days to prevent arachnoiditis. To allow dose-intensive treatment and potentially evade resistance most patients received alternating courses of liposomal cytarabine and etoposide. Toxicities following liposomal cytarabine were headache ($n = 10$), nausea/vomiting ($n = 8$), ataxia ($n = 1$), meningism ($n = 2$), and visual disturbance ($n = 3$), which were connected to benign cerebral hyperextension in 4 children that improved after one time of pressure release by lumbar/intraventricular CSF removal. Etoposide was generally well tolerated, although mild toxicities occurred (headache, $n = 2$; nausea/vomiting, $n = 2$). Since all patients received some sort of concurrent anti-cancer therapy, the efficacy of intrathecal therapy cannot be assessed independently. In conclusion, liposomal cytarabine alternating with etoposide appears to be feasible and well tolerated. Known side effects of liposomal cytarabine might occur less frequently if the time intervals of treatment may be extended and bridged with etoposide.

P.130. OPTIC NERVE LOCALIZATION OF INTRACRANIAL GERM CELL TUMORS

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Intracranial germ cells tumors are usually localized along the midline (pineal ± suprasellar) in Caucasians. Para axial tumors are mostly reported in Asiatic patients. Optic pathway germ cell tumors have been exceptionally reported. We report 3 of such cases in Caucasian patients. A 23-year-old male presented a diplopia with raised intracranial pressure, requiring ventriculostomy. The MRI showed a localized pineal tumor associated with raised serum bHCG (700 UI/L). The treatment included chemotherapy (BEP) + 50 Gy focal radiotherapy. A progressive visual loss occurred 6 years later, with unilateral swelling of optic nerve. Pure germinoma was confirmed by a biopsy. A 34-year-old male presented a diminution of vision predominantly on the right side, a left lateral hemianopsia, and a bilateral atrophy of the optic nerves. The MRI showed a swelling of the right optic nerve, extending to the chiasm. Biopsy showed a pure germinoma, no dissemination was found on MRI and markers were negative. Strategy was according to GCT 96 SIOP protocol: chemotherapy (Etoposide ifosfamide and Carboplatin) followed by a 24-Gy radiation to the ventricular system and tumoral bed. Six years later, he relapsed in the bulbomedullary junction, out of the irradiation field. A 13-year-old boy presented headache, diabetes insipidus, a growth hormone deficit, a bilateral diminution of vision, and bilateral optic atrophy. MRI showed a pineal region tumor. CSF HCG was raised (90 UI/L). This “bifocal” secreting tumor was treated according to GCT 96 SIOP protocol: chemotherapy (Etoposide Ifosfamide and Cisplatin) followed by 54 Gy radiation of hypothalamus and pineal region. He relapsed 6 years later as a ventricular seeding with chiasmatic, right optic nerve bulbular and pituitary localizations. AFP and HCG were elevated in CSF and serum. All 3 patients are currently in second remission after standard and high-dose chemotherapy followed by a 24-Gy craniospinal irradiation with respectively a 1-, 2-, and 3-year follow-up. These cases emphasize the need of careful evaluation of optic pathway in patients with CNS germ cell tumors, both at time of diagnosis and relapse. Visual symptoms may be misleading when patients present with raised intra cranial pressure. Isolated involvement of optic pathway at diagnosis is a rare disease and requires a biopsy. Decreased vision after radiation therapy is not always because of radiation.

P.131. RESULTS OF TREATMENT PEDIATRIC RECURRENT HIGH GRADE GLIOM (HGG) WITH COMBINATION OF BEVACIZUMAB AND IRINOTECAN

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Recurrent HGG in children have a dismal prognosis with minimal improvements in survival seen following currently available salvage therapy. This study was conducted to determine if the combination of a novel antiangiogenic therapy, bevacizumab, and a cytotoxic agent, irinotecan, is safe and effective for pediatric patients with recurrent HGG. From February 2008 till December 2009, 22 patients with progressive HGG were enrolled in this study (11 girls and 11 boys). Age 12.5 years (range, 5–17 years). Previously all patients received resection of the tumor followed by RT with parallel temozolomide and monotherapy of temozolomide. Relapse was documented by CT/MRI/PET. Median of follow-up until PD was 6 months (range 2–17 months). In 19 patients (86.3%), the glioblastoma (GB) was histologically verified, and in 3 patients (13.7%) anaplastic astrocytoma (AA) was verified. Karnovsky was 50–100 (with median 80). All patients received 6-week cycles of combined therapy: bevacizumab 5 mg/kg i.v. 1, 15, and 29 days + Irinotecan 125 mg/m²/day (in case of together with anticonvulsant, 340 mg/m²/days) 1, 8, 22, 29 days. Median of follow-up was 6 months (range 1–18 months). Median of number of cycles for 1 patient was 3.8 (range 1–10). Objective response (complete and partial) observed in 10 patients (45.5%): CR in 4 patients, PR in 6, SD in 6 patients, also PD in 6 patients (27.3%). Six-month PFS in all patients with HGG was 0.46, 12-month PFS was 0.19, 18-month PFS was 0.10; 6-month OS was 0.60, 12-month OS was 0.24, 18-month OS was 0.24. For the analyzed moment, 9 patients are still alive (40.9%), 13 died (59.1%); 1 patient with CR died in remission in 1.5 month after finishing therapy from leukoencephalopathy. The 18-month PFS in patients with GB was 0.11 and OS was 0.28. Objective response observed in 15 patients (78.9%); PD in 4 patients (21.1%). Median of OS was 10 months, median of PFS was 5 months. In all, 3 patients (100%) with AA was PD. Median of OS was 10 months and median of PFS was 7 months. No central nervous system hemorrhages occurred, but 1 patient developed leukoencephalopathy. Combination of bevacizumab and irinotecan is an effective in relapsed pediatric GB with acceptable toxicity. PFS in this group is higher than in patients with AA, what statistically proved ($P = .05$).

P.132. OVERVIEW OF CHILDREN WITH ANAPLASTIC ASTROCYTOMA

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INTRODUCTION. Anaplastic astrocytoma (AA) is a rare tumor of CNS in children, which differs the adverse prognosis if only surgical treatment performed. **MATERIALS AND METHODS:** From 2000 to 2005 37 pts at the age from 5 months to 16 years (median 8 years) with the first time verified AA were observed. 4 patients received only resection, 8 pts - resection and radiotherapy (RT), 25 pts - complex treatment (combination of resection, RT and chemotherapy (CHT)). Total resection of a tumor performed in 15 pts, subtotal - in 7 pts, partial - in 12 pts, biopsy - in 3 pts. 33 pts received RT in a dose of 50–60 Gy (median 55 Gy). CHT was carried out under various schemes depending on age. The pts under 3 years old ($n = 6$) received CHT by the protocol “Baby” POG. Pts older than 3 years received after RT: Temodal 200 mg/m² ($n = 11$), protocol HIT-91 ($n = 5$) or PCV ($n = 3$). **RESULTS.** The median of follow up was 46 months (7–150 months). 5-years PFS and OS for all group of pts was 40 ± 8 % and 50 ± 9

% respectively. Medians of PFS and OS - 24 and 60 mths respectively. PFS in pts with total resection was 69 %, subtotal- 42 %, partial- 10 %, biopsy- 0 % ($p = 0.01$). 5-year PFS was 56 % in pts with complex treatment, 10% - with surgical treatment and RT or surgery alone ($p = 0.02$). In pts under 5 years 5-year PFS - 80 %, older 5 years - 24 % ($p = 0.002$). The PFS in pts older 5 years who received different schemes of CHT was NS (not significant). CONCLUSIONS. The best indicators of PFS associated with complex treatment, total and subtotal resection of a tumor, age of pts older 5 years. The results permit to consider CHT as an effective and obligatory element of complex treatment of AA. The scheme of CHT choice depends on age of the patient.

P.133. MARKED REGRESSION OF CERVICAL PARANGLIOMA WITH ANTIANGIOGENIC METRONOMIC THERAPY

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BACKGROUND: Parangliomas of the head and neck are usually benign, hypervascular neuroendocrine tumors of the autonomic nervous system. Management is difficult, because such tumors are often inoperable and radiotherapy remains controversial, especially in young age. **CASE REPORT:** A 15-year-old girl was admitted to our hospital with a cervical mass. Imaging revealed bilateral paranglioma, deemed inoperable because of location and size. The patient was observed with repeated MRI scans, until she developed Horner's syndrome and hoarseness because of tumor progression 10 months after first presentation. Metronomic antiangiogenic therapy was initiated with daily oral thalidomide 3 mg/kg, twice daily oral celecoxib 100 mg, daily oral fenofibrate 70 mg/m², and alternating 21-day cycles of daily oral etoposide 50 mg/m² and cyclophosphamide 2 mg/kg, augmented with biweekly intravenous bevacizumab 10 mg/kg. **RESULTS:** Our patient showed an impressive response to therapy. After 8 weeks, MRI revealed response to treatment with regression in size and central necrosis. Additionally, the palpable cervical mass decreased. Therapy was well tolerated, and side effects included lymphopenia and peripheral neuropathy, requiring dose reduction of thalidomide and switch to lenalidomide after 1 year. With ongoing therapy, the patient could continue school. **CONCLUSION:** Antiangiogenic therapy may present a promising approach in cervical paranglioma.

MENINGIOMAS

P.134*. CRANIAL BASE MENINGIOMAS: THE ESSENTIAL ROLE OF STEREOTACTIC RADIOSURGERY

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INTRODUCTION: Surgical resection of cranial base meningiomas continues to present an enormous surgical challenge because of the complexity of the approaches and mainly of the possibility of disabling sequelae. In the past 2 decades, stereotactic radiosurgery has been advocated as the definite treatment in the management of these tumors, as a first-line treatment or after incomplete resections, changing the current management protocols in the treatment of these meningiomas. **MATERIALS AND METHODS:** A retrospective review of 615 patients treated for single cranial base meningioma at our Gamma Knife Unit during the period 1993-2009 was performed, particularly selecting 233 cases followed for more than 2 years. **RESULTS:** Two-thirds of the patients were females, with a global median age of 55 (25-84). The most frequent location was the cavernous sinus (37%), followed by clival and petroclival regions (16.3%). Forty-five percent of patients had previously been operated on, and 6 patients had received previous radiotherapy. The mean treated volume was 11 cm³ (0.6-85). The mean follow-up time was 50 months (24-163), with 60 and 7 patients followed during more than 5 and 10 years, respectively. Volumetric control was obtained in 97% of patients, with a reduced volume in 71.2%, and stabilization in 26%. Clinically, 91% of patients remained stable, with an improvement in 2.5%. **CONCLUSIONS:** Gamma-knife radiosurgery is a safe and efficient technique in the control of cranial base meningiomas, with an excellent preservation of neurological function. It has displaced conventional radiotherapeutic treatments and is

becoming a definite alternative to aggressive and potentially disabling surgical resections.

P.135. A PREDICTIVE GENETIC MARKER FOR TUMOR RECURRENCE IN MENINGIOMAS

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INTRODUCTION: Meningiomas are tumors that arise from the coverings of the brain or the spinal cord. They are mostly benign and can often be surgically cured. However, in about 8% of the cases, an increased risk of tumor recurrence and a more aggressive clinical behavior are observed. Cytogenetically, meningiomas are well characterized with either a normal karyotype or monosomy of chromosome 22 in most of the tumors. Clinically relevant are also most common losses of the autosomes 1p, 9p, 14, and 18. The order of accumulating genetic aberrations has previously been estimated with oncogenetic tree models, and a genetic progression score (GPS) derived from these models was shown to be more predictive for tumor recurrence than WHO classification. **MATERIALS AND METHODS:** Fifty meningioma patients were operated by open surgery between 2008 and 2010. Tissue specimens from tumors were obtained after surgery and prepared for FISH analysis. According to our previous results, we used two-color FISH for chromosomal alterations of chromosomes 1, 9, 14, 18, and 22. **RESULTS:** In our cohort, 52% (26 of 50) correspond to WHO I, 40% (20 of 54) to WHO II, and 8% (4 of 50) to WHO III meningiomas. The average age of all patients was 59.6 years (± 11.4), 58.5 years (± 11.4) of the female patients (32 of 50) and 63.4 years (± 15.1) of the male patients (18 of 50). FISH analyses were performed in these 50 cases and for each meningioma up to 200 nuclei were evaluated. In 32 tumors, we found deletions of chromosome 22. In 22 cases, the deletion of the short arm of chromosome 1 was detectable. Loss of chromosome 14 was found in 13 cases, of chromosome 18 in 6 cases, and of 9p in 4 cases. On basis of these results, 27 cases correspond to a GPS of ≤ 1 , 6 cases to a GPS of ≥ 1 and < 6.02 and 17 cases ≥ 6.02 , indicating a higher risk of tumor recurrence. **CONCLUSION:** Grading and prognostic assessment of meningiomas can be controversial. The biological behavior of meningiomas can obviously not be reflected in histological parameters alone. Cytogenetic characterization of meningiomas by FISH analysis or by karyotyping can provide an insight into their potential of recurrence by GPS classification and are therefore a valuable criterion for the neurosurgeon's postoperative management protocol.

P.136. SURGICAL TREATMENT OF CENTRAL NERVOUS SYSTEM HEMANGIOPERICYTOMAS

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INTRODUCTION: Hemangiopericytomas (HPC) are rare, highly vascularized tumors derived from pericapillary cells or Zimmerman's pericytes, which tend to recur locally and metastasize extraneurally. Treatment includes complete surgical resection followed by radiotherapy to optimize local recurrence control. We present our experience in the treatment of patients with HPC. **MATERIALS AND METHODS:** Retrospective analysis of clinical data from patients with HPC treated at the Department of Neurosurgery between June 1995 and February 2010 was reviewed to establish lesion location, associated symptoms, radiological features, preoperative embolization, intraoperative findings, postoperative complications, extent of resection, recurrences, and need for adjuvant radiotherapy. **RESULTS:** A total of 14 patients with HPC were subjected to surgery during this period, of which 9 were females (64%) and 5 males (36%). Mean age of patients in this series was 44 years (range 21-75), and mean follow-up duration was 50 months (range 7-147). Lesions were supratentorial in 7 patients (50%), infratentorial in 2 (14%), falco-tentorial in 2 (14%), skull base in 2 (14%), and dorsal spine 1 (8%). Headache was the most frequent symptom in 8 cases (57%) followed by neurological deficits in 7 (50%). Endovascular therapy was used in 5 patients (35%). Complete surgical resection was achieved in 11 patients (78%) and subtotal resection in 3 (22%). Eight patients received postoperative radiotherapy (57%). Recurrences were observed in 5 patients (35%), 4 at the primary site, and 1 at the craniospinal axis. Four of these patients were reoperated, and subsequently

treated with radiotherapy (75%). Five patients (36%) presented profuse intraoperative bleeding, and at most recent follow-up 1 patient had died (mortality 7%). **DISCUSSION:** HPC accounts for <1% of primary CNS tumors and about 2.2% of all meningial tumors. Clinical presentation varies according to tumor size and location. The main differential diagnosis remains meningioma. Radiologically, irregular margins and heterogeneous enhancement have been associated with aggressive behavior. Surgical resection is the treatment of choice followed by radiotherapy with doses over 50 Gy. Local recurrence incidence ranges from 26% to 80% depending on the extent of primary resection and administration of radiotherapy. Extraneural metastasis rates range from 14% to 30% and are found predominantly in the bone, lungs, and liver, making strict follow-up mandatory.

P.137. INTRACRANIAL MENINGIOMA WITH LEPTOMENINGEAL DISSEMINATION

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PURPOSE: Intracranial meningiomas comprised of 15%–20% of all primary brain tumors. The spread of meningioma through cerebrospinal fluid (CSF), although very rare, was reported primarily in nonbenign subtypes. The authors report rare cases of meningioma with leptomeningeal dissemination (LD) after surgery. **METHOD:** Four females (age ranged 21–72 years, mean 56.5) of 332 consecutive patients (1.2%) with surgically resected intracranial meningioma manifested CSF dissemination. The initial tumor location was posterior fossa in 2 cases and lateral ventricle and parasagittal convexity in 1 case each. Pathological examination revealed 2 cases of WHO grade II and 1 case of grade I and III, respectively. All patients received Simpson grade I/II resection at the first operation. Adjuvant focal radiotherapy was performed in a case of atypical meningioma with Simpson II resection and a case of malignant subtype. **RESULTS:** Each LD occurred after the first operation with the time interval of 2.5 month–6.9 years. A patient with malignant subtype showed the shortest time to LD. Metastasis was confined to intracranial CSF space in 2 patients, and was extended to both cranial and spinal subarachnoid space in 2. One patient also showed multiple extraneural metastases. Treatment included decompressive surgery for rapidly progressive motor weakness in 3 patients and radiosurgery with or without focal radiotherapy in 2. Three patients showed disease progression with the survival ranged from 1 month to 3.5 years after LD. One patient with WHO grade I meningioma was alive in stable disease at the time of last follow-up. **CONCLUSION:** LD of meningioma after surgery is very rare; however, it should be considered as a cause of unusual presentation. Further study for more effective systemic and local treatment for patients with CSF disseminated recurrent meningioma is needed. Whether surgery is the iatrogenic cause of metastasis of meningioma is further needed to be discussed.

P.138. IMPROVED PREDICTION OF TUMOR RECURRENCE IN MENINGIOMAS WITH INTRATUMORAL CYTOGENETIC HETEROGENEITY

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OBJECTIVE: Meningiomas are tumors that arise from the coverings of the brain or the spinal cord. They are mostly benign and can often be surgically cured. However, in about 5% of the cases, they turn into malignant forms with aggressive clinical behavior and increased risk of tumor recurrence. **METHODS:** Meningiomas are cytogenetically well characterized, with normal karyotype or monosomy of chromosome 22 in most tumors and clinically relevant secondary losses of other autosomes in a subset of potentially malignant tumors. The order of accumulating genetic aberrations has previously been estimated with oncogenetic tree models, and a genetic progression score derived from these models was shown to be predictive for tumor recurrence. **RESULTS:** Although more homogeneous than other cancer types, meningiomas show considerable intratumoral cytogenetic heterogeneity, particularly in their malignant form. We observed different cytogenetic patterns in tumor cells of 221 out of 661 (33.4%) meningiomas. The present investigation demonstrates that it is not sufficient to consider only the most frequent cytogenetic pattern observed in a set of cells derived from the same tumor. **CONCLUSION:** Even a single clone with more advanced genetic progression also indicates clinical progression. Cox regression analysis reveals that the

clone with most advanced progression is a superior marker for recurrence in meningiomas. It is expected that for other cancer types with higher intertumoral heterogeneity the selection of single genetically advanced cells improves the prediction of clinical tumor progression in an even more drastic manner.

P.139. TREATMENT OF RECURRENT MENINGIOMAS WITH IMATINIB MESYLATE: A SINGLE-INSTITUTION EXPERIENCE

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BACKGROUND: Some irresectable and symptomatic meningiomas recur after conventional radiation therapy or stereotactic radiosurgery and present a therapeutic challenge. Evidence-based data for medical therapy for patients with malignant or recurrent and irresectable meningioma can be deemed as insufficient. Because of the prevalent expression of PDGF receptors in meningiomas, imatinib mesylate, a tyrosine kinase inhibitor of PDGFR- α , β , c-kit, abl, and arg (Glivec-targets), has gained interest as a treatment of choice in this patient group. **METHODS:** We selected 18 patients with recurrent meningiomas, aged 30–74 years (median 53.5 years), treated at our institution from 1996 to 2008. Nine patients (4 males, 5 females, median age 54.0 years) with positive immunohistochemical staining of at least one of the “Glivec®-targets” were initiated with a daily oral dose of 400 mg imatinib mesylate as first-, second-, or third-line systemic therapy. MRI was performed every 3 months or at clinical suspicion of progression. The median time from diagnosis to start of imatinib treatment was 36 months. Immunohistochemical staining was performed on formalin-fixed and paraffin-embedded tumor tissue with antibodies against PDGFR- α , β , c-kit, arg, and abl. **RESULTS:** Imatinib mesylate at a dose of 400 mg/day was well tolerated. Out of 9 patients treated with imatinib, 7 had stable disease and 2 progressed at the first scan after 3 months. We did not observe any complete or partial responses, though prolonged disease stabilization with a progression-free survival rate of 66.6% at 6 months could be observed. Overall median progression-free survival in the imatinib-treated cohort was 16 months (range 1–31 months). Interestingly, imatinib treatment had a favorable impact on overall survival of meningioma patients (log-rank test, $P = .034$). **CONCLUSION:** Single-agent imatinib mesylate is a well-tolerated therapeutic option with a high rate of disease stabilizations in recurrent meningiomas. Imatinib treatment seems to correlate with survival in our patients despite heavy pretreatment with other therapeutic agents. Although these data clearly need verification in a larger, randomized clinical trial, our observation favors the use of imatinib mesylate in recurrent meningiomas, particularly in lack of other therapeutic options.

P13 SPINAL CORD TUMORS

P.140*. EPENDYMOMAS OF THE FILUM TERMINALE: A SINGLE-INSTITUTION EXPERIENCE

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BACKGROUND: Filum terminale ependymomas form a specific variant of spinal cord tumors. Histologically, most are myxopapillary (WHO grade I), although grade II tumors also occur. Controversy exists about the role of surgical removal (total vs subtotal) and technique (“en bloc” vs piecemeal) and about adjuvant radiotherapy. **METHODS:** We performed a retrospective analysis of 22 cases treated from 1992 until 2008 (average follow-up 84 months). Possible prognostic factors (dimensions, metastasis, surgical aspects, and radiotherapy) were analyzed. **RESULTS:** In 5 patients, metastases were found at diagnosis. Surgery was complete in 18 (including 2 with metastasis resection, in 5 “en bloc,” the others piecemeal), partial in 4. Histology showed myxopapillary type in 16 (4 metastasized), grade II in 6 (1 metastasized). Adjuvant radiotherapy was performed after incomplete resection and/or metastasis. Delayed radiotherapy was given in 2 cases after local recurrence and was given in 2 on a metastasis. At last follow-up, 1 patient died of progressive disease and 1 died of unrelated cause (tumor free). Five patients had been treated for recurrences. Of the 20 patients alive, 17 were tumor free and 3 had stable disease. The most important prognostic factor was initial tumor presentation: all 9 tumors smaller than 4.5 cm did not have metastasis or recurrence, were not irradiated, and had excellent functional outcome. In larger tumors, there were more metastases and recurrences, radiotherapy was performed and functional outcome was worse. **CONCLUSION:** Initial tumor characteristics, associated with the possibility to obtain complete surgical resection, are more important than histology or factors influenced by treatment.

P.141*. SPINAL CORD COMPRESSION FROM NEUROFIBROMAS IN NEUROFIBROMATOSIS TYPE 1 PATIENTS

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INTRODUCTION: Neurofibromas are characteristic tumors of Neurofibromatosis type 1 (NF1). They arise as discrete or more diffuse plexiform tumors, growing along cutaneous and subcutaneous nerves and spinal roots. Spinal neurofibromas are present in at least 50% of NF1 patients, and in most cases are asymptomatic. They are very different from sporadic or Neurofibromatosis type 2-associated schwannomas in their cell composition, location, and behavior. There are scarce case reports and two small series with limited information, about clinical presentation and management of NF1 patients with spinal cord compression from spinal neurofibromas. The aim of the study was to review our experience in the management of these tumors. **MATERIALS AND METHODS:** A retrospective review of clinical data has been performed in our series of 205 NF1 patients, followed up, and treated in a neurofibromatosis referral center based in a neurosurgical unit. Pre- and postoperative neurological status has been evaluated following McCormick's classification in patients with myelopathy secondary to spinal root neurofibromas. Demographic, clinical, anatomical, and surgical performance result data have been analyzed. **RESULTS:** Six surgical procedures for treatment of symptomatic spinal cord compression from neurofibromas have been performed in our series. Two surgical procedures at distant levels in 2 patients have been evaluated as 4 separated events. The age at presentation ranged from 29 to 51 years. Clinical presentation was quadriparesis in 3, paraparesis in 1, and monoparesis in 2 cases. The anatomical level was cervical in 4 and thoraco-lumbar in 2. Tumor was growing from either anterior or posterior roots. Large intraspinal tumors were radically resected in every case, sparing rootlets with small tumor nodules, through laminectomies or osteoplastic laminotomies. Five surgical events were followed by an upgraded performance, with full recovery in 2 from 4 patients. One patient, suffering from a long-standing severe paraparesis, still presents a severe deficit. In no cases the tumor recurred or progressed after surgery. No kyphotic deformity was observed at follow-up. **CONCLUSIONS:** Spinal cord compression secondary to spinal root neurofibromas is a known but infrequently reported complication in NF1 patients. The risk of becoming symptomatic does not decrease with age. The cervical area is where more neurofibromas appeared, but caudal spinal cord was also affected. Resection of spinal root neurofibromas producing symptomatic spinal cord compression is followed by excellent functional results, except in patients with long-lasting severe neurological deficit. Although asymptomatic spinal neurofibromas should not be treated, surgery should be considered and planned as soon as symptoms appear for the best functional results.

P.142. PARAGANGLIOMA OF THE CAUDA EQUINA: A REPORT OF 3 CASES

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INTRODUCTION: Cauda equina paraganglioma (CEP) is a rare tumor. The first case was described in 1970 and since then less than 200 cases have been reported. The origin of CEP is uncertain as the existence of paraganglia cells in the central nervous system remains a point of debate. There is a male predominance, and low back pain is the main symptom in more than 90% of patients, with sciatica in 72%. MRI is the study of choice and treatment consists of total excision if feasible. Definitive diagnosis can only be made after immunohistochemical investigation. CEP is classified as Grade I WHO and the prognosis is excellent. Nonetheless, tumor recurrence rate after subtotal removal is 10%. **CASE REPORTS:** We present 3 cases of paraganglioma of the cauda equina, preoperatively diagnosed as an intradural mass on MRI. In 1997, a 41-year-old man presented with low back and right-sided leg pain. Neurological examination revealed only sensory-loss in the right leg, and MRI showed an intradural mass at L2-L3. In 1999, a 37-year-old man presented with low back pain and bilateral sciatica without neurological deficits. MRI showed an intradural mass at L3-L4. In 2005, a 51-year-old man presented with low back pain with bilateral irradiation to the gluteal region. No neurological deficits were present and MRI showed an intradural mass at L4. In all cases, a total resection of the tumor was performed after which all patients fully recovered. There is no recurrence after 13, 11, and 5 years, respectively. **CONCLUSION:** Paraganglioma of the cauda equina is a rare tumor and we diagnosed 3 of such tumors on a total of 104 intradural extramedullary tumors (1994-2005). All 3 cases presented with low back pain, as most often reported in

the literature. No recurrence was seen in these 3 patients after total resection. In retrospect, MRI was not completely typical for Schwannoma or ependymoma, but the final diagnosis can only be made histologically.

P.143. MALIGNANT SPINAL CORD COMPRESSION IN A PATIENT WITH GLIOBLASTOMA

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INTRODUCTION: Spinal metastases in patients with malignant gliomas are rare, but a well-known complication in the advanced stage of the disease. Literature on the treatment of malignant spinal cord compression in patients with malignant gliomas is limited. **CASE REPORT:** A 65-year-old female patient was diagnosed with glioblastoma in July 2008. After gross total resection, she received a standard concomitant radio-chemotherapy according to the STUPP protocol. At first local relapse 10 months after diagnosis, she was treated by gamma-knife and subsequent, dose-intensified temozolomide chemotherapy. Fourteen months after diagnosis, she was admitted because of an acute deterioration of gait function within 48 hours. Neurological examination revealed a paresis of the right leg. Clinically, the neurological deficit was attributed to a progressive left temporal glioblastoma. On the next day she developed paraplegia of the lower limbs. Cranial MRI showed a multicentric bilateral glioblastoma. When compared with the previous MRI scan 2 months ago, multicentric supratentorial tumor progression in the left and right hemisphere could be detected, but not compatible with neurological signs and symptoms. MRI of the spinal cord exhibited contrast-enhancing lesions at the spinal level T3/4 and T6/7. Although steroids were administered and acute local radiotherapy (5 × 4 Gy) was applied, no improvement in neurological function could be achieved. Urinary and anal incontinence as well as diffuse abdominal pain occurred. Considering the progressive disease, no further antitumor treatment was started and the best supportive care was established at discharge. **CONCLUSION:** Reviewing the literature, in glioblastoma patients with malignant spinal cord compression, local radiotherapy can provide a temporary relief from pain and mild improvement of neurological deficits without survival advantage. However, no evidence-based treatment guidelines are presently available. Although our patient did not benefit from the therapeutic interventions, an early diagnosis and subsequent treatment seems mandatory to prevent loss of neurological function.

P.144. SPINAL CORD AND BRAINSTEM HEMANGIOBLASTOMAS IN PATIENTS WITH VON HIPPEL-LINDAU DISEASE: MICROSURGICAL RESULTS IN A REFERRAL CENTER SERIES

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INTRODUCTION: Spinal cord hemangioblastomas make up for 5% of primary spinal cord tumors, and are associated with von Hippel-Lindau disease (VHL) in more than 75% of cases, where they can be found at multiple levels. Brainstem hemangioblastomas are present in up to 20% of VHL patients, and their discovery is almost pathognomonic of the disease. Management of these tumors is controversial, having in mind that these patients are not affected bearers of isolated hemangioblastomas, but are affected by a genetic multi-neoplastic condition. The aim of this paper is to present the microsurgical management results of spinal cord and brainstem hemangioblastomas in a VHL referral center. **MATERIALS AND METHODS:** We reviewed a series of 11 patients (mean age 40 ± 15.4 years, range 21-66 years) who underwent 13 operations to remove 17 hemangioblastomas: 3 in brainstem, 3 in the bulbo-medullary junction, 4 cervical, 6 thoracic, and 1 lumbar hemangioblastomas. All surgical procedures were performed by the same neurosurgeon (JMdC) in a VHL referral center. The indication for surgery was established by the appearance of clinical symptoms or evident growth of hemangioblastoma. **RESULTS:** Sensory deficit was the most frequent symptom, present in 10 patients (76.9%), followed by motor deficit in 7 (53.8%). Complete resection was possible in all cases. In the pre- and postoperative functional assessment, according to McCormick's scale, clinical improvement was obtained in 1 (7.7%), clinical stability in 12 (84.6%), and clinical deterioration in 1 from I to II functional

grade (7.7%). Early in the postoperative assessment, a functional deterioration occurred in 4 (30.8%) patients, all fully recovered after 3 months, except in the abovementioned case. **CONCLUSIONS:** Complete microsurgical resection of spinal cord and brainstem hemangioblastomas in VHL patients can be achieved with good surgical results and a very low rate of neurological complications, when performed in a VHL referral center with surgeons particularly involved in the management of patients with this disease.

P.145. BURKITT-LIKE LYMPHOMA REVEALED BY SPINAL CORD INVOLVEMENT

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Intramedullary spinal lymphoma accounts for only 3.3% of CNS lymphoma. It was mainly reported with immunodeficiency.¹ Burkitt-like lymphoma (BL), also called atypical Burkitt lymphoma, is a rare high-grade lymphoma with characteristics on the borderline between large B-cell lymphoma (LBCL) and classical Burkitt lymphoma (BL). We report a case of primary intramedullary BLL in an immunocompetent adult. **CASE REPORT:** A 62-year-old immunocompetent white man presented in November 2006 left leg weakness and unsteadiness. Initial neurological examination showed only paraparesis. Immediate evolution was characterized by occurrence of an acute urinary retention and weakness of both hands. Spinal cord MR examination showed multifocal intradural nodular lesions, from C1 to C6, and upper thoracic spinal cord, diffusely enhanced by gadolinium injection. The same lesions were found in the brainstem and cerebellar lobes. Standard biological parameters, LDH, β 2-microglobulin, tumor markers were normal. Serological studies were negative. Blood protein immunoelectrophoresis found monoclonal lambda and kappa IgM. An extensive investigation, including chest and abdomen CT scan, bone marrow examination, ophthalmologic examination was negative. Protein level in CSF was increased (4.8 g/L), with a moderate lymphocytosis (19/mm³) without any abnormal cells. Surgical exploration showed involvement of spinal cord, intradural and arachnoidal tissue sparing epidural spaces. The diagnostic histological was high-grade B-cell lymphoma. The tumor had 2 populations, 1 of medium sized lymphoid cells with high nucleocytoplasmic ratio and 1 with irregular nuclei, with phagocytic macrophages giving a typical starry sky appearance (Figure 1b). Immunohistologically, the tumor cells expressed B cell antigen CD 20 and CD 45. The Ki 67 proliferative rate was near 100%. Bcl 6 was positive and Bcl 2 negative. No Epstein-Barr virus antigen was detected. These features led to the diagnosis of Burkitt-like lymphoma. The patient was treated by general polychemotherapy and intrathecal methotrexate. Treatment led to a decrease of the lesions size on further MR. The patient died from aplasia and respiratory distress syndrome after the third treatment. **DISCUSSION:** BL accounts only for 1%–2% of lymphoma in adult, and is described as a variant of classic BL. It was mainly described in immunodeficient patients. BLL are high-grade, and are characterized by a poor initial survival compared with diffuse LBCL. Spinal cord involvement by BLL mainly consists of epidural infiltration with meningitis and extensive nodular lesions. Rapid diagnosis is of major importance as evolution is severe and immediate treatment important. BL cells are known as extremely chemosensitive tumors. Survival rate at 5 years is <20%. Poor prognostic factors consist of older age, CNS, or bone marrow involvement.²

BRAIN AND LEPTOMENINGEAL METASTASES

P.146*. ROUTE OF INTRACEREBROSPINAL FLUID LIPOSOMAL CYTARABINE ADMINISTRATION, SAFETY, AND EFFICACY OF THERAPY IN NEOPLASTIC MENINGITIS

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BACKGROUND: Recently, it has been reported by Glanz et al. that there was no difference between route of intracerebrospinal fluid chemotherapy administration, intraventricular vs intralumbar, with different drugs (eg, methotrexate or liposomal cytarabine) in terms of progression-free survival or overall survival. We present our experience in one single-center with liposomal cytarabine administered to patients with neoplastic meningitis. **METHODS:** We reviewed 22 patients with cytologically documented neoplastic meningitis because of solid tumor or haematological malignancies. All of them were

treated with liposomal cytarabine. We examined the type of tumor (solid or haematological), overall survival (time from diagnosis till death or date of study), route of administration, and adverse events. **RESULTS:** Twenty-two patients were examined since December 2006 to March 2010. Seven of them received liposomal cytarabine by intraventricular administration; 15 by intralumbar infusion. Five had solid tumors and the rest haematological malignancies. Global overall survival was 9.04 months (6.01 for the intraventricular group and 9.86 for the lumbar group). In the intraventricular group, only 1 patient had serious adverse event (ventriculitis). In the intralumbar group, 2 patients developed chemical cauda equine syndrome; 1 developed toxic optic papillitis; and 1 developed both toxic papillitis and chemical cauda equine syndrome. **CONCLUSIONS:** Site of intra-CSF liposomal cytarabine is clinically relevant with fewer adverse events by intraventricular route.

P.147*. CENTRAL NERVOUS SYSTEM (CNS) COMPLICATIONS OF MULTIPLE MYELOMA (MM): MYELOMATOUS MENINGITIS AFTER ALLOGENIC STEM CELL TRANSPLANTATION (ASCT)

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INTRODUCTION: Neurologic manifestations are not uncommon in multiple myeloma (MM). They are represented by a broad spectrum according to the difference in pathological mechanisms, clinical presentation, and therapeutic implication. CNS invasion in MM is a rare event with <1% frequency in the small published series and account for about 70 cases reported in English literature. **PURPOSE AND METHODS:** We report a case of a 50-year-old Caucasian male who underwent chemotherapy and ASCT that controlled the disease for a number of years. For headache complaints, he undertook diagnostic procedures that established the leptomeningeal involvement by myeloma. IgA1 MM was diagnosed at age 42 (November 2002) and submitted at VAD regimen with a partial response. In June 2003, he has been offered ASCT from an HLA-matched sibling obtaining a complete response that lasted until October 2005 when he recurred first at the sacral level, with neurological compression, increased paraprotein levels and bone marrow infiltration. He has been submitted to local radiotherapy RT and subsequently to thalidomide, and donor lymphocytes with persistence of the disease. Started on bortezomid attained the 2nd complete remission until September 2007 when new recurrence occurred. The patient was rechallenged with bortezomid with normalization of analytical parameters. After one more recurrence at the spine, he was diagnosed with leptomeningeal disease and started an intrathecal, systemic, and whole-brain radiotherapy 30 Gy/10 # in May 2009. He obtained a complete response at CNS level and is alive and free of disease at 11 months after RT was done. **CONCLUSIONS:** Among the rather uncommon localizations of MM in the CNS, myelomatous meningitis may also occur. Different modalities of treatment are used, including intrathecal chemotherapy, cranial irradiation, and systemic chemotherapy. Patients with CNS myeloma even with aggressive treatment have extremely poor prognosis with <3 month disease-free survival. However, the patient is still alive at 11 months after the involvement of CNS by MM has been diagnosed.

P.148*. THE ROLE OF TEMOZOLOMIDE FOR PATIENTS WITH METASTATIC BRAIN DISEASE

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BACKGROUND: Standards of chemo- and chemo-radiotherapy for treatment of brain metastases is not available till present time. Purpose of this trial is to assess the efficacy of temozolomide (TMZ) as monotherapy, TMZ combined with whole brain irradiation (WBI), or combined chemotherapy of TMZ and irinotecan (I) and TMZ and cisplatin (DDP) in patients with brain metastases (BM) from non-small cell lung cancer (NSCLC), breast cancer (BC), or melanoma. **METHODS:** Ninety-seven patients were included in this study. Patients with BM from NSCLC (21 patients), melanoma (17 patients), and BC (17 patients) were treated with WBI (3 Gy/30 Gy) and concomitant TMZ therapy (75 mg/m²/day orally on Days 1–14). Patients with BM from NSCLC (7 patients), melanoma (17 patients) were treated with TMZ (150–200 mg/m²/day orally on days 1–5 every 4 weeks) as monotherapy. Eleven heavily pre-treated NSCLC patients (after I–II lines of chemotherapy and/or WBI) were treated with combined chemotherapy of 1 (250 mg/m² day 6 intravenous, every 4 weeks) and TMZ (150 mg/m²/day orally on days 1–5, every 4 weeks). Seven patients with melanoma were treated with combined chemotherapy of DDP (20 mg/m²/day intravenous on days 1–5, every 4 weeks) and TMZ (150 mg/m²/day orally on days 1–5, every 4 weeks). **RESULTS:** Observations of the

study were as follows: in the TMZ + WBI-treated patients with BM from NSCLC, 3 (14.3%) complete response (CR), 8 (38.1%) partial response (PR), 8 (38.1%) stabilization of disease (SD). The median overall survival (mOS) was 7.5 months. In the TMZ + WBI patients with BM from melanoma, 3 (17.6%) PR, 9 (52.9%) SD. The mOS was 6.6 months. Among patients with BC brain metastases, 2 (11.8%) CR, 11 (64.7%) PR, 3 (17.6%) SD. The mOS was 11 months. In the TMZ monotherapy patients with BM from NSCLC, there were 4 SD, 3 patients progressed. The mOS was 8 months. In the TMZ as monotherapy patients with BM from melanoma, 1 (5.9%) CR, 4 (23.5%) PR, and 7 (41.2%) SD. The mOS was 6 months. In the TMZ + I patients with NSCLC brain metastases, 7 (63.6%) SD. The mOS was 8 months. In the TMZ + DDP patients with melanoma brain metastases, 2 PR, 4 SD, 1 patient progressed. The mOS was 8 months. CONCLUSIONS: TMZ with WBI showed high efficacy in patients with BM from NSCLC, BC, and melanoma. TMZ as monotherapy showed efficacy and good tolerability in patients with BM from melanoma and SD in patients with BM from NSCLC. Combined chemotherapy of TMZ and I in heavily pre-treated patients with NSCLC BM showed prolonged SD and good tolerability. The TMZ + DDP combination has pronounced high anticancer activity in patients with brain metastases from melanoma.

P.149*. IRRADIATED TUMOR VOLUME INFLUENCES LOCAL CONTROL AND PROGRESSION-FREE SURVIVAL IN PATIENTS WITH 1–3 BRAIN METASTASES TREATED BY RADIOSURGERY
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OBJECTIVE: To evaluate the efficacy and clinical and radiological follow-up (r-FU) of patients with brain metastases (BM) treated with radiosurgery at a single institute. **MATERIAL AND METHODS:** Between 2003 and July 2009, 150 patients with BM (61.6% solitary, 61.9% lung) were treated with either single-fraction (SRS) or hypofractionated radiosurgery (SRT); the majority received SRS. SRT was indicated if the BM diameter was >3.5 cm. Patients were followed using a prospective radiological schedule at a 3-monthly interval. Patients lost to FU because of early death (within 3 months) were assumed to have local failure. Endpoints were local control (LC, defined as no enlargement of the metastasis on MRI or CT scan), overall survival (OS), and progression-free survival (PFS, defined as no local or distant intracranial progression and/or clinical neurological deterioration). Prognostic factors known from literature were evaluated and comparisons between SRS and SRT treatments were made. All imaging was fused to the baseline scan to obtain 3D information. All data were analyzed using univariate survival analyses. **RESULTS:** A total of 260 BM were irradiated; for 66% r-FU was available (23% had no r-FU because of early death). The median OS, PFS, and LC was 7.0 months (range 3 days–46 months), 4.0, and 6.0 months, respectively. There was a significant difference in OS between RPA classes ($P < .001$). Median PFS was 5.0 and 3.0 months for SRS and SRT treatment, respectively ($P = .001$). The 6 and 12 months, the LC rate was 46% and 37%. The sum of all target volumes irradiated per patient (PTV) was a significant prognostic factor for PFS (HR = 1.12/5 cc increase in PTV; 95% CI 1.01–1.23). PTV was a significant prognostic factor for LC (HR = 1.15/5 cc increase in PTV; 95% CI 1.04–1.27). The maximum dose, corrected for fraction dose (EQD2, $\alpha/\beta = 10$) was a significant factor for LC (HR = 0.98/Gy; 95% CI 0.97–0.99). Toxicity (acute or late) grade ≥ 3 was observed in 15 patients, there was no significant difference between patients treated with SRS or SRT. Full 3D radiological evaluation of LC is ongoing and the results will be presented. **CONCLUSIONS:** There is a clear correlation between the total irradiated target volume on PFS and local control; with increasing metastasis volume, a decrease of local control and PFS is obtained. The shorter PFS for patients treated by SRT also reflects this volume effect as SRT is applied for patients with larger metastases.

P.150. MULTIDISCIPLINARY TREATMENT OF LEPTOMENINGEAL METASTASIS IN PATIENTS WITH LUNG ADENOCARCINOMA

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BACKGROUND: Leptomeningeal metastasis (LM) is a devastating complication of systemic cancer. New therapies that effectively treat primary cancers outside the CNS have underscored the significance of LM. Intrathecal chemotherapy plus radiation (RT) are less effective for LM in lung cancer. We retrospectively studied outcome of patients with LM from lung adenocarcinoma underwent multidisciplinary treatment in our institute. **METHODS AND RESULTS:** Between December 2004 and

August 2009, 29 patients with LM from solid cancer underwent treatment. Eleven of 29 patients had lung adenocarcinoma; 7 of 11 presented with increased intracranial pressure, and other 3 with truncal ataxia. Treatment was indicated when LM was confirmed on MR images or cytology, Karnofsky performance score was more than 40, and life expectancy was more than 3 months if LM was controlled. The choice of treatment was based on clinical symptoms depending on the individual situation. Seven patients underwent intrathecal chemotherapy plus RT, EGFR-TKI plus RT, or VP-shunt plus RT (group A). Four patients underwent all of EGFR-TKI, RT, and VP-shunt (group B). Mean time to LM onset from diagnosis of lung adenocarcinoma was 24 (8–36) months. Mean survival time from LM onset was 4 months in group A and 9 months in group B ($P = .029$). Ten of 11 patients died: 9 of CNS metastases and 1 from pneumonia. No patients suffered from peritoneal carcinomatosis after VP-shunt. **CONCLUSION:** Combination of triple modalities (EGFR-TKI, RT, and VP-shunt) is a safe treatment, and may improve outcome of patients with LM from lung adenocarcinoma.

NEUROTOXICITY AND NEUROPROTECTION

P.151*. SUBACUTE NEUROLOGICAL SYNDROME WITH IMAGING CORRELATE FOLLOWING INTRATHECAL METHOTREXATE IN AN ADULT

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BACKGROUND: Methotrexate (MTX) is an important chemotherapeutic agent in the management of oncological and autoimmune diseases. It can be given intravenously or intrathecally and is frequently given in combination with other drugs. Methotrexate has been implicated in a variety of neurological complications. The acute or subacute neurotoxicity occurs within days to weeks after MTX therapy and is characterized by acute onset of focal neurological symptoms and mimic cerebrovascular stroke. While this syndrome has been described in several case report series in children, it is much less frequently seen in adults. **OBJECTIVES:** To describe the clinical course of a patient with subacute toxicity after intrathecal MTX and the corresponding abnormalities on imaging. **METHODS:** Case presentation and literature review. **RESULTS:** We report the case of a 29-year-old woman with acute systemic T-cell leukemia receiving systemic low-dose MTX, cytarabine, cyclophosphamide, doxorubicin, vincristine, and Peg-Asparaginase, as well as intrathecal methotrexate. Eighteen days after intravenous chemotherapy and 8 days after her seventh intrathecal MTX application, she presented with acute onset severe aphasia and difficulty mild right face and arm weakness. Cerebrospinal fluid was unremarkable. Magnet resonance imaging (MRI) of the brain revealed confluent restricted diffusion in the left greater than the right centrum semiovale and focally within the splenium of the corpus callosum. These lesion had no significant mass effect, no associated enhancement and were only minimally hyperintense on T2 sequence. The patient's neurological symptoms resolved completely over the next 24 hours without specific treatment. Repeat MRI 6 days later showed resolution of the diffusion abnormalities. **DISCUSSION:** Our case is unusual given the age of the patient. Most reports in the literature of subacute MTX toxicity describe children. Whether children are more likely to suffer MTX toxicity as a result of different susceptibility or children are simply more commonly treated with intrathecal or high-dose methotrexate requires more investigation. It is particularly important to be aware of this chemotherapeutic side effect in adults, as they are more likely to be misdiagnosed as presenting with an acute cerebrovascular ischemic or hemorrhagic event. The mechanisms of subacute MTX toxicity are poorly understood. Imaging abnormalities obtained in our case suggest a transient cytotoxic white matter edema as the underlying mechanism in subacute methotrexate toxicity.

P.152. VERIFICATION FOR HEMATOLOGICAL TOXICITY OF TEMOZOLOMIDE

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INTRODUCTION: Although 4 years has passed after temozolomide started to be sold on the open market, there are a few reports regarding the hematological toxicity of temozolomide in Japan. We have examined for our own experiments in 62 cases. **METHODS AND RESULTS:** Sixty-two cases of initial and

recurrent malignant glioma (25 males, 37 females, average age 58.1 years old) were included in the study. The observation period was from February 2004 to June 2008. Seven cases for administered group on consecutive days (temozolomide 75 mg/m²; oral administration once daily for 42 consecutive days). Thirty-eight cases for administered group on alternate days (temozolomide 150–200 mg/m²; oral administration once for 5 consecutive days and then cessation of the drug for 23 days. Including 18 cases of private import.) Fifteen cases took temozolomide in five days alternate regimen after forty two days continuous regimen. The grade classification was conducted for hematological toxicity at Common Terminology Criteria for Adverse Events (CTCAE) as follows. For leukocytopenia, Grade 2 had 19 cases (30%), Grade 3 had 16 cases (26%), and Grade 4 had 2 cases (3%). For lymphopenia, Grade 2 had 18 cases (29%), Grade 3 had 21 cases (34%), and Grade 4 had 9 cases (15%). For decrease in platelets, Grade 2 had 9 cases (15%), Grade 3 had 3 cases (5%), and Grade 4 had 6 cases (10%). In addition, 21 of 22 cases for daily administration indicated lymphopenia with Grade 2 or more and the lowest value was appeared at around the time of the post daily dose for 42 consecutive days. It is indispensable for the preventive administration of ST drug combination. There are 2 death cases to be considered as being related to temozolomide. (i) Male (84 years old) died with a combination of lymphopenic complication and carinii pneumonia by administered group on alternate days. (ii) Male (74 years old) has shown to decrease in leukocyte and platelets of Grade 4 by administered group on consecutive days and died with indication of a brain hemorrhage in approximately 1 month. It is needless to mention of aged people, and there are strong cases of hematological toxicity for young people; therefore, we consider it is indispensable to follow up for blood collection, including a differential count of leukocytes.

PARANEOPLASTIC NEUROLOGICAL SYNDROMES

P.153. HLA-DQ2 + INDIVIDUALS ARE SUSCEPTIBLE TO HU-ANTIBODY ASSOCIATED PARANEOPLASTIC NEUROLOGICAL SYNDROMES

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BACKGROUND: Hu-antibody-associated paraneoplastic neurological syndromes (Hu-PNS) are diagnosed in patients with severe and subacute neurological syndromes through detection of high-titer Hu antibodies (Hu-Ab) in serum. Hu-Ab are directed against the neuronal HuD-antigen that is aberrantly expressed by all small-cell lung-cancers (SCLC). This ectopic expression hypothetically causes an autoimmune response, which has a positive antitumor effect, but also causes devastating neurological damage. The precise immunopathogenic mechanism of the neurological damage is unknown, although occurrence of both B- and T-cell-mediated immune responses in Hu-PNS indicates a role for cellular immunity. **OBJECTIVE:** We aimed to identify genetic risk factors for Hu-PNS and further investigate the role of T cells by determining whether human leukocyte antigen (HLA) association plays a role in Hu-PNS. **PATIENTS AND METHODS:** Frequencies of HLA-A, B, DR, and DQ alleles were determined in 53 Caucasoid Hu-PNS patients with histologically proven SCLC and high-titer Hu-Ab. These were compared with the HLA types of 24 Caucasoid SCLC patients without Hu-Ab or neurological symptoms and 2440 healthy, unrelated, Dutch Caucasoid blood donors (HC). Odds ratios with 95% confidence intervals according to the Woolf test and two-sided Fisher exact test were used to compare the frequencies of the different HLA antigens in patients and controls. **RESULTS:** The frequency of HLA-DQ2 was significantly higher in Hu-PNS patients (33 of 53; 62%) than in HC (881 of 2360; 37%) ($P = .0015$). Although there also was a trend towards a higher prevalence of HLA-DQ2 in Hu-PNS patients than in SCLC patients (7 of 24; 29%), this difference did not reach statistical significance, probably because of the small size of the SCLC patient group. Additionally, the HLA-DR3 frequency was significantly higher in Hu-PNS patients (25 of 53; 47%) than in HC (599 of 2395; 25%) ($P = .0082$). **DISCUSSION:** This study indicates an association between Hu-PNS and presence of HLA-DQ2 and DR3 antigens. Both HLA-DQ2 and DR3 are part of the 8.1 ancestral haplotype (HLA-A1, Cw7, B8, DR3, and DQ2), which is a highly conserved HLA-complex associated with a wide range of autoimmune diseases. In Hu-PNS, the presence of HuD-specific T cells is suggested. Knowledge of the involved auto-antigen together with specific disease-associated HLA class II alleles may lead to detection of HuD-specific CD4⁺ T cells in HLA-DR3⁺/DQ2⁺ Hu-PNS patients and subsequent epitope identification. As a substantial proportion of Hu-PNS

patients does not express HLA-DQ2 and DR3, we suggest that additional factors must be involved in susceptibility to developing Hu-PNS.

P.154. A FIVE-YEAR FOLLOW-UP OF NEUROLOGICAL PARANEOPLASTIC SYNDROME PATIENTS IN WESTERN POLAND POPULATION

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INTRODUCTION: Neurological paraneoplastic syndromes (PNSs) are considered as rare, immune-mediated remote effects of systemic malignancy on nervous system. Long-term observations are not commonly performed and until now never—in Polish population. The aim of this study was to evaluate the survival of PNS patients and its association with diagnosed malignancy as well as with onconeural antibodies (ONAs). **MATERIALS AND METHODS:** The study included 177 NPS patients originating from Western Poland. In sera of all subjects, indirect fluorescence (EUROIMMUN, Germany) was performed as a screening and Western blotting (EUROIMMUN) as a confirmation test for the presence of ONA. The diagnosis of PNS was based on Graus' criteria. Five years after estimation of onconeural antibodies, the follow-up was performed in 57 patients after obtaining informed consent. Data for follow-up originated from medical records, personal (out-patients clinic) or telephone contact. **RESULTS:** A 5-year follow-up was performed in 57 patients, and malignancy was found in 11 cases. In 8 patients, primary tumor was diagnosed before or at the time of onset of PNS symptoms. The most common was lung cancer (32%), breast cancer (16%), ovarian cancer (16%), Hodgkin lymphoma (10.5%), and prostate cancer (10.5%). In the studied group, 16 patients had anti-Hu antibodies, 14 had anti-Ri, 14 had anti-Yo, 7 were unidentified, and 6 were seronegative. During the follow-up period, 8 patients died, 4 with diagnosed neoplasm, 6 had well-defined onconeural antibodies (3 with anti-Hu and 3 with anti-Ri). The number of patients with well-defined onconeural antibodies who survived 5-year period was higher ($n = 38$; $P < .0001$), than those without well-defined antibodies ($n = 11$). Also more patients ($P = .0192$) without diagnosed malignancy ($n = 34$) survived when compared with cancer patients ($n = 15$). **CONCLUSION:** The presence of well-defined onconeural antibodies in PNS patients is associated with better prognosis. Among well-defined onconeural antibodies, anti-Yo are predisposing to 5-year survival in Western Poland population.

P.155. NEUROLOGICAL PARANEOPLASTIC SYNDROMES AMONG WOMEN IN WESTERN POLAND: A STUDY FOCUSED ON OVARIAN TUMORS

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INTRODUCTION: The spectrum of primary malignancies in neurological paraneoplastic syndromes (NPS) patients differs among males and females. In females, gynecologic and breast cancers are most frequently diagnosed. The aim of this study was to evaluate underlying cancer in female patients with suspicion of NPS and neurological deficits or onconeural antibodies in ovarian tumor patients. **MATERIALS AND METHODS:** We included in the study 201 women from 395 patients with suspicion of NPS hospitalized in Department of Neurology in Poznan (Poland) in a time period 2002–2006. Based on Graus criteria, NPS were diagnosed in 113 females. In sera of all subjects, indirect fluorescence (EUROIMMUN, Germany) was performed as a screening and Western blotting (EUROIMMUN) as a confirmation test for the presence of onconeural antibodies. Eighty-five patients with ovarian tumors originated from subjects hospitalized between 2007 and 2009 in the Department of Gynecological Surgery in Poznan. **RESULTS:** Classical NPS were diagnosed more frequently ($P < .000001$) in patients with ovarian tumors (17%) than in subjects without clinical manifestation of malignancy (6%). However, in patients with other malignancies, it was higher (30%, $P = .0072$). Odds ratio for the presence of classical NPS in ovarian tumor patients was higher than in cases without malignancy (3.16; CI 1.10–9.053, $P = .0323$). In females with nonovarian carcinomas, odds ratio of classical NPS was higher (6.65; 1.87–23.63, $P = .0034$). Well-defined onconeural antibodies were found mainly (43%) in malignant ovarian tumors, and

anti-NMDA antibodies in teratoma patients without neurological deficit. CONCLUSIONS: Classical NPS were found both in patients with neurological deficits preceding clinical diagnosis of malignancy and in cases with ovarian tumors causing NPS. Anti-NMDA antibodies can appear in ovary teratoma patients without neurological deficit. Anti-CV2 antibodies were not found in ovarian tumors patients.

SUPPORTIVE AND PALLIATIVE CARE

P.156*. CLASSIFICATION OF HEADACHE IN PATIENTS WITH MALIGNANT GLIOMAS ACCORDING TO THE INTERNATIONAL HEADACHE SOCIETY (IHS) CRITERIA

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BACKGROUND: Approximately 50% of patients with malignant primary brain tumors suffer from headache. However, well-designed clinical studies concerning this frequent and heterogeneous neurological symptom are rare. The aim of the study was to investigate the frequency and clinical features of headache in the course of disease of patients with malignant gliomas. **METHODS:** We included 36 consecutive patients with supratentorial malignant gliomas in a prospective consecutive study. All patients were recruited from a Neurooncology outpatient unit. Using a standardized protocol, information concerning different aspects of brain tumor headache and general descriptive data were obtained. Patients were investigated at the time of diagnosis of the brain tumor, during concomitant radio/chemotherapy, and at time of tumor progression. **RESULTS:** At diagnosis, 47% of all patients reported headache. Among these, according to the IHS criteria, tension-type headache was as frequent as migraine-like headache (each 41%). Headache as the first symptom of the brain tumor was present in 39% of patients. During the concomitant treatment period, 56% of all patients reported headache. The proportion of tension-type headache increased to 70%, whereas migraine-like headache decreased to 15%. At the time of tumor progression, all patients reported tension-type headache. Diagnostic criteria for "headache attributed to increased intracranial pressure or hydrocephalus caused by neoplasm" (IHS 7.4.1) were not fulfilled at any time. At diagnosis, 76% of the patients met the IHS criteria 7.4.2 for "headache attributed directly to neoplasm." Only 10% of patients fulfilled these IHS criteria at the time of concomitant treatment, and no patient during tumor progression. **CONCLUSIONS:** This investigation reveals that according to the clinical diagnostic criteria (IHS: 7.4.1), "headache attributed to elevated intracranial pressure", and also "headache attributed directly to neoplasm" (IHS: 7.4.2), can rarely be a diagnosed in patients with malignant gliomas. We recommend a modification of the diagnostic criteria of the IHS classification system for headache in patients with malignant gliomas.

P.157*. INTRAVENOUS AND ORAL LEVETIRACETAM IN PATIENTS WITH A SUSPECTED PRIMARY BRAIN TUMOR AND SYMPTOMATIC EPILEPSY UNDERGOING NEUROSURGERY: THE HELLO STUDY

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BACKGROUND: Levetiracetam (LEV) is a newer anticonvulsant with a favorable safety profile. There are no relevant drug interactions, and an intravenous formulation is available. Therefore, LEV might be a suitable drug for the perioperative anticonvulsive therapy for patients with suspected brain tumors undergoing neurosurgery. **METHODS:** In this prospective study (NCT00571155), patients with suspected primary brain tumors and tumor-related seizures were perioperatively treated with oral and intravenous LEV up to 4 weeks before and until 4 weeks after a planned neurosurgical procedure. **RESULTS:** A total of 30 patients with brain tumor-related seizures and planned neurosurgery were included. Three patients did not undergo the planned surgery after enrolment, 2 patients were lost for follow-up. Therefore, 25 patients were fully evaluable. After initiation of therapy with LEV 100% of

the patients were seizure-free in the pre-surgery phase (3 days up to 4 weeks before surgery), 88% in the 48-hour post-surgery phase and 84% in the early follow-up phase (48 hours to 4 weeks post surgery). Treatment failure after dose escalation to 3000 mg/day occurred in 3 patients. No serious adverse events related to the treatment with LEV occurred. **CONCLUSIONS:** Our data show the feasibility and safety of oral and intravenous LEV in the perioperative treatment of tumor-related seizures. Although this was a single-arm study, the efficacy of LEV appears to be promising. Considering the side effects and interactions of other anticonvulsants, LEV seems to be a favorable choice in the perioperative treatment of brain tumor-related seizures.

P.158. INTRACTABLE HEADACHE BECAUSE OF NEOPLASTIC MENINGITIS IN TWO PATIENTS WITH GLIOBLASTOMA

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INTRODUCTION: Neoplastic meningitis in patients with malignant gliomas is rare complication in the advanced stage of the disease. Diagnosis of neoplastic meningitis can often be time consuming and misleading. From the clinical point of view, patients suffer from rapid neurological deterioration and intractable headache. In accordance with neurological signs and symptoms, the diagnosis can be established with MRI of the total neuroaxis. **CASE STUDIES:** One 37-year-old male and a 50-year-old female patient with glioblastoma received standard surgery and adjuvant radio chemotherapy. First patient developed headache 5 months after end of adjuvant treatment, and second patient during concomitant treatment. Both patients experienced severe headache, not responding to steroids or conventional analgesics. High dosages of opiates showed only little clinical efficacy. The diagnosis of neoplastic meningitis was established by means of MRI of the neuroaxis, showing typical enhancement of the meninges and contrast-enhancing bulky lesions together with neurological signs and symptoms. Because of rapid clinical decline, only supportive management was applied in both patients. Patients died shortly after the diagnosis of neoplastic meningitis. **CONCLUSIONS:** Neoplastic meningitis in patients with malignant glioma represents a fatal complication and control of its signs and symptoms are challenging. Malignant glioma patients with rapidly progressing intractable headache without showing clinical and radiological signs of increased intracranial pressure are highly suspicious for neoplastic meningitis. Only high-dose opiates may show some clinical benefit.

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL)

P.159*. LYMPHOMATOSIS CEREBRI (LC) PRESENTING WITH ORTHOSTATIC HYPOTENSION, ANOREXIA, AND PARAPERESIS

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INTRODUCTION: Primary central nervous system lymphoma (PCNSL) usually appears to be enhancing mass lesions on neuroimaging. In rare instances, PCNSL occurs as a diffuse infiltrative variant without mass lesion or blood-brain barrier compromise termed LC. In all published cases, LC presented as a rapidly progressive dementia. **RESULTS:** A 58-year-old woman presented with subacute onset paraparesis, orthostatic hypotension, and 80-pound weight loss. Her symptoms progressed over 6 months and she developed headache. Brain MRI revealed venous sinus thrombosis and nonenhancing foci of FLAIR hyperintensity in periventricular white matter (WM), subcortical WM, and pons. MRI spine revealed enhancement of lumbar nerve roots. Cerebrospinal fluid suggested malignancy. Cytology was negative despite multiple samples. Bone marrow biopsy, body CT, and body PET were unremarkable. She had subtle radiologic response, but no clinical improvement following steroids. Two months later, she developed encephalopathy, quadraparesis, and progression of the WM lesions. She developed fluctuating hypotension and expired. Systemic autopsy was unremarkable. Gross appearance of the brain and spinal cord was normal, without discrete mass. Microscopic inspection revealed diffuse infiltration of the brain by large B-cell lymphoma. **DISCUSSION:** Instead of dementia as in all prior published cases, this patient with LC presented with anorexia and orthostatic hypotension, symptoms usually associated with systemic disease. Diencephalic infiltration is a neurologic cause of anorexia. Entities such as lymphoma, leukemia, or histiocytosis should be considered in patients with weight loss and WM lesions. Rarely, orthostatic hypotension has a CNS cause. Recognition that the brainstem WM lesions

were tumor infiltration rather than chronic vascular disease may have prompted earlier diagnosis. LC has a variable presentation. A high index of suspicion is necessary to make the diagnosis. Early recognition is important since treatment can lead to prolonged survival or cure.

P.160*. PROGNOSTIC VALUE OF SERUM SOLUBLE INTERLEUKIN-2 RECEPTOR IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Soluble interleukin-2 receptor (sIL-2R) is well known as a disease marker of systemic malignant lymphomas. However, clinical significance of sIL-2R in primary central nervous system lymphoma (PCNSL) is still unclear. We investigated relationships between serum sIL-2R or other clinical values and the prognosis in patients with PCNSL. The patients were 14 men and 12 women, mean age 66.9 years, treated in our hospital from November 2002 to August 2009. Mean follow-up period was 16.8 months ranging from 1 to 86 months. For the statistical analysis, the patients were grouped by the age (cut off: 70 years), the serum level of sIL-2R (cut off: 550 IU/mL) and LDH (cut off: 220 U/mL) before the treatment, and the treatment with or without methotrexate-based chemotherapy. The serum level of sIL-2R was not affected by the age. Kaplan–Meier analysis exhibited the tendency of poor survival among the aged population ($P = .103$). Higher serum level of sIL-2R related to the poor survival ($P = .015$), whereas no significant impacts of serum LDH level and chemotherapy were observed on the prognosis. Multivariate analysis using Cox proportional hazard model showed the serum level of sIL-2R was significantly associated with the prognosis ($P = .025$). Our study suggests that the measurement of serum sIL-2R might be useful as a prognostic indicator for PCNSL patients.

NEW DEVELOPMENTS IN SURGERY

P.161. EXTENT OF RESECTION AND OVERALL SURVIVAL AFTER INTRAOPERATIVE IMAGE-GUIDED BRAIN TUMOR SURGERY

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OBJECTIVE: The use of intraoperative MRI (iMRI) has been reported to improve the extent of resection in glioma surgery, indirectly influencing survival. Yet, randomized or at least comparative studies to prove its value are lacking. With this analysis, we aim to assess the influence of iMRI guidance on the extent of resection and survival of patients with glioblastoma (GBM). **METHODS:** We analyzed data of all consecutive patients with GBM undergoing intended complete tumor resection in our department between October 2007 and September 2009. All patients had a preoperative KPS of 70 or greater. Surgeries were performed using conventional microsurgical techniques with or without iMRI guidance, employing a mobile 0.15 T device. An independent neuroradiologist, blinded for the surgical treatment modality, assessed MRI data to determine the extent of resection. It was classified as complete if no, and incomplete if any residual contrast enhancement was detected on early postoperative MRI obtained at 3 T. All patients received adjuvant treatment and were followed on a 3-monthly basis. **RESULTS:** Of the 101 patients meeting the inclusion criteria, 87 had a primary and 14 had a secondary GBM. Overall, the extent of resection was complete in 68.3% and incomplete in 31.7% of cases. Intraoperative MRI guidance was used in 28 patients. In 7 of these patients (25%), intraoperative imaging depicted residual enhancing tissue leading to further tumor resection. In the iMRI group, a complete resection was achieved in 25 patients (89.3%) compared with 43 (60.3%) in the conventional group ($P < .01$). Mean age was 55.8 years, which did not differ between the iMRI guided and conventional microsurgery group (54.9 vs 56.2 years, $P = .8$). Until March 1, 2010, 25 patients have died. Mean follow-up was 50.5 weeks. Kaplan–Meier estimates rendered a mean overall survival of 93.1 weeks. There was no statistically significant difference between patients with primary vs secondary GBMs ($P = .5$). Young age ($P < .01$) and complete resection ($P < .05$) were associated with a better outcome. **CONCLUSION:** Even in dedicated centers, intended radiologically complete tumor resections cannot always be achieved in GBM surgery. In terms of extent of resection, the use of

iMRI improves the surgical success rate compared with conventional microneurosurgical techniques. These results need further confirmation by a randomized trial.

NEW DEVELOPMENTS IN RADIOTHERAPY

P.162. DELIVERY OF WHOLE CEREBRO-SPINAL AXIS (CRANIOSPINAL) RADIOTHERAPY USING A SUPINE, INVERSE-PLANNED INTENSITY-MODULATED RADIOTHERAPY (IMRT) TECHNIQUE: CLINICAL EXPERIENCE IN 16 PATIENTS

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BACKGROUND: A new technique using inverse-planned IMRT to treat the whole CNS in a supine position was introduced into routine clinical practice in 2008. Initial technical and clinical experience is described. **METHODS:** Sixteen patients have been treated: medulloblastoma (7 patients), supratentorial PNET (5), ependymoma (3), and germ cell tumor (1). Median age was 18 years, range 8–49, 5 patients were <16. Treatment was planned using PHILIPS Pinnacle³ IMRT software and delivered in a head-first supine position. Pediatric patients required 2 isocenters (cranial-spinal junction) and adults require 3 isocenters (cranial-spinal and spinal junction). Staggered junctions were incorporated into a single inverse IMRT plan, so that one standard daily set-up was employed throughout treatment. Clinical target volume (CTV) to planning target volume (PTV) margins were 0.5 cm (cranium) and 1.5 cm (spine). Median prescribed dose was 35 Gy in 20 fractions to whole CNS, with sequential tumor boost of 20 Gy in 10 fractions. Verification was performed with cone beam (XVI) imaging daily for fractions 1–3, then weekly, using an off-line no action level (NAL) protocol. Dose delivered to the CTV was assessed via direct recalculation of the plan on the XVI images. **RESULTS:** All patients completed treatment with acceptable toxicity. The use of a single plan significantly reduced complexity of treatment set-up and delivery. The use of supine IMRT improved PTV conformity and minimized dose to OARs. Dose homogeneity was comparable with a posterior wedge pair technique and improved compared with a single posterior field. Uniformity of dose across the cranial and spinal junctions was also improved. Data quantifying these findings will be presented. Cone beam imaging allowed increased visualization and quantification of positioning including rotations. Although routine use of XVI highlighted discrepancies in the AP direction in the lumbar spine, all fields were delivered within the planning margins employed, following a systematic correction protocol. **CONCLUSIONS:** Introduction of an inverse-planned IMRT technique to deliver craniospinal radiation with a single plan in a supine position resulted in increased dose homogeneity and minimized dose to OARs, with improved workflow and reduced risk of operator error. Routine use of on-treatment XVI ensured that set-up corrections were employed only when there was a risk of CTV compromise. Scope to reduce CTV–PTV margins is being investigated.

P.163. MEDULLOBLASTOMA IN ADULTS: LONG-TERM SURVIVAL AND TOXICITY IN 47 PATIENTS TREATED WITH SUPINE WHOLE CEREBRO-AXIS (CRANIOSPINAL) IRRADIATION

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BACKGROUND: Since 1972 craniospinal irradiation (CSI) at The Christie has been delivered supine with a parallel pair of cranial fields and matching posterior wedge pair fields to the spine. This is delivered conformally to reduce the dose to the thyroid, heart, and gonads. We report the survival and late effects of all adult medulloblastoma patients treated between 1980 and 2007 with this technique. **METHODS:** Medical records of patients ≥ 16 years old treated for medulloblastoma were analyzed retrospectively. Prescribed CSI doses were 30 or 35 Gy in 20 fractions, with primary tumor boost of 20 Gy in 10 fractions. Ten-to-twenty-gray boost was given to metastases. Kaplan–Meier method was used to calculate overall survival (OS), time to relapse and relapse-free survival (RFS). **RESULTS:** Forty-seven patients were identified (19 females, 28 males). Median age was 25 (range 16–56). Twenty-two patients had MRI staging, 2 had myelograms, and 4 were metastatic at diagnosis. Surgery was complete in 8 patients, subtotal in 36, and 3 had biopsy only. Median time from surgery to RT was 33 days (range 11–107). Forty patients received 30 Gy to CSI, 5 received 35 Gy, and 2 received <30Gy. Three had concurrent vincristine only, 3

had concurrent vincristine and maintenance chemotherapy with CCNU and cisplatin. Median follow up is 12.2 years. Nineteen of 47 relapsed (6 outside the radiotherapy treatment fields). Longest interval to relapse was 10 years. Five of 19 patients who relapsed are alive and in remission. The 5-, 10-, and 20-year RFS are 61%, 52%, and 49%, and OS are 68%, 52%, and 43%, respectively. At last follow-up 27 live independently, 11 are employed, 9 are married, and 5 (3 males, 2 females) have had children post treatment. All survivors had endocrine assessments and 3 needed hormone replacement (3 growth hormone, 1 thyroxine, and 1 hydrocortisone). One patient developed epilepsy, 5 hearing impairment, and 2 second malignancies (breast, prostate). None developed meningioma, thyroid malignancies, or secondary BCCs. No cardiac events were noted. **CONCLUSION:** We report the longest follow-up data in adult medulloblastoma treated with a supine conformal radiotherapy technique. Our series is one of the largest and demonstrates survival comparable with published literature. Supine CSI is well tolerated by patients and reduces the long-term sequelae on the heart, thyroid, and gonads.

P.164. BORON NEUTRON CAPTURE THERAPY (BNCT): CLINICAL OPTIMIZATION OF UPTAKE PARAMETERS OF BORONOPHENYLALANINE (BPA)

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INTRODUCTION: MGMT shies have demonstrated that a limited number of patients with glioblastoma benefit from Temozolamide. The remainder of the patients depend on radiotherapy at a maximum radiation dosage of 60 Gy, with no safe means of dose escalation. Boron neutron capture therapy (BNCT) is a binary treatment modality which represents a biologically targeted means of radiation dose escalation targeting both cyclical and noncyclical glioma cells¹ without precluding other therapies. **METHODS:** We report on a Cancer Research UK clinical pharmacokinetic study of Boronophenylalanine (BPA) in patients with high-grade glioma to optimize uptake parameters for clinical trials of BNCT.

The goals of the study were:

- to investigate the pharmacokinetic profile of BPA in the new mannitol-based formulation;
- to evaluate the toxicity profile of BPA-mannitol; and
- to optimize the dose and uptake parameters for BPA-mannitol for use in future clinical trials of BNCT by integrating the tumor-handling data based on LAT-1 distribution and activity into the final pharmacokinetic model for clinical studies.

The study investigates the route of infusion and, in each case, will assess the effect of administration of mannitol as a blood-brain barrier disrupter.

Measurements were made by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) of 10B concentration in blood, urine, extra-cellular fluid (via brain microdialysis), brain tissue around tumor and tumor tissue. Additional analysis was performed using Secondary Ion Mass Spectrometry (SIMS). **RESULTS:** Peak Boron (10B) levels in blood were in keeping with previously published data but were significantly enhanced by the addition of mannitol. Tumor concentrations were variable, reflecting the heterogeneity of glioblastoma. Peak concentrations were not achieved in some patients until as late 6 hours after infusion, later than previously shown. This peak concentration correlated with concentrations in extracellular fluid. Administration via the intra-arterial route enhanced the tumor concentration, peaking 2 hours after blood BPA levels. **CONCLUSIONS:** Previous clinical studies into BNCT for glioblastoma have instituted early irradiation at 1hr after the end of BPA infusion.² Our study shows delayed peak boron levels in brain and ECF suggesting that the optimal window for delivery of the radiation dose may be approximately 4 hours after infusion. Escalation of tumor boron dose without additional dose to normal brain is possible and likely to further facilitate therapeutic response.

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P.165. A COMPARISON OF VOLUMETRIC MODULATED ARC THERAPY (RAPIDARC) AND CONVENTIONAL EXTERNAL BEAM RADIOTHERAPY IN TEMPORAL HIGH-GRADE GLIOMAS

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INTRODUCTION: Patients treated for high-grade gliomas in the temporal region with external beam radiotherapy are at risk of significant cognitive deficits, including short-term memory loss which has a significant impact on daily functioning and quality of life. This is thought to be related to dose received by the hippocampi. **Objectives:** A feasibility study was done using RapidArc™ (Varian medical systems), a volumetric arc-based intensity-modulated radiotherapy technique (IMRT) to treat high-grade gliomas in the temporal region and to establish if there was a dosimetric advantage of RapidArc compared with conventional radiotherapy with particular reference to dose to the hippocampi. **METHODS:** Ten patients previously treated with conventional 3D conformal radiotherapy for high-grade gliomas in the temporal lobe region were replanned using Varian RapidArc. Target volumes, organs at risk (OAR), and dose constraints defined for conventional planning were unchanged, but in addition hippocampi, inner ear, and temporal lobes were outlined using MRI fusion. No additional dose constraint was added for hippocampi. Comparisons of doses to the PTV and organs at risk including hippocampi were then made. **RESULTS:** The conformality index was much improved with RapidArc (typically 1.5 with conventional and close to 1 with RapidArc). Conformality was further increased by the use of more than 1 arc. Maximum doses to nearby critical structures such as optic nerves and optic chiasm were reduced with the use of RapidArc. There was a reduction in the volume of normal brain receiving a high dose. As these gliomas were within the temporal lobe, the ipsilateral hippocampus was within the PTV, so dose could not be reduced. Dose to the contralateral hippocampus varied from patient to patient. In some the contralateral hippocampus, dose was larger because of the increased volume receiving low doses with RapidArc. **CONCLUSION:** RapidArc is effective at reducing dose to OAR near to the PTV, improving conformality and reducing the volume of normal brain receiving a high dose of radiation in patients with temporal lobe gliomas. The use of RapidArc to improve conformality does not lead to hippocampal sparing per se. If low dose to the hippocampi is thought to be relevant to long-term cognitive function, these organs need to be regarded as dose-limiting structures.

MISCELLANEOUS

P.166. AWAKE SURGERY DURING RESECTION OF INSULAR GLIOMAS

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PURPOSE OF THE STUDY: Insular gliomas are by many still considered inoperable, because of anatomical localization, vascular supply, and the potential devastating complications. We present our experience with the operative treatment of patients with insular gliomas in an “awake craniotomy” setting. **PATIENTS AND METHODS:** Nineteen consecutive patients with an insular tumor were operated awake during the period 2003–2009. Pre-operatively, an extensive neuropsychologic/linguistic workup was performed. All patients underwent MRI with neuronavigation and when possible fMRI. After cortical stimulation, the Sylvian fissure and periinsular sulci were opened. Tumor resection was performed under speech and motor surveillance. **RESULTS:** The patients' average age was 41.4 ± 10.3 years. Pure insular lesions were seen in 2 patients, a medial temporal base-insular glioma in 1, insular fronto-opercular and orbitofrontal-insular-temporal polar in 6 and 10 patients, respectively. Presenting symptoms included epilepsy (95%), dysphasia (26%), and cognitive problems (26%). In 13 patients, the resection was near total (95–98%) and <95% in the remaining 6 patients. Histology confirmed 16 low-grade and 3 high-grade gliomas. The average follow-up was 2.1 ± 1.5 years. Perioperatively 9 patients clinically deteriorated. However, all patients with a low-grade glioma recovered to preoperative status. Two patients with a high-grade glioma have died during follow-up. **CONCLUSION:** Insular glioma surgery, facilitated by (sub)cortical stimulation in an awake setting, is feasible to acquire maximal cytoreduction in a safe manner. A dedicated surgical team is required, next to neurosurgeon, anesthesiologist, and patient interaction.

P.167. RADIATION-INDUCED OSTEOSARCOMAS AFTER TREATMENT FOR FRONTAL GLIOMAS: A REPORT OF 2 CASES
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Most reported radiation-induced osteosarcomas arise from the facial bone or paranasal sinus after radiotherapy for retinoblastoma and/or pituitary adenoma. We report 2 radiation-induced osteosarcoma cases occurring in the paranasal sinus after treatment for frontal glioma. **CASE 1:** A 56-year-old female underwent surgical resection of a left frontal tumor 16 years earlier in October 1990. The histological diagnosis was a low-grade glioma. Radiotherapy of 54 Gy was administered. In September 2006, the patient noted an enlarging subcutaneous mass in the right frontal region. CT showed an osteolytic mass in the right frontal sinus. An open biopsy was performed and a histological diagnosis of radiation-induced osteosarcoma was made, but the patient subsequently died of rapid tumor re-growth. **CASE 2:** A 58-year-old male underwent partial removal of a bifrontal tumor in May 1996. The histological diagnosis was anaplastic oligoastrocytoma. Radiotherapy of 56 Gy was administered. The patient was subsequently readmitted in March 2008 because of a marked deterioration in general health. As tumor recurrence was suspected in the left frontal lobe and a CT demonstrated an osteolytic mass in the left frontal and ethmoid sinus, a secondary operation was performed and the histological diagnosis was radiation-induced osteosarcoma. Radiotherapy was re-administered, but the subject died of rapid tumor re-growth. Radiation-induced osteosarcomas appeared 16 years after radiotherapy in Case 1, and 12 years after radiotherapy in Case 2. Given that the prognosis of radiation-induced osteosarcoma is poorer than that of primary osteosarcoma, careful attention is needed in considering the long-term survival of patients with glioma.

P.168. CEREBRAL VENOUS SINUS THROMBOSIS IN A PATIENT WITH METASTATIC GERM CELL TUMOR

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INTRODUCTION: Cerebral (venous) sinus thrombosis (CVST) in cancer patients is a rare complication, accurately diagnosed by MRI and MR venography (MRV). It has multiple etiologic factors with variable symptoms and signs at presentation and often with unpredictable outcome. We represent a young patient with metastatic germ cell tumor and a complication of CVST with good outcome. **CASE REPORT:** A 27-year-old male patient with primary retroperitoneal nonseminomatous germ cell tumor and metastases in the mediastinal and left scl lymph nodes and bone (L3, direct extension from retroperitoneal mass) was admitted for initial chemotherapy (CTh). A week after the completed first cycle of CTh according to BEP (bleomycin, etoposide, cisplatin) regimen, he returned because of repeated focal epileptic seizures progressing to epileptic status and left-sided hemiparesis. On admission, the patient had afebrile neutropenia, without clinical or laboratory signs of infection. During diagnostic procedures, urgent CT of the head disclosed no abnormalities, while MRI revealed a cortical thickening of both parietal and right frontal regions without any contrast enhancement or signs of expansion. Signs of CVST and cortical venous thrombosis were found retrospective on CT and MR images. EEG showed diffuse slowing down of background activity and focal slow-wave activity over the right frontal region. EEG findings were compatible with the signs of diffuse encephalopathy or encephalitis accentuated over the right frontal region. Diagnostic tests for excluding other causes of the condition, such as progression of malignant disease, metabolic, toxic, infectious and immune causes, were performed. After a few days, repeated MRI with flair, DW MRI, spectroscopy, and MRV disclosed focal changes in the fronto-parietal regions with surrounding edema containing white matter. MRI findings were compatible with the signs of venous sinus thrombosis of the right transversal sinus and partial thrombosis of the superior sagittal sinus with ischemic and already partly hemorrhagic cortical infarcts. After symptomatic treatment with antiepileptics and low-molecular-weight heparin, the patient slowly improved, and so did MRI findings. He continued with initial cisplatin-based CTh. After complete regression of mediastinal and scl metastases, the residual retroperitoneal mass was excised. No vital malignant cells were found. He received postoperative irradiation (L3) and is in complete remission for more than half a year. **CONCLUSION:** The complication of CVST in the presented patient was probably related to cancer and CTh with hypercoagulability and dehydration. Although this complication is rare, we should be aware of it. According to published data, the diagnosis of CVST can be quick and accurate by MRI and MRV, which can indicate how to treat the underlying cause, thereby giving better chances for a good outcome.

P.169. THE USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINES (CAM) IN BRAIN TUMOR PATIENTS

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INTRODUCTION: Complementary and alternative medicines (CAM) are known to be highly useful among cancer patients, but there is very little literature specifically addressing their use in brain tumor patients and the incidence of CAM use in this group is unknown. We designed a questionnaire study to assess the use of CAM in brain tumor patients treated at University College Hospital, London between April and September 2009. **METHODS:** Questionnaires were distributed to patients in neuro-oncology outpatient clinics, the radiotherapy department, and the chemotherapy suite. Patients were asked to fill out the questionnaire anonymously and return it in a prepaid envelope. The questionnaire included demographic information and diagnostic categories. Patients were asked to record the use of CAM by selecting treatments from a predefined list and give information about how they accessed and funded this. **RESULTS:** Thirty-three questionnaires were analyzed. Diagnoses included meningioma, high- and low-grade glioma, germinoma, and posterior PNET. Fifty-five percent of patients questioned reported the use of CAM. There was almost no gender difference with a higher than previously reported use in men. The majority of patients (79%) were in the younger age group (21–40 years). In addition, there was a positive correlation between CAM use and higher educational level. There was an association between the severity of the diagnoses and the use of CAM, with a particularly high use in the GBM group (70%). A range of different CAM were used, the most common being vitamins, reiki, reflexology, and acupuncture. Sixty-four percent of patients reported the use of CAM during treatment with either radiotherapy or chemotherapy. Only 22% of patients disclosed CAM use to the treating oncologist. Most patients learnt about CAM from family and friends, and funded themselves for treatments. Seven patients spent considerable amounts of money on CAM, 3 exceeding a thousand pounds. **CONCLUSION:** A very high incidence CAM use was reported in brain tumor patients, including males, which suggests a different pattern of use than has been documented in other cancer patients. A minority disclosed CAM use to the treating team. There have been reports of negative interactions between some CAM and radiotherapy and chemotherapy. This highlights the need to specifically question brain tumor patients about CAM use and to be able to advise patients on potential interactions.

P.170. THE ROLE OF A SPECIALIST THERAPEUTIC RADIOGRAPHER WITHIN THE MULTI-PROFESSIONAL RADIOLOGY TEAM

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The role of Clinical Nurse Specialists (CNS) is well established in Neuro-Oncology teams, given that radiotherapy remains central to the management of most brain tumors, the knowledge and skills incumbent in a radiographer's role place them in an ideal position to manage many of the radio-therapeutic aspects of care. However, paradoxically, there are few specialist radiographers in this discipline. At the Beatson West of Scotland Cancer Centre, we examined the patient treatment pathway and key elements were identified where the input of a dedicated Radiographer was felt liable to improve and optimize the delivery of care. Consequently, the role of a Neuro-Oncology Specialist Radiographer (Sp Rad) was established in November 2007. Elements were prioritized for introduction and protocols created incorporating ongoing assessment of competencies. The fundamental aspects of the Sp Rad role were identified early and quickly established: patient education regarding the process and delivery of radiotherapy; on-treatment assessment and management of toxicity; treatment verification with portal image review after training in anatomy recognition; and managing setup and immobilization issues for individual patients. More specialized tasks were gradually introduced, including identification and voluming of initially OAR's then tumor volumes on the radiotherapy planning system (with subsequent checking by the neuro-oncology consultant); also a protocol was developed establishing CT-MR fusion for all Glioma patients receiving radical radiotherapy. The Sp Rad played a pivotal role in the development and implementation of the stereotaxy service and delivery of IMRT for selected glioma patients. The Sp Rad was instrumental in the drafting of clinical protocols (compliant with IR(ME)R regulations) and related quality documentation for both of these technologies. The Sp Rad was also responsible for delivering a training package for radiographers and assessment of competence. The Sp Rad is currently the lead coordinator for radiosurgery services, liaising with the various diagnostic (MR) and therapeutic departments (simulation, planning, therapy delivery) as well as the patient to ensure rapid and efficient treatment delivery. The Sp Rad has been involved in other areas of service development, in particular creating a Pre-Treatment Assessment Clinic (PTAC), promoting cross boundary working with the CNS. The PTAC addresses the increasing complexity of

multi-modality therapy facing most glioma patients, including issues of patient and carer education, psychological adaptation, symptom control, the ongoing appropriateness of the therapeutic strategy, detailed planning and preparation for treatment, and obtaining informed consent. Future objectives include forging closer ties with the physics department to develop stereotactic IMRT, and supine craniospinal therapy delivery.

P.171. CRANIAL BASE PARAGANGLIOMAS: GAMMA-KNIFE RADIOSURGERY

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INTRODUCTION: Paragangliomas are highly vascular neuroendocrine tumors, usually benign and well encapsulated. In their cranial base location, microsurgery is associated to high morbidity (50%–80%) especially in relation to low cranial nerve damage. Gamma-knife treatment is emerging as a definitive therapeutic option in the treatment of these lesions. **MATERIALS AND METHODS:** We present a series of 57 patients bearing cranial base paragangliomas treated with Gamma-knife radiosurgery from February 1995 to January 2010. Forty-seven patients with a follow-up exceeding 2 years are analyzed in detail. There were 15 males and 32 females, with a mean age of 53.7 years (range 19.9–82.3). In 31 cases, there was a neuroimaging diagnosis exclusively, the other 16 had been operated on and had a pathologically confirmed diagnosis. In the surgical group, 3 patients had their lesions previously embolized, and 2 had received fractionated radiotherapy while in the nonsurgical group 5 had received endovascular treatment, and 1 had fractionated radiotherapy. At the time of treatment, 6% of the operated patients were asymptomatic, and 81% and 94% had low cranial nerve and VIII cranial nerve deficits, respectively. In the nonsurgical group, 3% were asymptomatic, with low cranial nerve involvement in 74%, and 71% and 23% with VIII and V, VI, or VII cranial nerve deficits, respectively. The mean tumor volume was 14.2 cm³ (1.4–62.2 cm³), with a mean marginal dose of 13.6 Gy (12–15 Gy) and maximal doses between 20 and 55 Gy. **RESULTS:** Mean follow-up was 65.5 months (12–175). Volumetric control was obtained in 93.6% (reduction in 68.1% and stabilization in 25.5%). Tumors progressed in three cases (6.4%). The volumetric reduction ranged from 0.75 (5%) to 15.55 cm³ (60%) (mean 5.6 cm³, median 3.4 cm³). No clinical complications were observed. **CONCLUSIONS:** Gamma-knife radiosurgery is an effective, safe, and efficient therapeutic option in the treatment of these tumors, as a first line treatment or associated to surgery, endovascular treatment, and/or conventional fractionated radiotherapy.

P.172. GAMMA KNIFE RADIOSURGERY IN NEUROFIBROMATOSIS TYPE 2 (NF2) PATIENTS

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INTRODUCTION: NF2 is an autosomal-dominant genetic disease with an incidence of 1 in 50,000 births and a prevalence of 1 case in 150,000 inhabitants. It is characterized by the simultaneous or consecutive development of intracranial or spinal meningiomas or schwannomas. The presence of bilateral VIII cranial nerve schwannomas is a main feature, with high surgical risks of cranial nerve deficits. **OBJECTIVE:** Analysis of our results of Gamma Knife Radiosurgery in this group of patients. **METHODS:** Between February 2004 and January 2008, 70 treatments in 33 NF2 patients have been performed, 13 patients were treated in more than one occasion (1–4 treatments, mean 1.6). Seventy-eight percent of patients have a complete follow-up. Two-thirds were females. The mean age was 36.5 (12–79). Forty patients had been previously operated (mean surgical procedures: 1.8, range: 1–4) and 22 had received previous radiotherapy. The mean number of treated lesions in one procedure was 3.9 (1–18), with a mean marginal dose of 12.7 Gy and a mean treated volume of 10.4 cm³. There was a known family history for only one-third of patients. **RESULTS:** The mean follow up time was 4 years (5–188 months), with 20% of patients followed for more than 5 years. The local volumetric control was obtained in 72.8% of cases with reduction in 31%. One hundred and forty-nine meningiomas and 62 schwannomas were treated. In 15 cases, the treated lesions grew (12 schwannomas and 3 meningiomas). In 39 cases, new tumors appeared during follow-up. From a clinical point of view, 28 patients remain stable, in 5 their symptoms improved and, in those where there was a clinical deterioration, hearing worsened in 11 cases. Three patients died as a result of progression of NF2. **CONCLUSIONS:** Gamma Knife

radiosurgery is an effective option to surgery in the treatment of NF2 patients, because of the minimal peripheral irradiation of healthy tissues, especially in meningiomas of patients previously operated on or with contraindications for new surgical procedures. Having in mind the genetic condition of this disease, where the potential oncogenic effect of radiotherapy should be taken into account, any therapeutic decision must be evaluated individually. This treatment must be used in those patients with lesions with evident growth or with progressive symptoms, when surgery is not a safe option in an NF2 experienced neurosurgical unit.

P.173. EXPLORING A NEW THERAPY FOR NEUROBLASTOMA: SILENCING OF DOUBLECORTIN-LIKE KINASE USING RNA-INTERFERENCE

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Neuroblastoma is one of the most common childhood cancers. Microtubule-destabilizing agents are used in the treatment of these tumors. However, resistance to chemotherapeutic agents and systemic toxicity make neuroblastoma a difficult drug target. In our previous work, we found that doublecortin-like kinase (DCLK) gene transcripts are crucial microtubule-associated proteins for correct proliferation and differentiation of neuroprogenitor cells. Gene expression profiling revealed a high expression of these transcripts in neuroblastomas and also in gliomas. Furthermore, these transcripts are endogenously expressed specifically in neuroblasts, but are not found in other cell types. Suppression of DCLK by short-interfering RNA (siRNA) disrupted the mitotic spindles in neuroblastoma cells and gene expression profiling revealed numerous differentially expressed genes indicating apoptosis. Apoptotic cell death of neuroblastoma cells by DCLK knockdown was further confirmed by several assays. Interestingly, mitochondria were the most affected cell components after DCLK-long knockdown. We also found in human neuroblastomas a significant correlation between DCLK expression and genes related to mitochondria activity. Furthermore, we showed a successful delivery of siRNA-targeting DCLK to neuroblastoma cells by using specific peptide-siRNA conjugates. In conclusion, silencing of the DCLK gene by siRNA interference is a novel potential therapeutic approach for neuroblastoma with the promise of combining high specificity with fewer side effects. Peptide-siRNA conjugates might be the tool needed for specific neuroblastoma delivery.

P.174. USE OF SHORT BATTERY FOR COGNITIVE, ANXIETY, DEPRESSION, AND QUALITY OF LIFE EVALUATION (BATCOG) IN PATIENTS WITH GLIOMAS: A FEASIBILITY STUDY

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INTRODUCTION: Cognitive difficulties (CDs) are very common in patients with gliomas, and their origin is multifactorial: tumor, surgery, radiotherapy, chemotherapy, antiepileptic drugs, steroids, and anxiety and depression are commonly described factors. However, the prevalence of CD is difficult to estimate. Discrepancies among studies are frequently explained by methodological differences. Performance status scales (KPS, ECOG) and short screening tests for CD (MMSE, MDRS) have a low sensitivity to detect CD in patients with gliomas, particularly in those with mild impairment and/or high pre-morbid function. A better approach is to use a battery of tests directed to evaluate the cognitive domains more frequently impaired in these patients. **METHODS:** Patients with primary brain tumors and CD were recruited from the Neuro-oncology Clinic. All subjects were evaluated with a selected battery of tests that examine the cognitive domains more frequently affected by cancer and its treatment (attention, memory, and executive function); similar batteries have shown usefulness to evaluate cognitive function in patients with gliomas. Tests are standardized for Spanish population. A screening test for anxiety and depression and a quality of life tool were also included. The battery comprises: Rey Complex Figure Test, Word list (WMS-III), Digit-Span Test, Symbol Digit Modalities Test, Trail Making Test A&B, FAS, STROOP, HADS, and EORTC QLQ-C30. **RESULTS:** A total of 7 patients were evaluated up to now. Median age was 43.5 years (28–68); 2 were men and 5 were women; all patients had at least primary studies. Tumor diagnosis was grade III glioma (2), grade II glioma (3), grade I glioma (1), and meningioma (1). Test results show more deficits in delayed

recall, working memory, mental flexibility, and verbal fluency compared with normalized population, in the absence of anxiety or depression. Completion time test was ~1 hour. Recruitment of further patients and a control group matched by age, sex, and level of education is currently ongoing. CONCLUSIONS: The introduction of a battery of cognitive tests for cognitive and quality of life evaluation was feasible and well accepted by patients. This experience will serve as a basis for future development of cross-sectional and longitudinal studies of cognitive function and quality of life in patients with gliomas in our environment. There is a need for specialized neuropsychological assessment in neuro-oncology patients.

P.175. A LITERATURE REVIEW OF FIBRO-OSSEOUS PSEUDOTUMOR: THE ROLE OF SURGICAL EXCISION

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BACKGROUND: Fibro-osseous lesions are a rare clinical entity. We present the case of a 65-year-old female with infrequent partial seizures. Electroencephalography revealed Grade I dysrhythmia of the right hemisphere and an MRI revealed a calcified lesion in the right precentral frontal lobe. The patient underwent surgical resection and histopathology revealed a fibro-osseous pseudo-tumor. No further therapy was required and the patient is symptom-free 1-year post-operatively. **METHODS:** A review of all existing cases of fibro-osseous lesions was undertaken. The clinical and radiographic characteristics, histopathology, management and outcome of each case were evaluated. The suspected pathophysiology of these lesions was reviewed. **RESULTS:** Only 44 cases (28 intracranial, 16 spinal) have been reported to date. The majority occur in adults over 40, with a slight male predominance. Neurological symptoms result from local mass effect; however, seizures are also frequently observed. Imaging demonstrates central calcification with variable contrast enhancement. Histopathologically, a variably calcified chondromyxoid matrix with surrounding gliosis and without mitoses or cellular atypia is characteristic, suggesting a reactive origin to the lesion. In all reported cases, surgical excision is curative with only one reported case of recurrence. No further treatment is required, although routine surveillance may be of benefit. **CONCLUSION:** Although uncommon, awareness of this entity and its inclusion in the differential diagnosis of long-standing calcified lesions along the craniospinal axis has practical importance in preventing unnecessarily aggressive investigation and treatment.

P.176. INVESTIGATION ON THE INCIDENCE, PREVALENCE AND MORTALITY RATE IN FIVE CITIES/DISTRICTS IN EASTERN AND NORTHERN CHINA

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PURPOSE: To provide accurate, population-based incidence rate, prevalence rate, and mortality rate of primary brain tumor in the Chinese population. **METHODS:** We investigated 5 big communities in China. They were Bao-shan district of Shanghai city, Long-nan district of Daqing city, Ma'an-shan city, Shi-yan city, Pu-yang city. The incidence, prevalence, and mortality rates from October 1, 2005 to September 30, 2006 were measured. **RESULTS:** The incidence rate, prevalence rate, and mortality rate of primary brain tumors in the investigated areas were 10.5/100,000, 24.5/100,000, and 4.3/100,000 respectively. Among males, these rates were 8.3/100,000, 20.3/100,000, and 3.6/100,000 respectively, and for females, 12.8/100,000, 29.0/100,000, and 5.1/100,000. The incidence rate for glioma was 3.13/100,000. **CONCLUSIONS:** This is the first report on the incidence rate and prevalence rate of primary brain tumors in China. Further study on the different histological subtypes and the etiology and risk factors of brain tumors are warranted.

P.177. POTENTIATING EFFECTS OF PARP INHIBITION ON CHEMO- AND RADIOTHERAPY TREATMENT IN SERUM-FREE GLIOMA CULTURES

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INTRODUCTION: The dismal prognosis of patients diagnosed with glioblastoma multiforme (GBM) urges researchers to investigate new treatment modalities for targeted drug regimens. Recent reports on glioma tissue cultured under serum-free (SF) conditions show that glioma stem cells (GSC) are preferably selected, and that these cultures preserve a good resemblance to the original tumor on DNA and RNA level. Several publications have illustrated GSC's to be more resistant to chemo- and/or radiation therapy. This led us to develop a high throughput model for screening promising combination therapies. By isolating GSC's from freshly isolated high-grade glioma (HGG) samples, we were able to test the effect of poly(ADP-ribose) (PARP) inhibitor ABT-888 in combination with temozolomide (TMZ) and radiotherapy (RT). **METHODS:** Freshly isolated HGG samples were cultured under SF conditions as neurospheres. SNP analysis of both low (p1-p4) and higher passages (p7-p11) illustrated the genetic stability and resemblance to the parental tumor tissue. The samples that were successfully expanded were tested in several cytotoxicity studies. Cells were seeded in monolayer on 96-well plates coated with extracellular matrix. Treatment consisted of TMZ monotherapy (50 and 100 µM) or RT (3 and 6 Gy). The combined effect with ABT-888 was tested with aforementioned dosing of TMZ or RT, combined with 2.5 or 10 µM of ABT-888. Read out of therapeutic effect was assessed on Day 5 and 8 by performing the Cell Titer GLO assay (Promega) in triplicate. We validated the data by parallel testing of TMZ resistant (T98) and sensitive (U373) glioma cell lines. MGMT expression was investigated by Western blotting (WB) of the cell cultures. **RESULTS:** We tested 9 SF cultured primary GSC cultures for TMZ or RT + ABT-888 combination therapy. Of these samples, the clinical histological diagnosis was: GBM (n = 6) and anaplastic OD (n = 3). ABT-888 did not sort out any effect as a single agent. TMZ resistance at 100 µM dosing was found in 7 out of 9 cell cultures (<25% decrease in viability). Of these 7 samples, we found a potentiating effect (>25-75% decrease in viability) of ABT-888 addition in 6 cultures at a 2.5 µM ABT-888 (n = 1) or 10 µM ABT-888 (n = 5). We observed no detectable MGMT expression in TMZ sensitive cultures on WB. TMZ-resistant cultures expressed MGMT in 4 of 7 cases. ABT-888 reversal of TMZ resistance appeared in both MGMT-positive as well as -negative cultures. For RT, we found resistance at 6Gy in 2 out of 9 cell cultures. Both cell cultures did not show any potentiating effect of ABT-888. **CONCLUSIONS:** We were able to culture and expand GSC's from HGG samples. These cultures were found to genetically resemble the parental tumor tissue. We found that TMZ-resistant cultures could be sensitized by adding ABT-888 to the medium. The RT resistance could not be reversed. On the basis of these findings we are further elucidating the synergy of alkylating agents in combination of PARP inhibitors.

P.178. DEVELOPMENT OF A DRUG SCREENING ASSAY BASED ON PATIENT-DERIVED GLIOBLASTOMA CELL CULTURES WITH GENOTYPIC RESEMBLANCE TO THE PARENTAL TUMOR

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INTRODUCTION: The culturing of cells that mimic the molecular and cellular aspects of gliomas is essential for the development of more reliable models for testing new drugs. We set up a protocol to efficiently grow low passage serum-free (SF) and serum-supplemented (SS) cell cultures from patient tumor material. We tested various coatings to allow growth of serum-free cultures in monolayers instead of neurospheres. The genotypic profiles of both SF and SS cell cultures were compared with the parental tumor. **METHODS:** Tumor tissue was enzymatically dissociated and split at equal concentration into either SF or SS conditions. SS cultured cells were split at 80%-90% confluence. SF cultured cells were grown as neurospheres (NS). NS cultures were dissociated with accutase and reseeded as NS or as monolayer cultures on various extracellular matrix (ECM) coatings. Expansion was scored as successful when cultures reached up to 5 passages, with expansion sufficient for pellet harvesting and low passage drug screening assays from p4 onward. DNA was isolated from snap frozen tumor sample or cell

pellets. For 3 individual patient series, we analyzed for copy number aberrations (CNAs) on Affymetrix SNP 6.0 arrays. RESULTS: In 12 months, a total of 59 glioma samples were collected; of which, 31 (52%) were propagated successfully. The success rate of SS cultures was solely dependent on the tumor size whereas the success rate in SF cultures was dependent on both sample size and initial amount of NS formation. SF tumor neurosphere cultures could be successfully transferred to monolayers in 96-well plates by seeding the cells on growth factor-reduced ECM coating, thereby attaining a model for drug screening. Successfully propagated tumors had similar genetic aberrations as the primary tumor. Genetic aberrations include high copy amplification of Chr.7p11 (EGFR) and loss of Chr. 9p (CDKN2A) and Chr10, all of which are common genetic aberrations in gliomas. Some CNA became more apparent in SF cultures through selective clonal expansion. Importantly, SS cultures showed a gradual loss of CNAs in higher passages. CONCLUSIONS: We developed an efficient protocol for SS and SF culture derivation of surgically removed tissue. Using growth-factor reduced ECM coating, we are able to culture monolayers of GBM cells under SF conditions, which allows high throughput screening of patient-derived tumor cells with genetic profiles resembling the parental tumor up to high passages. However, the lower success rate of obtaining viable SF cultures remains a disadvantage. Moreover, we have determined the genetic aberrations of SS cultured material to be similar to tumor tissue in low passages (up to p4). This is, for practical and financial reasons, an attractive option next to SF cultures.

P.179. IDENTIFICATION OF MAGNETIC RESONANCE IMAGING SURROGATES FOR GLIOMA GENE-EXPRESSION MODULES

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OBJECTIVE: To combine magnetic resonance imaging with microRNA detection and analysis to create a multidimensional map of gene-expression patterns in glioblastoma multiforme (GBM) that provided clinically relevant insights into tumor biology. **METHODS:** Eighty cases of GBM were studied. Tumor contrast enhancement and mass effect predicted activation of specific hypoxia and proliferation gene-expression programs, respectively. In total, 1147 miRNA were detected and analyzed. **RESULTS:** The relationship between MRI phenotype and the respective gene-expression profiles were identified. MiR-21, MiR-181,

and MiR-221 were found related to tumor infiltrative. **CONCLUSION:** An in vivo portrait of genome-wide gene expression in GBM offer a potential strategy for noninvasively selected patients who may be candidates for individualized therapies.

P.180. A NOTE-BASED STUDY OF HOW PATIENTS FIRST PRESENT WITH PRIMARY BRAIN TUMORS

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INTRODUCTION AND OBJECTIVES: GP guidance on the presenting symptoms of brain tumors is based on data sourced from tertiary care centers. As GPs will see, on average, only 4 patients with a new primary brain tumor during their career, they are reliant on these guidelines rather than experience. Small selective studies by Salander and Davis on early symptoms show a difference between reported initial symptoms and the symptoms at confirmed diagnosis. By collecting data on the presenting symptoms of an unselected, prospectively defined population of patients with incident brain tumors we aim to refine the guidance. **METHODS:** From May 2009, data were collected weekly from a neuro-oncology MDT meeting. The MDT supported a population base of ~3 million people (includes the following PCTs and district hospitals: Wandsworth; Frimley Park; East Surrey; Sutton and Merton; Kingston; Richmond and Twickenham; Croydon). Information on the presenting complaint was recorded for those patients meeting the inclusion criteria. The patient inclusion criteria were the first occurrence of a subsequently confirmed adult primary brain tumor (gliomas and meningiomas) whether benign or malignant. The exclusion criteria: metastatic brain tumors; spinal cord masses; pituitary tumors; peripheral nerve tumors; those with a previous history of brain tumors; pediatric cases (age <16). **RESULTS:** Data were collected for 137 patients. Cognitive loss was the most frequently reported symptom (39%). The red flag symptoms of headache and seizures were reported by 28% and 18% of patients, respectively. Of the 39 patients who reported headache symptoms, 4 of these patients reported headache as their only symptom. **CONCLUSION:** This study shows that the current beliefs about "red flag" symptoms (headaches and seizures) for brain tumors in the primary care setting, which are based on tertiary care data, may not be the most commonly reported symptoms in the community. The preliminary data show that cognitive loss and weakness are more frequently reported, and that headache alone does not seem to predict diagnosis of primary brain tumor.