ABSTRACTS

IL01

STEM CELLS AND CANCER STEM CELLS

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The quiescent state is thought to be a characteristic property for the maintenance of hematopoietic stem cells (HSCs). Interaction of HSCs with their particular microenvironments, known as the stem cell niches, is critical for adult hematopoiesis in the bone marrow (BM). We demonstrate that quiescent HSCs adhere to osteoblasts (OBs) in the niche through the Tie2/Angiopoietin-1 and/or mpl/thrombopoietin. Since Ang-1 enhanced the expression of N-cadherin in HSCs and induced the cell. adhesion to bone, we examined the role of N-cadherin in vivo. When HSC cells transfected with dominant negative N-cadherin, which lacks extracellular domain, HSC cells can reach bone marrow but not enter the osteoblastic niche, resulting in the defect in hematopoietic reconstitution.

When the BM is ablated during BM transplantation or after treatment with myelosuppressive agents, the quiescent HSCs enter the cell cycle and proliferate to supply progenitors of committed hematopoietic cells. We found that reactive oxygen species (ROS) induce the exit of HSCs from the niche after 5-FU injection through down-regulation of N-cadherin. Similarly, ROS was elevated after serial BM transplantation, and up-regulation of MAPK p38 and INK4A was detected only in HSCs. *In vivo* treatment of recipients with anti-oxidant or p38 MAPK inhibitor restored exhaustion of HSCs in the repeated BM transplantation.

We are now studying the cellular metabolic changes of HSCs. I will show that the metabolism of quiescent stem cells is glycolysis-dependent in the hypoxic niche, while that of proliferating progenitors are mitochondria-dependent, using HIF-1α/VHL-defficient mice.

Moreover, I want to discuss the therapeutic strategy targeting the niche of cancer cells.

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IL02

GLIOLAN IN THE THERAPY OF MALIGNANT GLIOMAS: QUO VADIS?

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Gliolan (ALA) has been developed and approved in Europe for intra-operative identification of malignant gliomas by inducing fluorescent porphyrins in these tumors. Data from the pivotal approval study has consequently increased the understanding for the principle value of cytoreductive surgery in malignant gliomas. Since then, novel data has been collected which underlines the value of cytoreductive surgery in the context of recently approved adjuvant therapies such as BCNU-wafers or concomitant radiochemotherapy with temozolomide. On the other hand, ALA-derived porphyrins are potent photosensitizers with efficacy for photodynamic therapy and demonstrate preliminary efficacy for stereotactic phototherapy of malignant gliomas. Preliminary experimental evidence indicates immnostimulation to play a role in mediating this efficacy. Finally, ALA-induced tumor porphyrins may be of value in other tumors relevant to neurosurgeons, such as pituitary adenomas, medulloblastoms, meningiomas or spinal chord tumors.

IL03

ENGINEERING T CELLS FOR ADOPTIVE THERAPY

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Adoptive cell transfer immunotherapy is based on the infusion of tumor reactive T cells into patients with cancer. This approach has multiple advantages compared to other forms of immunotherapy. It is possible to administer large numbers of highly selected cells with high avidity for tumor specific antigens that are required for CNS tumors, and the cells can be activated ex vivo to exhibit anti-tumor effector function and thus do not depend on activating stimuli at the tumor site. The exact cell subpopulations and effective functions that are required for cancer regression in vivo can be identified and it is possible to manipulate the host prior to cell transfer to provide an altered environment for the transferred cells. We are exploring the use of engineered T cells bearing chimeric receptors. As a model for non-immunogenic tumors, we are targeting the surface membrane glycoprotein mesothelin is a promising target for the immunotherapy of mesothelioma, ovarian, and pancreatic tumors due to the uniform overexpression of mesothelin and the benign phenotype of mesothelin null mice. We hypothesize that previous trials of adoptive immunotherapy for cancer that have used CTL have failed due to poor trafficking to sites of tumor, and insufficient effector functions to self antigens. Our preclinical data indicates that use of lentiviral engineered T cells with chimeric receptors that incorporate a 'tumor resistance genotype' should have improved function for cancer immunotherapy. We have tested mesothelin redirected T cells in humanized mouse models bearing tumor xenografts. The T cells are able to eradicate large, well established tumors at an E:T ratio of at least 1:70 in vivo. Three routes of administration with redirected T cells have been tested in mice bearing xenografted flank tumors. The intraperitoneal route was found to be inferior to intravenous or direct intratumoral injection of the redirected T cells. Long term engraftment of the redirected T cells correlates with antitumor efficacy. This strategy could be adopted to CNS tumors, where the engineered T cells could prove to have resistance to the highly immunosuppressive tumor microenvironment that is characteristic of glioblastoma.

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IL04

BRAIN TUMOR STEM CELLS: NEW INSIGHTS AND CHALLENGES IN HUMAN AND MOUSE MODELS

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Human brain tumors appear to have a hierarchical cellular organisation, suggestive of a stem cell foundation. We have recently derived adherent cell lines with high efficiency from human malignant glioma that display stem cell properties and initiate high grade gliomas following xenotransplantation. Significantly, these cell lines from different tumors exhibit divergent gene expression signatures and differentiation behaviour that correlate with specific neural progenitor subtypes. The diversity of gliomas may therefore reflect distinct cancer stem cell phenotypes.

Adherent brain tumor stem cell lines offer significant experimental advantages compared to 'sphere' based cultures, and provide a simplified model enabling refined studies of cancer stem cell behaviour.

In addition, it remains unclear if genetically engineered mouse models of cancer recapitulate the functional heterogeneity observed in their human counterparts. We demonstrate medulloblastomas arising from Patched1 (Ptc1+/-)deficient mice contain a subpopulation of cells that demonstrate a neural precursor phenotype, show clonogenic and multilineage differentiation capacity, wild-type Patched-1 expression, and the ability to initiate tumors following allogeneic orthotopic transplantation. The normal neural stem cell surface antigen CD15 enriches for the in vitro clonogenic and in vivo tumorigenic potential from uncultured medulloblastomas supporting the existence of a cancer stem cell hierarchy in this clinically relevant mouse model of cancer. Interestingly, the cell that drives the growth of these tumors has a stem cell phenotype, lending further support to a potential cell of origin of these tumors from normal cerebellar neural stem cells, or acquisition of a stem cell phenotype as part of the neoplastic transformation process from lineage restricted progenitors.

I will present these recent data along with my current perspectives on cancer stem cell research, particularly the challenges the field faces moving forward.

KN01

SURGERY ON PATIENTS WITH SYMPTOMATIC LOW GRADE GLIOMA IN CRITICAL BRAIN AREA

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Objective: Up to now there still has been no therapeutic standard for the heterogenic groups of low grade gliomas (LGG). Our tumor data bank demonstrates an incidence of 5 -10% LGG among averaged 180 primary surgically treated brain tumors per year with an increasing tendency. In this manuscript we describe our operative procedure and its results on patients with symptomatic LGG in critical brain area.

Methods: The analysis revealed 30 patients with LGG who were operated on in the last 3 years. Most patients had diffuse astrocytoma WHO grade 2 (15 patients). Rare tumors such as ganglioglioma (4), central neurocytoma (2) and subependymoma (2) were also diagnosed during this period. The tumor was mostly localized perisylvian (15) less in ventricle (5), brainstem (5), central region (4) or basal ganglia (1). All patients were operated on with navigation support, endoscopic- microsurgical technique and intraoperative neurophysiologic monitoring (IOM) of different modalities. Since 2007 the morphologic data ha been fused with the functional one (fMRI, DTI as well as PET) for navigation setting.

Results: Total excision could be achieved in 17 patients. Subtotal (11)or part resection (2) has had to be performed due to the functional data and the IOM results. Temporary worsening of the neurological finding occurred in 18 patients for several days. The examination 3 months after surgery and later on demonstrates, however, an improvement status in comparison to that before surgery in 25 patients. During this period no recurrence surgery was needed. The seizures improve in 10/ 13 patients with or without antiepileptic drugs but in decreased dosage.

Conclusion: Our results show that LGG in critical area can be operated on safely with good outcome thus improvement of the quality of life. This therapeutic option using modern morphologic and functional data should be offered to patients with symptomatic LGG

KN02

THE ROLE OF MICRORNAS IN MALIGNANT GLIOMA

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MicroRNA's (miR's) are recently described small non-protein-coding RNA molecules that are involved in the modulation of protein synthesis, via binding to complementary mRNA. They are also known to modify the activity of oncogenes and tumor suppressor genes of tumors, such as glioblastoma multiforme (GBM), the most common and deadly form of brain cancer. Using microarray analysis and quantitative RT-PCR, we have noted that miR-128, which is normally abundant in normal brain tissue, is significantly under-expressed in GBM tumor samples (18.75-fold reduction). Ectopic expression of miR-128 using oligonucleotide precursor or lentiviral vector reduced glioma cells growth considerably – both in vitro (U87, U251) in vivo (flank), as well as in models that enrich for glioma "stem-like" cells. Direct effects on several target genes functionally linked to regulation of oncogenic growth related processes, as well as impact exerted on stem cell – like self-renewal will be discussed. Another characteristic of gliomas is invasion into normal tissues. miR-451 was noted to have the most profound change in expression during the invasion process (relative signal strength drop from 2.753 to 0.000) when we compared invading vs. non-invading glioma cells. This down-regulation during invasion was corroborated in a number of glioma cell lines. In over-expression experiments, cells treated with pre-miR-451 or stably expressing pri-miR-451 demonstrated significantly reduced migratory behavior during invasion assay experiments. Potential targets of miR-451, involved in migration/invasion processes, will be discussed and validated. In concluiosn, miR's are important modulators of GBM proliferation and invasion, and have the potential to be used as diagnostic or therapeutic agents.

KN03

COMPREHENSIVE GENOMIC CHARACTERIZATION OF GLIOBLASTOMA: THE CANCER GENOME ATLAS EFFORT.

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Background: Advances in understanding the genetic basis of cancer have led to the identification of molecules that are specifically expressed or mutated in tumors. Such molecules represent targets for therapy whose targeting might be expected to have increased selectivity for tumor cells compared to normal tissue with resultant enhancements in effect and reductions in the non-specific toxicities that lead to chemotherapeutic side effects. In order to provide a large-scale comprehensive catalogue of such targets, the National Institutes of Health have initiated The Cancer Genome Atlas project and the first cancer to be subjected to this multi-center analysis is glioblastoma multiforme. The purpose of this lecture is to review the progress this effort has made.

Methods and Results: The purpose of the TCGA is to catalogue and discover major cancer-causing genome alterations in large cohorts of human tumors through integrated multi-dimensional analyses. To this end, a committee screened retrospective biospecimen repositories for possible inclusion and each of these was then reviewed at the Biospecimen Core Resource. This resulted in approximately 35% of candidates being included in the initial studies (n=206). These samples were considered to be of sufficient quality and size to be entered into the analysis stage. Various parameters were then examined: DNA copy number by high-resolution comparative genomic hybridization, gene and microRNA expression was determined on several platforms, loss of heterozygosity was determined using single nucleotide polymorphism arrays, cancer-specific DNA methylation in CpG islands and promoters of 2305 genes was determined using Golden Gate technologies, and 91 matched normal-tumor pairs were subjected to Sanger sequencing of 601 selected genes. All of this information was then entered into a large database for statistical analysis in the Data Coordinating Center (http://cancergenome.nih.gov/) that has various levels of public accessibility. The major findings from this effort are: 1) it is possible to perform a comprehensive, multi-institution, multi-investigator effort and the structure to accomplish this has now been established; 2) genes that were previously found to be targets of the oncogenic process such as EGFR, PDGFRA, MDM2, CDK4/6, CDKN2A/B, PTEN, RAS and AKT were found to have significant levels of alterations in this larger dataset; 3) new potential targets such as NF1, ERBB2, MET, PIK3R1 were discovered; 4) an interaction between MGMT promoter methylation and a hypermutator phenotype was uncovered with clinical significance for the application of alkylating tharapeutics.

Conclusions: These results demonstrate the value of a large comprehensive genetic analysis of tumors, particularly glioblastoma multiforme. The alterations that were uncovered in this way suggest a limited number of signaling/metabolic pathways that are likely to be defective in tumors and furthermore that targeting pathways rather than specific molecules may be a reasonable approach in future.

KN04

THE FUTURE TREATMENT OF ANAPLASTIC GLIOMA: LESSONS FROM NOA-04 AND BEYOND

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The common practice of care for anaplastic astrocytoma (AA) is to use postoperative radiotherapy alone or to embark on regimens developed for the treatment of malignant gliomas in general. Can and should the increase in survival gained by radiochemotherapy with temozolomide (TMZ) in glioblastoma be extrapolated to patients with AA. Taking the evidence on nitrosourea-based therapy from the glioma meta-analysis, it would seem reasonable to suggest that a similar survival benefit might be seen amongst AA patients. However, there are concerns regarding late neurotoxicity related to the administration of concurrent radiochemotherapy, especially for patients who are living long enough to experience these unwanted sequelae. In the early 1990s, physicians began treating most AO patients with procarbazine, lomustine (CCNU), and vincristine (PCV) chemotherapy not only at recurrence but also at diagnosis in combination with or without radiotherapy, despite the lack of convincing data supporting this practice. In the United States, the prospective randomized Phase III Radiation Therapy Oncology Group (RTOG) study (R9402) compared radiotherapy with and without preceding PCV. The contemporaneous study of the European Organization for Research and Treatment of Cancer (EORTC) (26951) used the same comparators but completed radiotherapy before commencing PCV. Both trials showed increased progression-free survival (PFS) but not overall survival (OS) in patients receiving radiochemotherapy, gained at the cost of relevant toxicity with the PCV regimen. Deletion of 1p and 19q may predict a superior outcome in AO and anaplastic oligoastrocytoma (AOA) in response to genotoxic treatments but not in patients with low-grade oligodendroglial tumors treated with surgery alone. To date, it has still remained unclear whether the combined1p/19q deletions simply represent a molecular signature in anaplastic gliomas, which reflects a favorable natural biological behavior, or whether these markers are mechanistically related to response to therapy. The NOA-04 phase III, multicenter, open-label trial compared the efficacy and safety of radiotherapy versus chemotherapy (PCV or temozolomide) in 318 patients with newly diagnosed, supratentorial anaplastic gliomas of WHO grade III. In addition, the clinical significance of 1p/19q deletion and MGMT promoter methylation in these tumors was assessed in a translational study. Median time-to-treatment failure (TTF), PFS, and OS were not different between arms. Patients with an astrocytic tumor had a worse TTF. Oligoastrocytic tumors share the same favourable risks of pure oligodendroglioma. Combined 1p/19q deletion and hypermethylation of the MGMT gene promoter provided a large risk reduction for TTF and PFS irrespective of histology and treatment. In conclusion, there is an identical clinical course of anaplastic oligodendroglioma and oligoastrocytoma. The favourable impact of an oligodendroglial component was as strong as detecting combined 1p/19q deletion in the tumor tissue. MGMT promoter methylation was associated with prolonged PFS also in the radiotherapy arm A. Initial therapy in all anaplastic gliomas could possibly be either temozolomide or radiotherapy. The next challenges include intensifying therapy for anaplastic gliomas with unfavourable prognosis (e.g. no MGMT methylation, no combined 1p/19q deletion) and optimizing treatment for anaplastic gliomas with favourable prognosis without increasing toxicity. Trials basically testing radiochemotherapy with temozolomide separate for these entities are ongoing. Further, it will be important to test novel agents also in anaplastic gliomas, especially if influence on differentiation, angiogenesis or invasiveness can be postulated with a potential of influencing malignant progression.

KN05

MR METABOLIC AND PHYSIOLOGICAL IMAGING OF PATIENTS WITH GLIOMA

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MR imaging is an important technology that is of interest for diagnosing, planning focal therapy, evaluating treatment effects and assessing prognosis for patients with glioma. Despite the widespread application of conventional anatomic imaging techniques, there is a critical need for more advanced methods that are able to characterize biological properties of the lesion and surrounding normal brain. This is particularly important for understanding the effects of combination therapies, which influence proliferation and angiogenesis, as opposed to having a direct effect on cell viability. A number of advanced in vivo MR imaging methodologies are being evaluated in the clinic to see which combination of parameters are the most relevant for evaluating patients with gliomas.

Anatomic Parameters: In addition to optimizing the acquisition times and resolution of anatomic images with standard contrast mechanisms, high resolution T2*weighted magnitude and phase images are being acquired with 3T and 7T whole body scanners. These images visualize heterogeneity in the region of T2 hyperintensity caused by local changes in susceptibility due to hemorrhage and other treatment effects. Using either the phase image alone or by the applying post-processing methods to generate susceptibility weighted data it is possible to visualize small veins and hence examine differences between the tumor and surrounding tissue.

Vascular Parameters: To further measures changes in vascular properties, data acquisition and analysis techniques have been developed to measure arterial spin labeling, Dynamic Contrast Enhanced (DCE) and Perfusion Weighted (PW) Images. The latter two methods utilize changes in T1-weighted or T2*-weighted images during and after an injection of Gadolinium to estimate parametric maps of with vascular density and permeability. This is particularly important for the evaluation and quantification of measures of response for patients receiving anti-angiogenic therapies.

Structural Parameters: Degradation in the normal architecture of the brain can be evaluated using Diffusion Tensor Imaging. This gives information about the change in the magnitude of the apparent diffusion coefficient of water (ADC), as well as utilizing the directional properties of the diffusion tensor to mapping of connectivity using tractography. The ADC measurements have been reported as being linked to tumor cellularity and as being an early predictor of treatment effectiveness. The tractography is being used routinely for pre-operative analysis of the disruption in normal tissue structure caused by the tumor and for surgical planning.

Metabolic Parameters: The use of MR spectroscopy methods has been a major focus of our brain tumor research. 1-H spectroscopy has been shown to highlight areas of metabolically abnormal, non-enhancing tumor that are important for assessing disease burden, treatment planning and assessing treatment response. Of particular interest are the spatial extent of areas where both the spectral peak corresponding to choline containing compounds and the peaks from lactate and/or lipid are elevated. Quantitative measures of these regions have been shown to be predictive of poor outcome in patients with GBM. More recent studies using very rapid spectroscopy acquisitions following the injection of hyperpolarized C-13 agents have shown promise for monitoring metabolically active lactate.

KN06

BRAIN TUMOR INITIATION AND DEVELOPMENT, INFLUENCE OF CANCER STEM-LIKE CELLS.

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The molecular events that lead to the cancer-initiating cell involve critical mutations in genes regulating normal cell growth and differentiation. Cancer stem cells, or cancer initiating cells have been described in the context of acute myeloid leukemia, breast, brain, bone, lung, melanoma and prostate. Yet, there is emerging evidence for that tumor initiation may occur also from tumor cells that are not defined as cancer stem cells. The origin of the cancer initiating cell and their mechanisms of progression are not clear. At present there are two main models of cancer development 1) The hierarchical model that suggests that tumours are generated and maintained by a small subset of undifferentiated cancer stem cells that are able to self renew and differentiate into the bulk tumour population, and 2) The stochastic model implying that tumor progression is the result of acquired genetic variability within the original clone allowing sequential selection of more aggressive subclones.

The strongest evidence for a hierarchical model comes from studies of Leukemia. For other tumors, however, the evidence is less clear. It has often been a problem to identify a clear-cut marker that defines the cancer stem cell. For brain tumors, recent evidence show that also CD133- cells can be tumorigenic indicating that CD133 is not a unique marker for cancer stem cells in the CNS. Therefore, it is not clear if brain tumor development follow a hierachical or stochastic model of progression..

What is clear is that within tumors, there are tumor cells that express and behave as stem cells. These cells can as normal stem cells, adapt to new micro-environments and may well represent the most resistant cells to therapy. Based on their adaptability and differentiation capacities they can most likely not be defined by a specific marker. To what extent the environment and epigenetic factors influence the cancer stem like cells deserves further studies. In conclusion, increased knowledge of developmental aspects in relation to self-renewal and differentiation, both under normal and deregulated conditions, will probably shed more light on the mechanisms that lead to tumor initiation and progression.

KN07

CAN WE DEVELOP MANAGEMENT STRATEGIES FOR ADULTS AND CHILDREN WITH PRIMARY BRAIN TUMORS IN LOW INCOME COUNTRIES?

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The Problem: About 200,000 primary brain tumors are diagnosed world-wide each year, 70% in low income countries.

The cure rates for several cancers of both children and adults have improved markedly in high income countries in recent decades. However, substantial improvements in the cure of patients with primary brain tumors remain an elusive goal even in high income countries, except for those with "pediatric" brain tumors such as medulloblastomas, ependymomas, pilocytic astrocytomas and germ cell tumors. Quality of life has improved for many brain tumor patients in high income countries, even in the absence of improved cure rates, through introduction of advanced imaging, neurosurgical and more focused radiotherapeutic technologies.

Concerted efforts in recent years in several low income countries throughout the world, mainly focused on children with leukemias and lymphomas, have produced remarkably speedy and dramatic improvements in cure rates. However, such tumors do not require neurosurgical, imaging and radiotherapeutic skills and technology to improve outcome.

No single approach can be employed in tackling the problem of patients with brain tumors in low income countries: first, there is tremendous heterogeneity of resources amongst low income countries: one cannot compare Paraguay with Brazil, the Congo with the Republic of South Africa, or Cambodia with Thailand. Any and all efforts must be placed within the context of the major prevailing health issues of the particular country: malnutrition, sanitation and water supply, infectious disease (including but not confined to HIV-AIDS), immunization. Efforts must recognize that in the majority of low income countries, some 35-60% of the population will be less than 21 years of age – necessitating a substantial "pediatric" focus. Finally, for several countries, one cannot even begin to consider definitive treatment of brain cancer when there is a total absence of potent analgesics, anticonvulsants or even dexamethasone…….One cannot address improving neurosurgical skills in so many countries who have fewer than one neurosurgeon for 12 million people (70% of African countries, at least) when WHO recommends one per 100,000 people. One cannot address radiotherapeutic techniques when 90% of the African continent has less than one machine for 30 million people –and where expertise lags even further behind availability of equipment.

The Solution: (a) The "Big Picture" - Creation of a global patchwork quilt……triaging of efforts, avoidance of duplication, maximizing economy of effort, serving to connect interested/enthusiastic individuals/programs with emerging countries' programs. (b) The details – Palliative care/quality of life strategies for malignant glial tumors; neurosurgical/neuro-oncological training in and establishment of regional centers; establishment of satellite centers affiliated with regional centers to facilitate decentralization of supportive and acute care; development and adaptation of radiotherapy avoiding treatment strategies for "pediatric" brain cancers.

The WFNO Mini-Symposium on Developing Strategies for Brain Tumor management will endeavor to address several aspects of this "solution" already in operation in different parts of the world.

KN08

INVOLVEMENT OF VEGF-VEGFR SYSTEM IN THE PROGRESSION OF CANCER INCLUDING BRAIN TUMORS.

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An endothelial cell-specific growth factor VEGF (VEGF-A) is deeply involved in physiological as well as most of the pathological angiogenesis detected in cancer and arthritis. VEGF-A binds and activates two tyrosine kinase (TK) receptors, VEGFR-1 (Flt-1) and -2 (KDR/Flk-1). VEGFR-2 has a 10-fold higher kinase activity compared to that of VEGFR-1, whereas VEGFR-1 has a strong affinity to VEGF-A. The major signaling pathway from VEGFR-2 has recently been clarified. However, the possible role of VEGFR-1 and its signaling are not fully understood yet.

1. A unique signaling of VEGFR-2 for angiogenesis.

First, I will briefly summarize the signaling of VEGFR-2, the major positive signal transducer for angiogenesis. We found that VEGFR-2 activates a unique signaling, PLCγ-PKC(C-kinase)-Raf-MAP kinase pathway for DNA synthesis in endothelial cells. Ras-activation was only minor for this downstream signaling from VEGFR-2. Also, we have shown that a single autophosphorylation site 1175PY (phosphotyrosine. 1173-position in mice) is critical for this pathway. 1173F/F mutant mice died at E8.5-9.0 with a severe defect on vasculogenesis, indicating that this pathway is important for angiogenesis *in vivo*, and a good target for suppression of tumor angiogenesis.

2. An important role of VEGFR-1 in tumor growth and metastasis.

VEGFR-1 gene knockout mice are embryonic lethal due to an overgrowth of blood vessels at E8.5, suggesting a negative role of VEGFR-1 in early embryogenesis. We showed that VEGFR-1 ligand-binding domain [VEGFR-1 TK-/-] rescues this lethality. Since VEGFR-1 TK-/- mice develop essentially normal angiogenesis, these mice are useful to study the role of VEGFR-1 signals at adult stages in disease models such as cancer. We found that in various tumors, tumor growth and angiogenesis are significantly lower in VEGFR-1 TK-/- mice compared with that in the wild type mice mostly due to a defect of macrophage recruitment from bone marrow. In collaboration with us, Kerber et al. also showed a slower growth of gliomas in VEGFR-1 TK-/- mice.

VEGFR-1 is expressed not only in vascular endothelial cells but in macrophages, suggesting that VEGFR-1 plays an important role in the promotion of tumors including glioma via activation of macrophage-lineage cells. Finally I will discuss on a possible increase in tumor aggressiveness after low oxygen-nutrition stress.

KN09

EGFR SIGNALING THROUGH AN AKT/SREBP1-DEPENDENT, RAPAMYCIN-RESISTANT PATHWAY SENSITIZES GLIOBLASTOMAS TO ANTI-LIPOGENIC THERAPY

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ABSTRACT
Glioblastoma, the most common malignant brain tumor, is amongst the most lethal and difficult cancers to treat. Although EGFR-mutations are frequent in glioblastoma, their clinical relevance is poorly understood. Unexpectedly, studies of tumors from patients treated with the EGFR-inhibitor lapatinib revealed that EGFR induces the cleavage and nuclear translocation of the master transcriptional regulator of fatty acid synthesis, SREBP1. We show that this response is mediated by Akt, but surprisingly clinical data from rapamycin-treated patients shows that SREBP1 activation is independent of the mTORC1, potentially explaining its poor efficacy in the treatment of such tumors. The importance of these observations are underscored by the finding that non-EGFR-activated glioblastomas are resistant to inhibition of fatty acid synthesis whereas introduction of an activated EGFR-allele sensitizes tumor xenografts in mice to cell death, which is augmented by the HMG-CoA reductase inhibitor atorvastatin. These results identify a novel EGFR-mediated pro-survival metabolic pathway, suggesting a promising approach for treating EGFR-activated glioblastomas.

KN10

MANAGEMENT OF YOUNG CHILDREN NEWLY-DIAGNOSED WITH CENTRAL NERVOUS SYSTEM (CNS) EMBRYONAL TUMORS: 18 YEARS OF THREE SEQUENTIAL IRRADIATION-AVOIDING CHEMOTHERAPY STUDIES - THE "HEAD START" PROTOCOLS

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Study Purpose: To improve the survival and quality of life for young children with newly-diagnosed primary malignant CNS embryonal tumors through a strategy of relatively brief and intensive chemotherapy (<5 months duration), followed by myeloablative chemotherapy in an effort to avoid CNS irradiation.

Study Design: Since 1991, three sequential prospective non-randomized multi-center feasibility studies have been conducted. In the first study, "Head Start" I (1991-1997) eligible children received a single treatment strategy: Induction chemotherapy of vincristine, cisplatin, cyclophosphamide and etoposide administered every 21-28 days for 5 cycles (Regimen A). This was followed by a single cycle of myeloablative chemotherapy (thiotepa, carboplatin and etoposide) and rescue with autologous hematopoietic progenitor cells. In the subsequent "Head Start" II (1997-2003) study, patients with localized medulloblastoma/PNET received the "Head Start" I Induction Regimen A, while patients with AT/RT or metastatic tumor received a modified Induction Regimen A2 intensified with high dose systemic methotrexate (HD-MTX, 400mg/Kg). In the current "Head Start" III study (2003 to present), all patients receive the same new Induction Regimen D, which includes 3 cycles of Regimen A2 (cycles 1, 3 and 5), alternating with two new cycles (cycles 2 and 4), in which HD-MTX and cisplatin are deleted, and replaced by oral etoposide and oral temozolomide.

Summary of Results: In children with medulloblastoma, gross total resection of localized primary tumor, absence of dissemination and desmoplastic histology conferred highly favorable irradiation-free 5-year overall survivals (OS) of 73% (classical) and 88% (desmoplastic). The use of HD-MTX in the "Head Start"II protocol produced markedly improved 5-year EFS (45%) and OS (53%) for children with disseminated medulloblastoma, exceeding even the 5-year EFS of 29% for children with localized but incompletely resected medulloblastoma who did not receive HD-MTX. For this reason, the "Head Start" III regimen incorporates HD-MTX for all children. For children with supratentorial PNET (sPNET), the only statistically significant prognostic factor was location; children with pineal region PNET (pineoblastoma) experienced poorer outcome than those with non-pineal sPNET (EFS of 15% versus 44% and OS of 25% versus 62%). The small group of patients with brainstem PNET represents the first reported cures (2/6 patients) of this rare entity. For children with AT/RT, the addition of HD-MTX in "Head Start" II resulted in an overall significant improvement in 5-year EFS (43%) and OS (54%), but only for patients with localized disease at diagnosis. Prospective longitudinal follow-up, including formal quality of life, neuropsychological, endocrine and audiometric assessments of the surviving patients is ongoing, and has now been completed for "Head Start" I and II. Neuropsychological studies document preserved quality of life and intellectual functioning for those children who avoided irradiation, including children who received HD-MTX. Hearing loss continues to be the major long-term morbidity of treatment, and seems magnified by the use of ototoxic antibiotics during treatment. Endocrine studies have identified only rare isolated examples of growth or thyroid dysfunction requiring hormonal replacement therapy. Second malignancies have not been identified in patients receiving only protocol chemotherapy without CNS irradiation.

Conclusions: The "Head Start" protocols have demonstrated significant improvements in both the survival and quality of life of young children with CNS embryonal tumors, compared with previously published treatment regimens.

KN11

THE CHEMOTHERAPEUTIC MANAGEMENT OF LOW GRADE GLIOMAS

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Low grade gliomas (LGG) account for the largest group of brain tumours in the paediatric age, representing approximately one third of all childhood brain tumours. These tumours can arise in any part of the nervous system. Subtypes of LGG are distinguished by their histological features, but all LGGs are classified as World Health Organization (WHO) grade I or II. LGG are usually slow growing although for reasons that are not completely clear, some of these tumours may show aggressive behaviour and/or have erratic growth rates, particularly in young children with hypothalamic/chiasmatic gliomas.

Surgery is the treatment of choice of resectable tumours and is often curative in these instances. However, a large number of paediatric LGG arise in areas where the role of surgery is limited, due to the risk of morbidity associated with attempts at aggressive resection. Radiation therapy to such tumours has demonstrated evidence of benefit, with objective radiographic responses often for long periods of time, although the use of radiation therapy in young children results in serious long-term sequelae, including permanent neurocognitive and neuroendocrine deficits, as well as an increased risk of vasculopathy and second malignancy. It is now clear that chemotherapy can delay the need for radiation therapy. Over the past decade, the upfront use of chemotherapy has become standard therapy for children and infants with unresectable tumours.

Various regimens have been tested, both in chemotherapy naïve patients and in patient with recurrence after previous radiation and/or chemotherapy. Carboplatin is one of the most effective agents against low-grade gliomas. The regimen of weekly carboplatin and vincristine has been extensively piloted and is considered by many to be the standard chemotherapeutic regimen for young children with unresectable low-grade gliomas. Other regimens have been suggested, and the choice between different options is often a challenge for paediatric oncologists. Beside the efficacy, the decision should take into account various factors such as short and long term toxicity, the route and schedule of administration and the cost.

Recent prospective studies have shown that a majority of children (up to 70%) will require additional treatments after a first line of chemotherapy. The management of these children who do not respond to upfront chemotherapy or show progression after a first line of treatment is challenging. Traditionally, radiation has been offered as a “salvage treatment”, regardless of the age of the patient. Increasingly chemotherapy is being been considered in this context, particularly when young age predicts significant neuro-intellectual deficits with the use of radiation. Several studies are ongoing, looking at new agents or combinations in children with recurrent LGG. How many lines of chemotherapy can be used without compromising long term outcome is still unclear. It appears however that the use of multiple lines of chemotherapy is possible and that the need for radiation can be reduced to a small proportion of children.

Recently, biological studies have suggested that it might be possible to predict the behaviour of paediatric LGG by evaluating the proliferation index (mib-1 or Ki-67), microvessel density, or telomere length. Evaluation of these parameters in prospective protocols is ongoing and the results of these studies may potentially influence the chemotherapeutic management of patients with low risk and high risk features.

KN12

NEUROPSYCHOLOGICAL FUNCTIONING AND OUTCOME FOR BRAIN TUMOR PATIENTS

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Survival rates for those diagnosed with brain tumors have generally improved with advances in surgery, radiation therapy, and chemotherapy. As a result, understanding the long-term impact of therapy including the neurocognitive and psychological effects of cancer treatment are important. Most brain tumor survivors who achieve durable survival often present with various deficits. Significant sequelae, including intellectual decline, as well as variable attention-concentration, slow processing speed and motor deficits have been observed as a result of treatment, with specific risk factors including young age at the time of treatment, higher dose and larger brain volume involved in radiotherapy, and longer follow-up. Cranial irradiation has been associated white matter changes, particularly in the frontal cortex. Consequently, a young child treated for Medulloblastoma with craniospinal radiation will likely experience more adverse late effects as compared with an adolescent treated for a CNS germinoma with focal radiotherapy. In an effort to preserve intellectual functioning and quality of life, many current treatment studies are utilizing reduced doses and volumes of radiotherapy, while others are employing postoperative chemotherapy to delay or avoid radiation therapy.

Despite "benign" histology and lower toxicity of treatments for low grade tumors that are commonly limited to maximal surgical resection, these tumors are associated with increased risk for late effects in both cerebellar and supratentorial locations given their slow growing and infiltrative presentation. In contrast, high-grade gliomas are a group of CNS tumors characterized by heterogeneity and a typically poor treatment response to surgery, chemotherapy and radiotherapy. Consequently, newly emerging treatment regimens often involve significant toxicities which necessitate the sequential measurement of quality of life by both patient- and caregiver-report as a primary outcome in conjunction with length of survival.

Two prominent pediatric multi-center intervention studies in the U.S. involved the use of Methylphenidate (Ritalin) medication and the other utilized a Cognitive Remediation Training Program in order to address symptoms such as attention, memory and academic achievement difficulties following treatment for pediatric cancer and the results have demonstrated modest effect sizes. Similarly, recent adult studies have reported encouraging pilot data in response to patients using substances such as Donepezil (Aricept), Modafinil (Provigil) and Ginkgo biloba.

Emotional effects from the treatment of a brain tumor vary depending on the patient, diagnosis, location of disease, treatment type, family dynamics and pre-existing psychological conditions. Some studies indicate that brain tumor survivors are at no greater risk for long term emotional sequelae than would normally be expected in a healthy population with lower levels of psychological distress, better psychological health, lower levels of aggressiveness, antisocial behavior and substance abuse than case controls. However, other survivor studies have identified social difficulties in withdrawal, isolation and poor social competence attributed to physical appearance, functional impairments, and reduced time with peers. Thus, while information about brain tumor patients' social-emotional and behavioral functioning continues to emerge, there does appear to be an increased risk for deficit in certain domains of physical and psychosocial functioning. Consequently, there is currently a change underway in research orientation from cancer treatment as a necessarily pathology-inducing experience but rather focusing upon the skills and resilience of patients and families who were struggling with a difficult situation. As such, it is important to identify risk factors in patients and families in order to tailor interventions to minimize the psychological sequelae in brain tumor survivors.

In sum, neuropsychological, social-emotional, behavioral and quality of life evaluations and interventions provide a range of benefits to the field of neuro oncology including the assessment of functioning prior to treatment, as well as measuring the neurocognitive impact between treatment regimens and facilitating the psychological transition through treatment and beyond into survivorship.

KN13

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Chemotherapy, with or without radiotherapy, is the mainstay of treatment for primary central nervous system lymphoma (PCNSL). High-dose methotrexate (MTX) is the most effective drug, and is used in doses of 1-8 g/m^2 either as a single agent or in combination with other drugs such as corticosteroids, cytarabine, procarbazine, vincristine, carmustine, lomustine, thiotepa, cyclophosphamide, temozolomide, and rituximab. To date, an overwhelming number of different regimens utilizing high-dose MTX have been reported. However, given the lack of randomized trials, the optimal treatment remains controversial. Varying methodology makes the comparison of available studies extremely difficult; however, some common themes can be found throughout literature. Treatment paradigms vary considerably according to the patient's age. Most studies support the use of chemotherapy-only treatments for elderly patients (> 60 years), given the high risks of neurotoxicity associated with radiotherapy. However, the prognosis remains poor independent of the chemotherapy chosen, and less toxic regimens might be preferable for such elderly patients. Conversely, the goal of treatment in younger patients (< 60 years) is a cure, and a more aggressive approach seems warranted. Growing evidence suggests that commonly used chemotherapy-only regimens for younger PCNSL patients are associated with increased relapse rates that may not justify deferral of radiotherapy. Thus, a significant focus of research has been the development of intensified chemotherapy regimens that could replace radiotherapy. Several ongoing studies are evaluating the role of high-dose chemotherapy with stem cell rescue utilizing drugs that penetrate the blood-brain barrier. An important feature of these studies is the inclusion of thorough neuropsychological evaluation in order to evaluate the long-term cognitive outcomes. Another important venue of investigation is trying to identify molecular predictors of aggressiveness, as well as markers of biological predisposition for neurotoxicity, which would allow more judicious therapeutic decisions.

O001

NG2 EXPRESSION ESTABLISHES A LINK BETWEEN NEURAL PRECURSORS AND CANCER STEM CELLS IN GLIOBLASTOMA

This Abstract nominated the Hoshino Award.
Please refer to P049 on page 146.

O002

A SINGLE CD133-POSITIVE CELL IS CAPABLE OF PRODUCING GLIOMA POPULATION DIVERSITY

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PURPOSE: Mounting experimental evidence suggests that malignant brain tumors contain and may arise from cancer stem cells (CSC). CSC are believed to play important roles in tumorigenesis and recurrence. CD133, a pentaspan transmembrane glycoprotein, is a putative marker of glioma-derived CSC based on the ability of CD133-positive cells to recapitulate the tumor in animal models. Glioma tumor tissues are known to be populated by a heterogeneous mix of cells with distinct genotypes and phenotypes, and different proliferative capacities. The cellular origin of such karyotypic heterogeneity is unclear. Whether the observed population diversity within a tumor has mono- or polyclonal origin has important implications for cancer progression. **METHODS:** To investigate the cellular origin of population diversity, we established 23 subclones from a single glioma CSC positive for CD133. These subclones were subsequently propagated and analyzed. **RESULTS:** The self-renewal and proliferative capacities of the subclones were highly distinct. Sensitivity to chemotherapeutic agents also differed widely. FACS analysis for expression of stem and differentiation markers (e.g. CD133, A2B5, CD44, CXCR4, and CD24) showed that each subclone was composed of distinct population of cells. Immunoblot showed different expression levels of cell cycle regulation proteins such as Bmi-1, p53, p27, and p21. CD133-negative cells failed to establish subclones. **CONCLUSIONS:** Our observations suggest that a single CD133-positive tumorigenic CSC is capable of producing phenotypically heterogeneous self-renewing progeny in an in vitro setting. Whether such observed diversity is regulated by genomic alteration or gene expression remains to be seen.

O003

ANALYSIS OF CANCER STEM CELLS IN GENETICALLY-INDUCED BRAIN TUMORS

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Tumors are thought to contain a small population of cells, designated cancer stem cells (CSCs), that possess the ability both to self-renew and to differentiate and that play a key role in the initiation and maintenance of cancer. However, whether CSCs arise from multipotent immature cells or differentiating cells has remained unclear. Moreover, the minimal repertoire of genetic changes required for the generation of CSCs is unknown. The establishment of CSCs from cells of various differentiation stages by genetic manipulation might be expected to provide insight into these questions.

We have now established mouse malignant brain tumor models by overexpressing c-MYC or RasV12 in neural stem cells/multipotent progenitor cells derived from subventricular zone of mouse with a homozygous deletion of the Ink4a/Arf locus (Ink4aKO mice). Tumors induced by c-MYC are histologically different from those induced by RasV12, suggesting that pathological phenotype of tumor is dependent on the gene(s) responsible for tumorigenesis. Tumor induced by RasV12 is highly heterogenous and invasive, and a fraction of the induced-tumors cells was found to exhibit properties of CSCs. Invading cells at the tumor margin expressed high levels of CD44, which plays a role in both cell migration in extracellular matrix and stemness of cancer cells. Furthermore, tumor cells formed cuffs around the pre-existing microvessels, and those invading area contained numerous microglial cells. Our genetically-induced cancer systems provide ideal models for the characterization of CSCs and the development of novel therapeutic approaches to malignant brain tumors.

O004

GLIOMA STEM CELLS ARE INVOLVED IN TUMOR TISSUE REMODELING IN XENOGRAFT MODEL

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Although tissue remodeling plays a crucial role in tumorigenesis and progression of human gliomas, its mechanisms, however, remain largely uncertain. In the current study, we investigate the potential role of human glioma stem cells (hGSCs) in tissue remodeling of gliomas. First of all, the GFP transgenic nude mice were obtained by crossing NC athymic nude mice with GFP transgenic C57BL/6J mice. As a result, GFP is expressed in essentially all tissues in the offspring. Then, hGSCs were orthotopically implanted into GFP nude mice in an effort to assess the interactions between hGSCs and host brain , thereby elucidating the roles of tissue remodeling during tumorigenesis and progression of huaman gliomas. We found all of the essential tissues in GFP transgenic nude mice including the brain fluoresced green under excitation light. Therefore, the hGSCs labeled with PE-conjugated anti-HLA antibody, which manifested red color can be unambiguously distinguished from bright green background composed of adjacent host GFP-expressing components. This technique enabled us to address the following concerns: (1) hGSCs were involved in the invasiveness in gliomas and adjacent stroma degradation of the host. (2) in vivo study demonstrated that cell fusion between hGSCs and host cells were present. (3) Vasculogenic mimicry, the formation of patterned, tubular networks of vascular channels formed by trans-differentiated hGSCs could be observed. (4) Differentiation mimicry, namely, differentiation direction of hGSCs bearing muiti-differentiation potentials seemed to be decided by local host cellular microenviroment. These results suggest that GFP transgenic nude mouse model, with GFP expression in essentially all tissues, could be obtained by crossing nontransgenic athymic nude mice with the transgenic GFP C57BL/6J mouse. The GFP transgenic nude mouse model should greatly expand our knowledge of glioma &host interactions. Our data indicated that hGSCs may play a decisive role in tissue remodeling of gliomas as well.

O005

ENDOTHELIAL CELLS AND DIFFERENTIATED TUMOR CELLS FUNCTION AS A STEM NICHE TO GLIOBLASTOMA STEM-LIKE CELLS BY PROVIDING NOTCH LIGANDS

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It has been reported recently that endothelial cells function as a stem cell niche to promote CD133-positive cancer stem cells (CSCs) self-renewal in glioblastoma (GBM). However, the mechanism that endothelial cells function as a CSC niche is largely unknown. It has been shown that endothelial cells promote neural stem cell self-renewal by providing Notch signaling and that Notch ligands are expressed in GBMs. To test if endothelial cells and differentiated tumor cells could function as a CSC niche by providing Notch ligands to CSCs, first we co-cultured GBM neurospheres with primary human endothelial cells (PHECs) which express high levels of Notch ligands Jagged/Dalta. We found the PHECs promoted GBM neurosphere growth in vitro. Then, we found the CD133-negative population expressed a higher level of Jagged/Dalta and glial differentiation marker GFAP, compared with the CD133-positive population. When GBM neurospheres were forced differentiation to grow as a monolayer culture, the CD133-positive population and CD133 expression were reduced, whereas expression of GFAP (glia marker), GalC (oligodendrocyte marker), and Notch ligands were significantly induced. Furthermore, treatment of GBM neurospheres with a Jagged peptide increases GBM neurosphere propagation in a dosage dependent pattern, indicating that Notch ligands expressed in both differentiated tumor cells and endothelial cells can function as a niche to promote GBM CSC self-renewal in vitro. Finally, we found that Notch ligands were highly expressed in the blood vessels of GBM primary tumor and intracranial xenograft, and that CSCs accumulated around the blood vessels within the tumor. In summary, our studies demonstrate that Notch activation is driven by juxtacrine signaling between tumor cells replicating the classical process of lateral inhibition and by stromal niche signals, suggesting that targeting both CSC and its niche may provide a profound strategy to deplete CSCs in GBM. This may also apply to other cancers.

O006

AN ESSENTIAL ROLE OF C-MET/HGF SIGNALING IN GLIOBLASTOMA INITIATING CELLS

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C-MET, preferentially expressed in stem/progenitor cells, plays critical roles in the regulation of cell growth and motility during embryogenesis and throughout the life. Deregulated MET signaling has been implicated in various types of cancers including glioblatomas (GBMs). Increasing experimental evidences support the hypothesis that a subpopulation of tumors, namely, cancer stem cell/tumor initiating cells (CSC/TICs), are responsible for tumor initiation/propagation and share many developmental pathways with their normal counterparts. However, the precise roles of MET signaling pathway operated in CSCs/TICs are largely unknown.Here we show evidence that some of Methigh GBM cells are indeed CSC/TICs. Methigh populations but not Metlow/- populations are highly tumorigenic and give rise to both Methigh and Metlow/- cells, thereby establishing tumor hierarchy. Methigh cell populations significantly overlap with cells positive for expression of CD133, a putative CSC/TIC marker, and these cells are highly resistant to radiation. Furthermore, we found that suppression of MET pathway in these GBM CSC/TICs achieved by siRNA or shRNA mediated knockdown of c-met gene results in significantly reduced cell survival in vitro and impaired tumorigenic potential in vivo, suggesting that C-Met activation is required to maintain cancer stem cell phenotype in these cells. Interestingly, suppression of MET activation also significantly reduces migration/invasion property of GBM TSC/TICs, in part by derepressing expression of E-cadherin. Taken together, our data demonstrate that MET activation in GBM is a functional requisite for "stemness" phenotypes in CSC/TICs. In summary, we provide experimental evidences supporting an essential role of Met signaling pathway in the survival and invasion of glioblastoma CSC/TICs. Not only will such insights pave the way for a more thorough understanding of CSC biology, but also they further implicate Met as a promising therapeutic target.

O007

DEVELOPMENT AND APPLICATIONS OF STEM CELL BASED PRE-CLINICAL MODELS OF GLIOMAS

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Gliomas are among the most frequent tumors prevalent in the adult and pediatric populations. Unfortunately, despite current therapies implemented for patients with high grade gliomas, the prognosis is extremely poor. There is a current sparsity of in- vitro pre-clinical models of gliomas that recapitulate the pathological and treatment responses reminiscent in human gliomas. Current in-vitro models such as glioma stem cells are very problematic due to having low clonality and variable therapeutic responses. In an effort to explore alternative robust in-vitro models of gliomas, we have developed the first non-neural stem cell based model of gliomas from murine embryonic stem cells. Specifically, we have noted that synthetic astrocytes derived from murine embryonic stem cells are more representative in the origin of gliomas, even at high clonality, compared to terminally differentiated somatic astrocytes. Similar findings are also documented with synthetic astrocytes differentiated from murine neural stem cells. With the use of molecular transcriptome fingerprinting, these stem cell derived synthetic astrocytes are most similar (R-value = 0.8, P-value less than 0.01) to murine somatic astrocytes from the embryonic cortex (E13.5) and newborn hippocampus (P4). Furthermore, these synthetic astrocytes have an intrinsic property of enhanced proliferation that may potentiate an ease towards transformation. We have noted that the genetic alterations of these synthetic astrocytes with candidate gliomagenesis genes including MDM2, AKT and H-RAS can potentiate intracranial high grade gliomas in Nod-Mice. Furthermore, we also undertook functional random mutagenesis screens on these synthetic astrocytes using gene trapping. We obtained a genome wide functional map of genes that are stringently correlated with a pathological spectrum of astrocytomas. This body of work augments the use of stem-cell based pre-clinical models to decipher the genetic and pathological basis of gliomagenesis. Moreover, these models can also be utilized for therapeutic targeting applications.

O008

IN VIVO TRACKING SPIO LABELLED MESENCHYMAL STEM CELL TROPISM TOIN VIVO TRACKING NANOPARTICLE-LABELLED STEM CELL TROPISM TO BRAIN MALIGNANT GLIOMA AFTER SYSTEMATICAL INJECTION BY A CLINICAL 1.5T MR

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Objective: Mesenchymal stem cells (MSCs) have been shown to migrate toward tumors, but their distribution pattern in glioma is not completely portrayed. The primary purpose of the study was to assay the tropism capacity of MSCs to glioma, to delineate the pattern of MSCs distribution in glioma after systematical injection, and to track the migration and incorporation of magnetically labeled MSCs with clinical 1.5T MRI. **Methods:** Fisher344 rats MSCs were co-labeled with superparamagnetic iron oxide nanoparticles (SPIO) and enhanced green fluorescence protein (EGFP). The tropism capacity of MSCs was quantitatively assayed by Transwell system in vitro. To in vivo track the migration of MSCs, magnetic resonance imaging (MRI) was performed at 7 days and 14 days after systematic administration of labeled MSCs. After scanned, the distribution patterns of MSCs in glioma burdened rats were examined by Prussian blue and fluorescence staining. **Results:** In vitro study showed MSCs possessed significantly greater migratory capacity than fibrablast cells, and lysis of F98 glioma/cultured F98 cells showed more capacity to induce migration of cells than other stimuli. At the 7 days after MSCs transplantation, the SPIO/EGFP co-labeled cells distributed throughout the tumor, where a well-defined dark hypointense region represented on gradient echo (GE) sequences. While at the 14 days, most of labeled MSCs were found at the border between tumor and normal parenchyma, which represented on GE sequence as diluted amorphous dark areas at the tumors edge. **Conclusion:** This result demonstrated systemically transplanted MSCs home to glioma with high specificity with a temporal-spatial pattern, which can be tracking by MRI.

O009

P53 INDEPENDENT ABROGATION OF G1 ARREST IN MURINE BRAIN TUMOR-DERIVED STEM LIKE CELLS AFTER IONIZING RADIATION

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Treatment and cure of medulloblastoma continues to be disappointing. Approximately 30% of cases recur and even when a cure is achieved, the treatment is highly morbid to the developing brain results in long-term sequelae. Recent evidence suggests that many solid tumors are sustained by a population of cells with stem cell-like properties that have extensive capacity for self-renewal. Tumor stem cells (TSC) are also thought to have increased radio- and chemoresistance and may be responsible for treatment failures. Mechanisms of DNA damage response have not been explored previously in neural stem cells derived from medulloblastoma. Identifying novel therapeutic targets that spare normal neural stem cells while eradicating the tumor-derived stem cell population is a long-term goal.

TSCs were isolated from medulloblastomas that spontaneously arose in mice haploinsufficient for Patched (Ptc), a component of the Sonic hedgehog receptor, and cultured in serum free media as free-floating spheres. Neural stem cells were derived from the early post-natal hippocampus and cerebellum of wild-type and Ptc+/- mice. Cultures were exposed to 2 Gy of ionizing radiation and cell cycle progression was characterized with flow cytometery. We found that normal neural stem cells had a similar phenotype with sustained accumulation in G1 within two hours of irradiation. In contrast, we found that TSCs failed to arrest in G1 and showed a marked but unsustained G2/M arrest eight hours after radiation. By 48 hours after irradiation TSCs had re-entered the cell cycle. The level of apoptosis was unchanged compared to controls. We repeated these experiments at 10 Gy of radiation and in the TSCs found a large increase in apoptosis over time. Accumulation of TSCs in G2/M was again noted at eight hours, however the G2/M arrest was sustained at 24 and 48 hours after irradiation. Sequencing of the open reading frame demonstrated that p53 is wild type in TSC's, appropriately phosphorylated on Serine15, p21 is induced and Rb phosphorylated, suggesting that the abrogated G1 arrest is not due to abrogation or mutation of p53 pathway.

We have characterized the cell-cycle and DNA damage response of murine NSCs and found that this population of undifferentiated cells undergo cell cycle arrest in G1 after DNA damage. This is in contrast to embryonic stem cells that lack a G1 checkpoint, do not initiate cell cycle arrest and undergo apoptosis after exposure to ionizing radiation. Additionally, we found that TSCs have escaped the G1 checkpoint in a p53 independent manner and proceed to replicate their DNA without adequate repair that likely contributes to genomic instability and propagation of new therapy-resistant clones through aberrant phosphorylation of Retinoblastoma gene. These data suggest that TSC and normal neural stem cells have distinct responses to ionizing radiation and DNA damage. Studies are ongoing to determine the molecular basis for the distinct phenotype observed in response to radiation in normal and neoplastic stem cells and to specifically increase the sensitivity of TSCs to apoptosis after DNA damage.

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O010

ANGIOGENESIS OF GLIOMAS: VASCULAR TUBE FORMATION FROM TRANSDIFFERENTIATION OF GLIOMA STEM/PROGENITOR CELLS.

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Object: Previous studies suggest that tumor cells might be the progenitor for tumor vasculature. Whether vascular tube formation from transdifferentiation of glioma stem/progenitor cells (GSPCs) contribute to angiogenesis of gliomas remain largely uncertain. **METHODS:** GSPCs were isolated from surgical specimens of gliomas and cultured in medium favored for stem cell growth. In viro trans-differentiation of GSPCs were performed under hypoxia. Expression of vascular endothelial cells (VECs) markers CD31, CD34 KDR, and vWF were analyzed with real-time quantitative RT-PCR and immunofluorescence techniques. Vasculogenic mimicry of GSPCs was evaluated in xenograft model in vivo. Relationships between content of GSPCs and expression levels of both VECs markers and proangiogenic factors in large number of clinical specimens were further investigated in glioma tissue microarray. **RESULTS:** GSPCs can transdifferentiated into VECs under hypoxia in vitro. Expressions of VECs markers including CD34, KDR, vWF and CD31 were significantly up-regulated after transdifferentiation. GSPCs manifested typical flagstone pattern when cultivated in medium containing VEGF for a few days; when cultivated on Matrigel they were capable of forming capillary-like structures. Human leukocyte antigen positively stained vessels were observed inside the xenograft tumors after intracerebral transplantation of human GSPCs in athymic nude mice, implied part of tumor cells with human origin were involved in formation of tumor vessels. In surgical specimens of human glioma, tumor vascular cells coexpressing the markers of early VECs (CD34) and markers of GSPCs (ABCG2 and nestin) suggest that these vascular cells stemmed from GSPCs. **CONCLUSION:** Our observations suggest the functional role of GSPCs as endothelial progenitors, which have properties that give rise to vascular endothelial cells, and have the ability to form vascular endothelial tubes.

O011

DUAL-TARGETED ANTITUMOR EFFECTS AGAINST BRAINSTEM GLIOMA BY INTRAVENOUS DELIVERY OF TRAIL GENE TRANSFERED STEM CELLS

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Brainstem gliomas are common in children. Despite recent attempts to optimize or combine radiotherapy and chemotherapy, the poor prognosis of these tumors has not changed in the past two decades. This poor outcome is due to relatively inoperable location and infiltration into the brain. Precisely targeting these tumor satellites may prove critical for the success of any potential therapeutic strategy. Based on the selective killing of malignant cells by TRAIL and targeted migration toward tumor of MSCs, it is logical to hypothesize that membrane-spanned TRAIL engineered MSCs may provide dual-targeted antitumor effects against brainstem glioma. We have demonstrated systemically transplanted MSCs immigrate to brainstem glioma with high specificity. MSCs can penetrate the vessels surrounding tumors, then stream as a chain pattern toward gliomas, eventually circumscribe the tumor. Membrane-spanned TRAIL engineered MSCs not only express full-length TRAIL in surface, but secrete some sTRAIL in medium. After infected with rAAV-hTRAIL, hMSCs show no increase in apoptosis, which suggest it is feasible to engineer MSCs with TRAIL for glioma therapy. After co-cultured hTRAIL engineered MSCs with U87MG cells, the apoptosis of U87MG cells significantly increase than sTRAIL treated U87MG cells. Systematic delivery of hTRAIL engineered MSCs into established human brainstem glioma xenografts results in the potent induction of apoptosis in gliomas, but not in normal brain. This confirms that hTRAIL engineered MSCs are dramatically effective at killing glioma cells, but no toxic to normal brain tissue.

O012

ALTERED EXPRESSION OF MICRORNA CLUSTERS IS ASSOCIATED WITH THE STEMNESS PROFILE OF GLIOMAS

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Background: Growing evidence suggests that the growth of brain tumours is maintained by cancer stem cells. Thus, normal neural precursor cells (NPCs) are attractive candidates for the origin of human gliomas. Objective: To explore the relationship between gliomas and stem cell properties, by comparing the microRNA (miRNAs) expression signature of glial tumours, embryonic stem cells (ESCs), NPCs and normal adult brain tissues of both human and mouse. Methods: We compared the expression profiles of 186 mature microRNAs in ESCs, NPCs, gliomas and normal brains. our panel contained RNA samples derived from glial tumours of various grades, ESCs, NPCs and normal adult brains from both humans and mice. The miRNA expression was quantified by RT real time PCR. Results: Human gliomas, regardless of their grade, displayed a miRNA expression profile that was reminiscent of that in NPCs. Of special note is the 80% identity observed between the miRNA expression signature of human gliomas and the methylcholanthrene-induced mouse glioma, GL-261. Interestingly, about half of the miRNAs in this shared profile were clustered in seven genomic regions susceptible to genetic/epigenetic alternations in different types of cancers. These clusters comprised the miR-17 family of miRNA clusters, mir183-182, and the stem cell-specific clusters mir367-302 and mir371-373, which are upregulated in gliomas, ESCs and NPCs. We also identified the bipartite cluster of 7+46 miRNAs on chromosome 14q32.31, which was down regulated; therefore, it might represent the largest tumour suppressor miRNA cluster. Conclusions: Our finding that all gliomas displayed a NPC-like microRNA signature might imply that normal NPCs that sustain cancer-promoting mutations are the cells of origin of gliomas. It might also suggest that cells within this differentiation window are particularly susceptible to gliomagenesis. Alternatively, a more differentiated progeny, that acquire aberrations in specific genomic regions, could have given rise to this characteristic "NPCs microRNA signature".

O013

THE ROLE OF SURGERY IN THE MANAGEMENT OF PEDIATRIC BRAIN TUMORS

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Introduction. Surgery is an integral component and typically the first line of therapy for children with central nervous system (CNS) tumors. The outcome with regard to surgical morbidity and disease control can be greatly influenced by the initial care that these children receive. The neurosurgeon's role in the care of children with newly diagnosed CNS tumors is reviewed with emphasis on general surgical principles, evolving philosophies, and technical innovations.

Management of Hydrocephalus. The management of hydrocephalus is typically the most urgent issue at time of presentation for most children with newly diagnosed brain tumors. The percentage of children that undergo removal of a posterior fossa brain tumor and who ultimately require CSF diversion by way of shunting ranges from 10-35%. Therefore, since the majority of children will not require postoperative treatment for hydrocephalus every effort should be made to avoid committing the child to such preoperatively. The evolution of hydrocephalus management has been rapid in the past couple of decades principally due to the introduction of endoscopic neurosurgery and the technique of endoscopic third ventriculostomy (ETV). Although it has been shown to be beneficial, ETV prior to 4th ventricular tumor removal should be reserved for children that require urgent treatment of hydrocephalus when tumor removal is not imminently possible. However, ETV for the management of hydrocephalus due to some brain tumors such as tectal gliomas and pineal reagion tumors is state of the art.

Cytoreductive Surgery. With rare exception, the degree of tumor remvoal positively impacts the eventual outcome with respect to disease control. Thus, whenever a total excision can be accomplished this should be the goal of the neurosurgeon. Due to the importance assigned to the degree of tumor removal, advanced strategies have been developed to maximize this goal. Techniques that are now readily available rely on intra-operative imaging including ultrasound, computed tomography (CT), and MRI. Other important operative adjuncts include functional MRI (fMRI), MR tractography, and stereotactic guidance. Fluorescence-guided surgery using 5-aminolevulinic acid has also been reported as a useful technique for differentiating neoplatic tissue from unaffected brain. With the advent of small caliber endoscopes, minimally invasive techniques are quickly evolving as an alternative method for some pediatric brain tumors. These lesions are characterized by a relatively small size (i.e. < 2 cm) and devoid of any significant intraparenchymal component. Another expanding arena of minimally invasive neurosurgery has been the integration of the endoscopic-assisted transnasal approach for tumors situated in the sella and suprasellar compartment.

Tumor Biopsy. As mentioned above, since most children's survival is positively influenced with total tumor removal, irrespective of tissue diagnosis, tumor sampling is rarely indicated. However, some clinical scenarios justify tumor biopsy as an initial neurosurgical alternative, chiefly when neo-adjuvant therapy is the preferred initial treatment. Primary CNS germ cell tumors (GCT), including both germinoma and nongerminomatous GCT (NGGCT), are renowned for being treated without aggressive up-front tumor resection. The diagnostic yield for endoscopic tumor biopsy has been reported to be as great as 90% with little morbidity.

Second-Look Surgery. Since total tumor removal is of such importance in many pediatric CNS tumors, a second attempt at total tumor removal is being used on an increasing basis. There are 3 noted scenarios in which second-look surgery is currently being explored as a feasible alternative to adjuvant therapy: 1) incompletely resected 4th ventricular ependymoma, 2) radiographic residual disease after induction chemotherapy with nongerminomatous germ cell tumors (NGGCT), and 3) malignant tumors of infancy.

Disease Staging. An important and unique physiologic feature of primary pediatric brain tumors is the potential for CSF dissemination. Whether staging information can be obtained at the time of initial surgery is being actively explored in two arenas. Preliminary data exists to support the notion that intra-operative techniques of arachnoid and CSF sampling could play a future role in contributing toward prognostic grading for children with medulloblastoma. Equally novel is the potential of using endoscopic inspection of the intraventricular system. Tumor dissemination observed during endoscopic surgery has recently been highlighted in patients with GCT in which the preoperative MRI failed to exhibit metastatic disease. These current concepts, both awaiting validation through cooperative group studies, would suggest that the pediatric neurosurgeon may play a future role in determining the degree of metastatic dissemination.

Tumor Procurement. An increasing vital role of the neurosurgeon is to submit tissue for investigative purposes. The molecular and genetic analysis of pediatric brain tumors is an exciting and burgeoning field. As an example, prognostic staging for medulloblastoma will soon incorporate molecular characterization to enhance outcome prediction and therapeutic planning. Neurosurgeons are increasingly being relied upon to participate in this vital process and need to be aware of proper tissue preservation and handling.

Therapeutic Delivery. Some surgical techniques are on the forefront of therapeutic delivery of antineoplastic agents. The limitation by the blood brain barrier (BBB) and the frequency of systemic toxicity using systemically administered agents is the basis for exploring the alternative method of local delivery. Local delivery capitalizes on the concept of avoiding systemic toxicity while maximizing local therapeutic concentrations. An additional appealing feature of this technology is the ability to successfully deliver macromolecules such as monoclonal antibodies or virus particles that would otherwise be restricted by the BBB. Biodegradable polymers have already been used extensively in adults with newly diagnosed and recurrent malignant hemispheric gliomas. An alternative form of local delivery, convection enhanced delivery (CED), relies on slow infusion of the agent directly into the brain tumor. The use of CED has gained wide popularity on the investigative preclinical and clinical fronts. Interestingly, CED in the brain stem has been recently and extensively studied as a potential means for treating children with diffuse pontine gliomas.

Conclusions. The neurosurgeon who is called upon to care for children with CNS tumors should be one who is comfortably familiar with these diseases. The experience of the neurosurgeon undoubtedly can have an effect on the peri-operative and long-term outcome of these children. The role of complete tumor removal with minimal morbidity has been the genesis of newer operative techniques and technologies. The role of the pediatric neurosurgeon in the care of children with CNS tumors is expanding to include unconventional responsibilities including disease staging, tissue procurement, and drug delivery. It is thus anticipated that the pediatric neurosurgeon will be increasingly relied upon for oncologic therapeutic strategies and should thus remain abreast of forthcoming information and technologies.

O014

SURGERY OF SUPRATENTORIAL PEDIATRIC BRAIN TUMORS

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Introduction:
The treatment of malignant brain tumors comprises sugery, radiotherapy and chemotherapy. Especially in childhood, patients with malignant brain tumors are enrolled in clinical studies. This allows uniform treatment protocols, comparable results and close follow-ups.

Patients and Results:
Children with malignant supratentorial tumors, e.g. PNETs, glioblastomas, malignant ependymomas, plexus carcinomas, and rarely occurring tumors, e.g. chondrosarcomas and anaplastic meningiomas were admitted to our department. Surgical resection was performed in all cases. Whenever possible, even recurrent tumors or metastases were resected. In highly vascularized lesions, preoperative embolization was considered. Besides surgical resection, the children were all enrolled in clinical studies, being treated according to study protocols.
All children survived the surgical procedures without major neurological deficits. Particularly in tumors of the ventricle (plexus carcinomas and a chondrosarcoma), intraoperative blood loss could be high. The surgical resection of unusually large neoplasms is possible in children without causing major neurological deficits. Especially at very young age, the plasticity of the brain compensates the resection of large intraparenchymal tumor masses, not compromising the normal development of the children. Besides well established radio- and chemotherapeutical protocols, multiple resections seem to be justified. They are well tolerated and help to prevent neurological deficits and prolong the survival time of the young patients.

Conclusions:
Surgical resection is well tolerated by most of the infants and children, independent on age. The inclusion into clinical studies should be mandatory, leading to optimal radiotherapeutical and chemotherapeutical management and a close follow-up. Re-operations should be considered, whenever possible.

O015

SURGICAL RESECTION STRATEGIES FOR OPTIMIZING GLIOMA REMOVAL

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There is still no general consensus in the literature regarding the role of extent of glioma resection in improving patient outcome. Although the importance of resection in obtaining tissue diagnosis and alleviating symptoms is clear, a lack of Class I evidence prevents similar certainty in assessing the influence of extent of resection. We reviewed every major clinical publication since 1990 on the role of extent of resection in glioma outcome. Twenty-eight high-grade glioma articles and 10 low-grade glioma articles were examined in terms of quality of evidence, expected extent of resection, and survival benefit. Despite persistent limitations in the quality of data, mounting evidence suggests that more extensive surgical resection is associated with longer life expectancy for both low- and high-grade gliomas

The prognostic role of extent of resection (EOR) of low-grade gliomas (LGGs) is a major controversy. We designed a retrospective study to assess the influence of EOR on long-term outcomes of LGGs. The study population (N = 216) included adults undergoing initial resection of hemispheric LGG. Region-of-interest analysis was performed to measure tumor volumes based on fluid-attenuated inversion-recovery (FLAIR) imaging. Median preoperative and postoperative tumor volumes and EOR were 36.6 cm(3) (range, 0.7 to 246.1 cm(3)), 3.7 cm(3) (range, 0 to 197.8 cm(3)) and 88.0% (range, 5% to 100%), respectively. There was no operative mortality. New postoperative deficits were noted in 36 patients (17%); however, all but four had complete recovery. There were 34 deaths (16%; median follow-up, 4.4 years). Progression and malignant progression were identified in 95 (44%) and 44 (20%) cases, respectively. Patients with at least 90% EOR had 5- and 8-year overall survival (OS) rates of 97% and 91%, respectively, whereas patients with less than 90% EOR had 5- and 8-year OS rates of 76% and 60%, respectively. After adjusting each measure of tumor burden for age, Karnofsky performance score (KPS), tumor location, and tumor subtype, OS was predicted by EOR (hazard ratio [HR] = 0.972; 95% CI, 0.960 to 0.983; P < .001), log preoperative tumor volume (HR = 4.442; 95% CI, 1.601 to 12.320; P = .004), and postoperative tumor volume (HR = 1.010; 95% CI, 1.001 to 1.019; P = .03), progression-free survival was predicted by log preoperative tumor volume (HR = 2.711; 95% CI, 1.590 to 4.623; P <or= .001) and postoperative tumor volume (HR = 1.007; 95% CI, 1.001 to 1.014; P = .035), and malignant progression-free survival was predicted by EOR (HR = 0.983; 95% CI, 0.972 to 0.995; P = .005) and log preoperative tumor volume (HR = 3.826; 95% CI, 1.632 to 8.969; P = .002). Improved outcome among adult patients with hemispheric LGG is predicted by greater EOR..

Language sites in the cortex of the brain vary among patients. Language mapping while the patient is awake is an intraoperative technique designed to minimize language deficits associated with brain-tumor resection. To study language function after brain-tumor resection with language mapping, we examined 250 consecutive patients with gliomas. Positive language sites (i.e., language regions in the cortex of the brain, 1 cm by 1 cm, which were temporarily inactivated by means of a bipolar electrode) were identified and categorized into cortical language maps. The tumors were resected up to 1 cm from the cortical areas where intraoperative stimulation produced a disturbance in language. Our resection strategy did not require identification of the stimulation-induced language sites within the field of exposure. A total of 145 of the 250 patients (58.0%) had at least one site with an intraoperative stimulation-induced speech arrest, 82 patients had anomia, and 23 patients had alexia. Overall, 3094 of 3281 cortical sites (94.3%) were not associated with stimulation-induced language deficits. A total of 159 patients (63.6%) had intact speech preoperatively. One week after surgery, baseline language function remained in 194 patients (77.6%), it worsened in 21 patients (8.4%), and 35 patients (14.0%) had new speech deficits. However, 6 months after surgery, only 4 of 243 surviving patients (1.6%) had a persistent language deficit. Cortical maps generated with intraoperative language data also showed surprising variability in language localization within the dominant hemisphere. Craniotomies tailored to limit cortical exposure, even without localization of positive language sites, permit most gliomas to be aggressively resected without language deficits. The composite language maps generated in our study suggest that our current models of human language organization insufficiently account for observed language function.

O016

GLIOMA SURGERY, ITS ADVANCEMENTS, PROBLEMS AND FUTURE

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It is well accepted that surgical management of malignant gliomas has dramatically changed by using new advanced modalities. Preoperative precise anatomical and functional information enabled safe and sure surgical resection of malignant gliomas around the eloquent areas. It could be mentioned that surgery contributed to get good QOL of glioma patients. However, improvement of survival duration has not been achieved on GBM, and more effective adjuvant therapy should be expected. This time, I would like to show how it is possible to promote to more effective surgery, including our new intra-operative MRI system (Surgical Suite) and review the problems with glioma surgery. Future glioma treatment, including pathophysiology of glioma and future advancement of technology against glioma, will be addressed in my talk.

O017

AWAKE SURGERY DURING RESECTION OF INSULAR GLIOMAS

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Purpose of the study. In glioma surgery, maximal cytoreduction followed by chemoradiotherapy is regarded standard of care for glioma treatment at present. Insular gliomas, however, are by many still considered to be inoperable. Anatomical localisation, vascular supply, and the potential devastating complications make a complete resection highly impossible. We present our experience with the operative treatment of patients with insular gliomas in an "awake craniotomy" setting. Patients and methods. Sixteen consecutive patients with an insular tumor were operated awake during the period 2003 and 2008. Pre-operatively, an extensive anaesthesiologic and neuropsychologic/linquistic workup was performed. All patients underwent MRI with neuronavigation and when possible fMRI. After cortical stimulation, de Sylvian fissure and periinsular sulci were opened. Tumor resection was performed under speech (dominant hemisphere) and motor movement surveillance. Results. The patients' average age was 41.6 (SD 10.2) years. Pure insular lesions were seen in two patients, a medial temporal base-insular glioma in one, insular fronto-opercular and orbitofrontal-insular-temporal polar in five and eight patients, respectively. Presenting symptoms included epilepsy (94%), dysphasia (19%), and cognitive problems (19%). In eleven patients, the resection was near total (95-98%) and less than 95% in the remaining five patients. At histological examination we encountered thirteen low-grade and three high-grade gliomas. The average follow-up was 1.8 (SD 1.4) years since surgery. Perioperatively seven 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, anaesthesiologist, and patient interaction.

O018

AWAKE CRANIOTOMY FOR THE PRESERVATION OF THE BRAIN FUNCTIONS INCLUDING LANGUAGE, MOTOR, AND HIGHER BRAIN FUNCTIONS

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OBJECTIVE: In the treatment of malignant brain tumors, maximum resection is recommended. However, aggressive resection tends to cause neurological deteriolation such as paresis, aphasia, and many deficits of higher brain functions. For the maximum tumor resection without any neurological deficit, we perfomed intraoperative neurological monitoring of not only language and motor function, but also various higher brain function in awake craniotomy. **METHODS:** From April 2003 to December 2008, 80 tumor resections under awake craniotomies were performed in Komagome Metropolitan Hospital. The tumors included 28 gliomas, 39 metastatic brain tumors, 4 radiation necroses, and 9 other brain tumors. Intraoperatively, we evaluated motor function in 47 surgeries, language in 26 surgeries, and higher brain functions such as imitation, writing, motor programming, visual memory, emotion etc. in 19 surgeries according to the location of tumors. Functional magnetic resonance imaging (f-MRI) and fiber-trackng integrated neuronavigation was also used during tumor resection. Neurological examination was performed before and after the surgery. **RESULTS:** The mean extent of tumor resection was not inferior to that of our prior series of surgeries under general anesthesia. Of 47 patients harboring tumor within or adjacent to motor area, only 4 patients experienced permanent deteriolation of paresis after surgeries. Among the 19 patients with tumors located nearby language area, only one patients suffered worsening of aphasia after surgeries. In the evaluation of higher brain function during the tumor resection, we found various symptoms such as imitation disorder, writing disturbance, memory loss and so on. But the symptoms were improved after discontinuation of the resection, and no permanent disorder was recognized. **CONCLUSIONS:** Awake craniotomy contributes to the preservation of neurological functions with acceptable extent of tumor resection and keeps high performance status of the malignant brain tumor patient after surgery.

O019

CRANIAL SURGERY AND NAVIGATION BY USING LOW-FIELD-STRENGTH INTRAOPERATIVE MAGNETIC RESONANCE IMAGING: EXPERIENCE IN 251 PATIENTS

Cancelled

O020

DEVELOPMENT OF THE COMPACT MAGNETIC RESONANCE IMAGING SYSTEM FOR INTRAOPERATIVE IMAGING.

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Introduction: Magnetic resonance imaging (MRI) during surgery has been proven to improve surgical resection rate and to reduce postoperative complication during brain tumor surgery. However, current commercially available intraoperative MRI system is relatively large and requires extra room for installment. To distribute such technique in world wide, we need to build a small intraoperative MRI system with good imaging quality. **Method:** With collaboration with Yoshida MFG. Co. Ltd., we created a new MRI system. Our concept in creating the machine is, 1) the 5 gauss line is with in the circle of 3m in diameter and 2) the system weighs less than 3 tons, which will be the limit of routine hospital elevator carriage. 3) Also, we desired imaging time to obtain essential intraoperative imaging series should be less than 30minutes. **Results:** Our system called "Vesalius", fulfilled our requirements. The size of the system is half of the currently available systems and the 5 gauss line was confined within 2.2m in diameter around the center of the machine. However, to reduce outer magnetic influence, we need limit the inter-polar gap to 35cm. With this limitation, we improved operative bed and head clamping system. High quality images (including T1, T2, Flair) with less image distortion was obtained within 30min. We are now starting clinical application. Conclusion; We have build new intraoperative small low magnetic field MRI system, with high imaging quality. We believe this system will significantly improve future brain tumor surgery.

Key word: intraoperative magnetic resonance imaging, brain tumor surgery, low magnetic field

Disclosure:
The first and second authors has no financial relation ship with the manufacturing company. The last author is an employee of the company involved in the development of this system.

O021

PRESURGICAL PLANNING FOR CEREBRAL GLIOMA WITH PYRAMIDAL TRACT INVOLVEMENT IN A STEREOSCOPIC DTI-BASED VIRTUAL REALITY ENVIRONMENT

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OBJECTIVE: Information of glioma mass and involved pyramidal tracts are invaluable clinically to surgical trajectory plan and therapeutic outcome. We used diffusion tensor tractography in a stereoscopic virtual reality(VR) environment to perform presurgical planning for cerebral glioma with pyramidal tracts(PT) involvement. **METHODS:** 45 glioma cases of Patient-specific Digital Imaging and Communications in Medicine(Dicom) data from diffusion tensor imaging(DTI) and three-dimensional magnetic resonance imaging scans were transferred to the workstation (Dextroscope; Volume Interactions Pte. Ltd., Singapore.). The tumor and adjacent PT were segmented and reconstructed for presurgical planning and intraoperative Guidance. The number of effective pyramidal tracts(EPT) and the motor strength after operation are compared with those before operation. **RESULTS:** On this VR workstation, diffusion tensor tractography and three-dimensional magnetic resonance imaging scans were integrated. The brain tissue,glioma mass and PT were obviously displayed. An individual presurgical project was meticulously planned in every case. There was no significant difference between presurgical and postsurgical motor strength,as well as the presurgical EPT and postsurgical EPT in our study. A positive relationship was found between presurgical EPT and follow-up motor strength. **CONCLUSION:** Diffusion tensor tractography in this VR environment exhibits the information of glioma mass and the involved PT, which is invaluable in designing a suitable presurgical trajectory. According to the presurgical tractography we can predict as well as optimize the therapeutic outcome. **KEY WORDS:** virtual reality; diffusion tensor imaging; tractography;outcome; glioma

O022

ROLE OF DTI FIBER TRACKING IN PREDICTING THE EXTENT OF RESECTION IN GLIOMA SURGERY

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Aim: To assess the ability of pre-operative DTI-Fiber Tracking (DTI-FT) to predict the extent of resection achievable during surgery of gliomas performed with intraoperative brain mapping. **Materials and methods:** 70 patients with lesions located in the frontal, temporal and insular lobes of the dominant hemisphere were included. All of the patients, after preoperative neuropsychological assessment, underwent DTI-FT of the Cortico-Spinal Tract (CST) and of the Inferior Fronto-Occipital (IFO) fasciculus. Each of the tracts (CST and IFO) was scored by two independent observer as being inside or outside of the tumor. Awake intraoperative language and motor brain mapping included EEG, ECoG, EMG and MEP monitoring. Surgery was carried out according to the functional boundaries of the lesion. Then, for each patient preoperative and postoperative MR images and DTI-FT were loaded into the neuronavigation software and image fusion was used in order 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 (inside/outside of the lesion) of each tract (CST, IFO) and the extent of resection was also investigated. **Results:** The CST was inside the lesion in 38 patients (54%) and the IFO in 23 patients (33%). In all the cases in which the CST (38 pts) or IFO (23 pts) was inside the lesion, we found only incomplete resections (subtotal 19, partial 31). When CST and IFO were both outside the lesion, complete resections was carried out (20 patients). **Conclusions:** DTI-FT of the CST and IFO can help to predict the possibility of total resection. In particular, when CST and IFO are inside the tumor a radical removal is rarely possible, while when outside of lesion, an extensive resection in the dominant hemisphere with the aid of brain mapping may be possible.

O023

OPTICAL COHERENCE TOMOGRAPHY FOR INTRAOPERATIVE ANALYSIS OF GLIOMA TISSUE MICROSTRUCTURE AND LIGHT ATTENUATION AS A NOVEL TECHNIQUE TO CONTROL THE EXTENT OF RESECTION

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Objective
Optical coherence tomography (OCT) is a non-invasive imaging technique with a micrometer resolution. It operates in no tissue contact and generates images based on the reflection of infrared light. We have demonstrated that OCT discriminates tumor and normal brain in experimental preclinical settings. We have developed an OCT-integrated operating microscope and we have performed a clinical study in 20 patients.

Method
The optical path of an operating microscope was optimized for the near-infrared and conventional light spectrum. The OCT-integrated microscope was used to analyse brain tumor tissue and areas of the resection cavity during resection of 20 malignant gliomas. We have used post image acquisition processing to compensate for movements of the brain and to calculate light attenuation factors for brain and brain tumor tissue.

Results
OCT imaging of cortex and white matter showed a typical light attenuation profile. Tumor tissue was identified by loss of the normal light attenuation and appearance of a prominent microstructure. OCT analysis allowed discrimination of normal brain tissue, invaded brain, and solid tumor tissue. In a clinical study OCT analysis of the resection cavity demonstrated residual tumor following macroscopically complete resections, which was confirmed by histological analysis. The blinded scoring of 41 tissue samples for OCT characteristics (microstructure and light attenuation) showed a high correlation with conventional histology (r = 0.99). The OCT integrated microscope remained fully functional for microsurgery and allowed OCT analysis of a 3 - 50 mm scan line at a zoom factor 2 - 14.3.

Conclusion
Integration of OCT into an operating microscope allows continuous tissue analysis of the resection edge during resection of brain tumors and identifies residual tumor prior to secondary changes such as induced by tissue contusion. OCT allows the detection of residual tumor and extents the information provided by conventional operating microscopes today.

O024

SYSTEMIC INHIBITION OF TRANSFORMING GROWTH FACTOR β IN GLIOMA-BEARING MICE IMPROVED THE THERAPEUTIC EFFICACY OF PEPTIDE VACCINATIONS TARGETING GLIOMA-ASSOCIATED ANTIGENS

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A variety of cancers, including malignant gliomas, overexpress transforming growth factor β (TGF-β), which helps tumors evade effective immune surveillance through inhibiting cytolytic activity of natural killer cells and CD8+ cytotoxic T lymphocytes (CTL), and enhancing the generation of regulatory T cells (Tregs). We hypothesized that inhibition of TGF-β would improve the efficacy of vaccines targeting glioma-associated antigen (GAA)-derived CTL epitopes by reversal of immunosuppression. In mice bearing intracranial GL261 gliomas, intraperitoneal (i.p.) administration of the TGF-β neutralizing monoclonal antibody (mAb), 1D11, was combined with subcutaneous vaccinations of synthetic peptides for GAA-derived CTL epitopes, GARC-1 (77-85) and EphA2 (671-679) emulsified in Incomplete Freund's Adjuvant (IFA). Mice receiving the combination regimen exhibited significantly prolonged survival compared with mice receiving either 1D11 alone, GAA-vaccines alone or mock-treatments alone. 1D11 administration enhanced the systemic induction of antigen-specific CTLs in GL261-bearing mice. Flow cytometric analyses of brain infiltrating lymphocytes (BILs) revealed that i.p. 1D11 administration increased GAA-reactive/IFN-γ-producing CD8+ T cells and reduced CD4+/FoxP3+ Tregs in the glioma microenvironment. Systemic 1D11 administration also up-regulated plasma levels of interleukin (IL)-12, macrophage inflammatory protein (MIP)-α and interferon (IFN)-inducible protein (IP)-10, suggesting a systemic promotion of type-1 cytokine/chemokine production. Furthermore, 1D11 treatment up-regulated plasma IL-15 levels and promoted the persistence of GAA-reactive CD8+ T cells in the GL261-bearing mice. These data suggest that systemic inhibition of TGF-β by 1D11 can reverse the suppressive immunological environment of intracranial tumor bearing mice both systemically and locally, thereby promoting the therapeutic efficacy of GAA-vaccines.

O025

INTERFERON-α SIGNALING IN THE BRAIN PLAYS A MAJOR ROLE IN IMMUNE SURVEILLANCE DURING GLIOMA-GENESIS IN MICE

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Recently, a novel spontaneous mouse glioma model has been developed, where *Sleeping Beauty (SB)* transposase-mediated stable transfection of oncogenes in the mouse brain induces central nervous system (CNS) tumors recapitulating human malignant glioma. This system allows us to address the mystery of early events in glioma-genesis. We have previously demonstrated critical roles of type-1 adaptive immunity in control of established intracranial gliomas in mice. Although decreases in type-1 cytokine levels have been reported in glioma patients, it has not been determined whether such changes merely reflect consequences of established glioma and/or whether pre-existing alterations play any roles in occurrence of gliomas. We hypothesized that the interferon (IFN)-α signaling pathway might play a pivotal role in immune surveillance protecting against glioma development. We induced gliomas in IFN-α receptor (R)1$^{-/-}$ or wild type neonatal mice via intracranial injections of plasmids encoding *NRas*, short hairpin RNA for *p53*, and *EGFRvIII*, along with the fourth vector encoding *SB* transposase. Spontaneous tumors in IFN-αR1$^{-/-}$ mice grew and ceased hosts more rapidly than those in immune-competent mice. When tumor-infiltrating lymphocytes were analyzed, IFN-αR1$^{-/-}$ mice showed increased accumulation of CD4^{+}FoxP3^{+} regulatory T cells and CD11b^{+}Gr-1^{+} myeloid-derived suppressor cells in tumors. Furthermore, following intravenous infusions of Pmel-I mouse-derived CTLs, IFN-αR1$^{-/-}$ mice exhibited reduced accumulation of transferred CTLs at the tumor site compared with wild type mice. Of great interest is that when primary tumor cells were cultured and subsequently injected into the brain of immune-competent hosts, IFN-αR1$^{-/-}$ glioma cells demonstrated a remarkably lesser degree of tumorigenicity compared with those from wild type mice, suggesting a possibility that IFN-αR1$^{-/-}$ hosts allowed growth of tumors that could be immunologically rejected or inhibited in immune-competent mice. These results suggest a potentially pivotal role of IFN-α reactivity in CNS immunological surveillance of gliomas, and warrant well-designed immuno-epidemiological studies in humans.

O026

AUTOLOGOUS DENDRITIC CELLS-BASED IMMUNOTHERAPY FOR MALIGNANT GLIOMAS- A PHASE II PROSPECTIVE RANDOMIZED CLINICAL TRIAL

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Background: To perform a prospective phase II clinical trial using adjuvant autologous dendritic cell-based immunotherapy for malignant gliomas. **Methods:** Patients aged 14 to 70 years with malignant gliomas and initial treatment Karnosfky Performance Scale (KPS) score of more than 50 were enrolled. Eighteen patients with newly diagnosed malignant gliomas underwent conventional treatments (surgery, radiotherapy, and chemotherapy) and adjuvant autologous dendritic cell immunotherapy. The control group (n=18) underwent conventional treatments only. **Results:** The follow-up time ranged from 18 to 50 months (median 30 months). Fourteen of 18 patients (78%) who received immunotherapy survived, but only 17% survived in the control group ($p<0.01$). The median survival time for the immunotherapy group was 25.5 months and for the control group 16.7 months ($p<0.05$). Four of 9 patients with glioblastoma multiforme (GBM) (44%) who received immunotherapy survived more than 24 months compared with none in the control group ($p=0.03$). The survival fraction was significantly higher in the immunotherapy group compared with the control group based on Kaplan Meier analysis ($p<0.01$). The remarkable response rate in the immunotherapy group was 56% but only 6% in the control group ($p<0.01$). The recurrence rate was lower in the immunotherapy group (8/18) than in the control group (17/18) ($p<0.01$). The KPS maintenance rate was significantly better in the immunotherapy group (80%) than in the control group (50%) ($p=0.02$). **Conclusions:** Adjuvant autologous dendritic cell immunotherapy may improve survival time and rate, reduce recurrence rate, and maintain better quality of life. It seems to be a safe and effective adjuvant treatment for malignant gliomas. Multimodality treatment for malignant gliomas is essential.

O027

PHASE II CLINICAL TRIAL OF WT1 VACCINATION AGAINST RECURRENT GLIOBLASTOMA WITH ANALYSES OF WT1 SPECIFIC CYTOTOXIC T LYMPHOCYTES

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(Purpose) To investigate the safety and clinical responses of immunotherapy targeting WT1 gene product, 31 patients with WT1/HLA-A*2402 positive recurrent glioblastoma (GB) were evaluated in a phase II clinical study. **(Material and Method)** All patients were intradermally injected with an HLA-A*2402-restricted, modified 9-mer WT1 peptide every week for 12 weeks. The responses on MRI were analyzed by RECIST criteria 12 weeks after the initial vaccination. Progression-free (PFS) and overall survival (OS) after initial WT1 treatment were estimated. The percentage of numbers of WT1 specific cytotoxic T lymphocytes (CTL) among those of all lymphocytes in the patients' peripheral blood were drawn using flow-cytometry, to clarify the effect of concomitantly used steroids and anti-epileptic drugs (AEDs). The same analyses were done with peripheral blood from another group of 14 patients with WT1/HLA-A*2402 positive malignant gliomas, who has been receiving oral administration of temozolomide, to give a rationale of the combined chemo- immunotherapy. **(Results)** The protocol of phase II study was well tolerated. The clinical responses included: none with complete response (CR), 2 patients with partial response (PR), 13 with stable disease (SD), and 16 with progressive disease (PD). The overall response rate (CR+PR) was 6.5%, and the disease control rate (CR+PR+SD) was 48.4%. The median PFS was 16.0 weeks and PFS at 6 months was 29.0%. The median OS after initial vaccination reached to 267 days. The analyses of WT1 specific CTL revealed that neither concomitantly used agents (steroids, AEDs) nor temozolomide does affect the percentage of WT1 specific CTL. **(Conclusion)** This study showed that WT1 vaccine therapy for patients with GB was safe and had a clinical response as compared with any other therapeutic modalities. Analyses of WT1 specific CTL raised the possibility of concomitant use of considerable dose of steroids and the rationale of combined chemo-immunotherapy planning in the future.

O028

PHASE I/IIA TRIAL OF AUTOLOGOUS FORMALIN FIXED TUMOR VACCINE IN TREATMENT OF NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME.

Yoshihiro Muragaki[1], Takashi Maruyama[1], Hiroshi Iseki[1], Kintomo Takakura[1], Masahiko Tanaka[1], Chie Shinohara[1], Koji Tsuboi[2], Tetsuya Yamamoto[3], Akira Matsumura[3], Masao Matsutani[4], Katsuyuki Karasawa[5], Tadao Ohno[6], Tomokatsu Hori[1]

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Origin of Study: Tokyo and Ibaragi, Japan

Topic: J immunotherapy

(Introduction) Autologous formalin-fixed tumor vaccine (AFTV) could induce killer lymphocytes in vivo and exhibited prophylactic effect against recurrence of hepatocellular carcinoma after the surgery in a randomized clinical trial. AFTV was combined with radiotherapy (RT) for primary glioblastoma (GBM) in a phase I/IIa trial. (Patients and Methods) Adults after resection of GBM were treated with standard RT and AFTV. AFTV was prepared with fixed GBM fragments, PPD, and BCG extracts suspended in saline. When the cumulative radiation dose reached 32-36 Gy, AFTV treatment was started and injected three times weekly. Second delayed type hypersensitivity tests (DTH2) were performed two weeks after third and last vaccination. The primary endpoint was overall survival (OS) and secondary endopoint was progression free survival (PFS). (Results) Twenty-two eligible patients from two hospitals were accrued with a median follow-up time of 18.9 months (M). Median OS and PFS were 18 M and 7.6 M, respectively. OS and PFS of RT with AFTV were not significantly different from those of RT with Temozolomide in the same hospital. The patients whose DTH2 reaction was more than 12mm showed significantly longer OS and PFS than those with DTH2 reaction less than 12mm (OS: 22.5M v.s. 18M, PFS: 14M v.s. 7.5M,). There was no significant adverse event whose grade was more than grade 2. (Conclusion) Concomitant use of AFTV in the late stage of radiotherapy resulted in comparable median times of PFS and OS when compared to our historical controls of RT with Temozolomide without severe adverse events.

O029

TYPE-1 DENDRITIC CELL VACCINES IN COMBINATION WITH POLY-ICLC - ASSOCIATION BETWEEN POSITIVE TETRAMER RESPONSE AND 6-MONTH PROGRESSION-FREE SURVIVAL

Hideho Okada[2], Frank S. Lieberman[1], Ryo Ueda[1], Mitsugu Fujita[1], Pawel Kalinski[2], Arlan H. Mintz[1], Deric M. Park[1], David L. Bartlett[2], Charles K. Brown[2], Herbert Zeh[2], Theresa L. Whiteside[3], Lisa H. Butterfield[3], Ronald L. Hamilton[1], Andres M. Salazar[4], Ian F. Pollack[1]

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Our previous preclinical studies have demonstrated that intramuscular (i.m.) administration of a Toll-like receptor 3 ligand poly-ICLC remarkably enhances induction of type-1 cytotoxic T-lymphocytes (CTLs) and improves therapeutic efficacy of vaccinations against glioma-associated antigen-derived CD8+ T cell epitopes. Based on these studies, we have developed a phase I/II trial. Human leukocyte Antigen (HLA)-A2+ participants with recurrent malignant glioma received intra-lymph-node injections of type-1 dendritic cells (DCs) loaded with HLA-A2 binding peptides EphA2 (883-891), IL-13Rα2 (345-353:1A9V), YKL-40 (202-211) and GP100 (209-217: 2M) at two-week intervals. Participants also received twice weekly i.m. injections of 20μg/kg poly-ICLC. Participants who demonstrated positive radiological response or stable disease without major adverse events were allowed to receive booster vaccines. Primary endpoints were assessments of safety and immunological responses. Clinical and radiological responses were also evaluated. To date, 13 participants (6 with glioblastoma multiforme [GBM], 5 with anaplastic astrocytoma [AA] and 2 anaplastic oligodendroglioma [AO]) have received vaccinations with no major adverse events. Increased CD8+ cells reactive to HLA-A2.1-EphA2 (883-891) or HLA-A2.1-IL-13Rα2 (345-353) tetramers were detected in post-vaccine peripheral blood mononuclear cells (PBMC) in 7 of 9 participants evaluated. These patients also demonstrated up-regulation of a chemokine receptor CXCR3 on CD8+ PBMC following vaccines, indicating that the vaccine regimen induced type-1 CTL responses. One of these participants with recurrent GBM exhibited partial radiological response, which persisted for 7 months with booster vaccines. Biopsy of tumor site after vaccination in this participant revealed intensive infiltration of CD8+ T cells and macrophages. Six patients achieved progression free at 6 months (2 GBM, 2AA and 2 AO). Fisher's exact test indicated an association between positive tetramer response and 6-month progression-free survival, suggesting a possible correlation between tetramer-detected immune responses and clinical response. These interim data demonstrate preliminary safety and immunological activity of poly-ICLC-assisted type-1 DC-based vaccines. Grants: 1R21-CA117152, 1P01-NS40923

O030

INDUCIBLE ONCOLYTIC TRANSFORMATION OF REPLICATION-DEFICIENT VIRUS FOR THE CELL-BASED VIROTHERAPY

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Replication-conditional viral mutants promise an alternative treatment modality for malignant gliomas because of their potential to selectively eradicate tumor cells through oncolysis and spread their progeny throughout the tumor tissues. Initial data obtained from clinical trials have demonstrated their clinical safety, but not their effectiveness, probably because of the still inefficient delivery, propagation and spread of such viruses in tumor tissues. Efficient delivery of oncolytic viruses to tumor regions is desirable due to the diffusely invasive property of malignant gliomas as a major obstacle in their treatments. Cell-based delivery of oncolytic viruses has become a promising strategy to target the diffused tumors. Some cell types like neural and mesenchymal stem cells have a homing ability to pathological regions including tumors. However, oncolytic viral carrier cells may not survive long enough to migrate to scattered tumor regions, because of viral lytic cycle.. To overcome this limitation, we have developed a novel strategy to improve carrier cell-based virotherapy. This new method consists of two parts: 1) transformation from replication-deficient pro-oncolytic virus to replication-competent oncolytic virus, 2) inducible viral transformation by tumor microenvironment. Our results showed viral gene expression was suppressed in pro-oncolytic virus, but activated by recombinase-based inducer in the infected cells. Once pro-oncolytic viruses changed to replication-competent viruses, these induced viruses efficiently propagated in glioma cells compared to non-induced viruses. This strategy enables viral carrier cells to survive for a longer period of time, compared to cells carrying general oncolytic viruses. We believe that this promising method will enhance the development of cell-based virotherapy.

O031

ONCOLYTIC VIRUS THERAPY OF GLIOMA SENSITIZES GLIOMA CELLS TO CILENGITIDE TREATMENT.

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Objectives: Oncolytic HSV-1 derived viruses (OVs) are being investigated as treatment modalities for many cancers. However, a clear understanding of the action of this biological therapy in human patients is currently not feasible. We have been investigating OV therapy induced changes in the tumor extracellular matrix. **Methods & Results:** Transcript profiling of secreted proteins involved in angiogenesis revealed a significant induction of Cystein-rich 61(CYR61) gene expression in tumors treated with OV in vivo. CYR61 is a secreted ECM protein that can bind to and activate integrins $\alpha v \beta 3$ and $\alpha v \beta 5$. Increased CYR61 in the ECM of breast cancer cells has also been shown to activate an autocrine loop resulting in upregulation of its own receptor $\alpha v \beta 3$ resulting and increased sensitization to integrin anatgonists (Menendez JA, et al. Oncogene 2005). Consistent with this we found that glioma cells stably over expressing CYR61 were much more sensitive to the integrin antagonist, Cilengetide, than control cells. Hence we hypothesized that OV treatment, which induces secreted CYR61, would increase integrins $\alpha v \beta 3$ on both infected and uninfected glioma cells, and sensitize them to Cilengitide. We tested the effect of OV treatment on glioma cells for the expression of integrin receptors $\alpha v \beta 3$. Moreover we detected synergistic killing of glioma cells treated with OV and Cilengitide compared to cells treated with either agent alone. We tested this hypothesis in immunocompromized mice with intracranial tumors (U87dEGFR). Five days after OV treatment mice were injected systemically with a single dose of cRGD (5mg/kg) or PBS. Mice treated with OV and Cilengitide survived significantly longer than mice treated with OV alone ($P < 0.01$ between OV and cRGD + OV). **Conclusion:** These results indicate that OV therapy can sensitize glioma cells to Cilengitide and lays a rationale for designing future clinical trials combining the two agents.

O032

MEDULLOBLASTOMA : THE ROLE OF TOTAL TUMOR REMOVAL TO AVOID RECURRENCE

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Medulloblastoma is one of the most malignant brain tumor. From 1995 to 2009 we have treated 80 cases of medulloblastoma. There were 48 female and 32 male in our series, ranging from 2 months old to 55 years. In all cases, total removal was attempted and achieved in 68 cases (85 %). Total removal was impossible with brainstem attachment.

Radiotherapy following surgery was routine procedure. (except in very young infants) and starting from 2005 we used chemotherapy.

In our series, recurrent rate was 10% with total removal, in comparison with 80% in the other group. Complications included lower cranial nerve palsy. Cerebellar dysfunction and mutism, which all recovered.

Keywords : Medulloblastoma – total tumor removal – recurrent rate

O033

OPTIMIZING THE PHYSICAL AND BIOLOGICAL PARAMETERS OF RADIOTHERAPY IN AVERAGE-RISK MEDULLOBLASTOMA

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Purpose: To optimize physical and biological parameters of radiotherapy in average-risk medulloblastoma. **Methods:** Twelve children with average-risk medulloblastoma were treated with hyperfractionated radiotherapy (HFRT). Radiotherapy consisted of 2 fractions daily of 1 Gy each, 6 hours apart, 36 Gy/36 fractions of craniospinal irradiation (CSI), followed by conformal tumor bed boost (32 Gy/32 fractions) for a total dose of 68 Gy/68 fractions over 6.5 weeks. Chemotherapy was reserved for relapse. **Results:** The median age of the cohort was 8 years (range 5-13 years). Acute hematologic toxicity was self-limiting, not requiring interruption of treatment, growth factor support or platelet transfusions in any patient. Grade II neutropenia and thrombocytopenia was seen in 33% and 16% patients respectively. No one developed grade III-IV thrombocytopenia. Five of 12 children had suboptimal mean Intelligence Quotient (IQ) scores (mean IQ less than 90) even before initiation of radiotherapy. Two patients relapsed, at 18 and 21 months from diagnosis, both supratentorially and in the leptomeninges, of which one died at 25 months despite salvage chemotherapy. With a median follow-up of 19 months (range 6-30 months), the 2-year event-free-survival and overall survival was 65% and 100% respectively. Mean IQ scores for all tested domains were preserved in all the 10 children evaluable at 1-year. Two children developed hypothyroidism necessitating thyroxine supplementation, while no patient developed significant sensori-neural deterioration. **Conclusion:** Preliminary analysis suggests that HFRT without chemotherapy for average-risk medulloblastoma is a promising option with acceptable self-limiting acute toxicity, without an unduly increased risk of relapse. Long-term results on larger number of patients are required to confirm its role in reducing the known late sequelae of radiotherapy.

O034

DO MEDULLOBLASTOMAS IN ADOLESCENTS HAVE A DISTINCT MOLECULAR SIGNATURE & DIFFERENT TUMOR BIOLOGY?

This Abstract nominated the Hoshino Award.
Please refer to P061 on page 151.

O035

AUTOLOGOUS HEMATOPOETIC STEM CELL TRANSPLANTATION FOR CHILDREN WITH HIGH RISK BRAIN TUMORS

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Purpose: Although the outcome of brain tumor in children has improved significantly over decades, the high-risk (HR) brain tumors such as recurrent, residual or infant disease still have a poor prognosis. Autologous hematopoetic stem cell transplantation (AHSCT) has been applied to improve the outcome of HR brain tumors. We report on the outcome for HR brain tumors receiving AHSCT at a single institution. **Method:** Between October 1999 and August 2008, 20 patients received AHSCT using peripheral blood stem cells for HR brain tumor at Asan Medical Center. Their medical records including transplant data were reviewed and the outcome was analyzed as of December 2008. **Results:** Of 21 patients, 8 had medulloblastoma (2 infant, 2 metastatic, 3 recurrent), 6 had supratentorial PNET (1 infant, 1 residual, 4 recurrent), 2 had pineoblastoma (1 infant, 1 recurrent), 2 had ATRT, and 2 had recurrent GCT. At transplantation, 13 patients were in CR and 7 in PR. Nine patients (CR 5 & PR 4) received conditioning with cyclophosphamide/melphalan (CM) and 11 (CR 8 & PR 3) with carboplatin/thiotepa/etoposide (CTE). After AHSCT, 5 patients (4 in CM, 1 in CTE) relapsed and 4 patients (3 in CM, 1 in CTE) died of causes other then disease progression. With a median follow-up of 33.7 months, 3-y EFS and OS were 50.8% and 56.1%, respectively. CR at the time of AHCT tended to have better EFS compared to PR (64.5% vs 24.6%, P=0.103). The 3-y EFS for 11 patients conditioned with CTE was better than that of 9 with CM (79.5% vs 22.2%, P=0.014). **Conclusion:** AHSCT with CTE is highly successful treatment modality for HR brain tumors in children. Given the limitation of this study including small number of patients as well as heterogeneous population, further study is warranted to clarify the role of AHSCT in pediatric brain tumors.

O036

CLINICOPATHOLOGICAL FEATURES OF ATYPICAL TERATOID/RHABDOID TUMORS: ITS DIAGNOSIS AND TUMOR GROWTH FACTOR

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Introduction: The clinicopathological features of Atypical Teratoid / Rhabdoid Tumors (AT/RTs) in Japan, and the importance of the differential diagnosis from PNET/medulloblastoma and germ cell tumor are described. **Methods:** Forty one patients with AT/RT in Japan were analyzed about clinicopathological features. **Results:** Forty one AT/RTs (23 male and 18 felame) were aged from 1 month to 37 years (mean 2.9 years) and tended to show increased intracranial pressure by obstructive hydrocephalus and/or tumor volume. This tumor locations were in the posterior fossa (63%), brain hemispheres (22%), spinal (12%), and pineal region (3%). Interestingly, younger patients (under 2 years) with AT/RT tended to located in posterior fossa or spinal region. Leptomeningeal dissemination showed over 50% of AT/RTs. Histologically, AT/RT is defined as a polymorphous neoplasm often featuring rhabdoid, PNET, epithelial, and mesenchymal components. AT/RTs usually include PNET components and occur mainly in the posterior fossa, so mimic medulloblastoma. AT/RT is characterized by the cytogenetic finding of monosomy 22 rather than i(17q). The tumor is similarly mistaken for PNET at supratentorial immunophenotypic diversity, particularly features indicative of epithelial and mesenchymal differentiation. Nonetheless, the remarkable spectrum of tissues that typify teratoma is absent in AT/RT. The prognosis of this tumor is far less favorable than that of PNET/medulloblastoma or germ cell tumor. For the diagnosis of AT/RT, Negativity of INI1 protein is important and IGF-1 receptor tended to express in this tumor cells. **Conclusions:** This study describes the clinicopathological features of 41 AT/RT in Japan and we emphasizes the necessity for distinguishing this unique tumor from other pediatric centran nervous system neoplasms. And INI1 is important for this tumor diagnosis. Moereover, IGF-1 may play a role as growth factor of AT/RT cells.

O037

PRE-CLINICAL TESTING OF COMBINATION RADIATION PLUS HISTONE DEACETYLASE INHIBITOR THERAPY IN TREATING ATYPICAL TERATOID RHABDOID TUMOR

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PURPOSE: The purpose of this study was to determine whether combination treatment involving radiation therapy (RT) and valproic acid (VA), a histone deacetylase (HDAC) inhibitor, provides superior anti-tumor effect than either therapy alone, when evaluated in the context of an intracranial xenograft model of atypical teratoid rhabdoid tumor (ATRT). **METHODS:** 300,000 ATRT cells, modified with luciferase for bioluminescence imaging (BLI), were injected into the supratentorial compartment in each of 35 mice. Tumor cells were monitored by BLI until exponential growth was indicated, at which time mice were randomized into four treatment groups: 1) Vehicle only treatment; 2) RT only (0.5 Gy/day x 5 days); 3) VA only (150 mg/kg/BID x 7 days); or 4) RT and VA administered concurrently as indicated for monotherapy administrations. BLI was used for continuous monitoring of tumor response to therapy, and all mice were followed until euthanasia was required due to presentation of symptoms associated with neurological compromise from tumor burden. **RESULTS:** Mice receiving concurrent RT + VA treatment experienced significant survival benefit relative to vehicle treated control group animals ($p = 0.0009$), as well as with respect to mice receiving either treatment as a monotherapy ($p = 0.0017$ in comparison with VA alone, and $p = 0.0223$ in comparison with RT alone). Bioluminescence imaging revealed that combination therapy reduced the incidence of neuraxis dissemination of intracranial tumor. **CONCLUSIONS:** Our results indicate that combination therapy using an HDAC inhibitor + RT has enhanced anti-tumor activity relative to either therapy used alone, and therefore warrants consideration for use in the young pediatric patients that are affected by this highly malignant central nervous system tumor.

O038

ATYPICAL CHOROID PLEXUS PAPILLOMA: 2008 INTERIM ANALYSIS OF THE CPT-SIOP-2000 STUDY

Johannes E.A. Wolff[1,2], Ove Peters[6], Peter Thall[2], Blanca Diez[3], Martin Hasselblatt[4], Anita Mahajan[5], Brigitte Wrede[6]

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Introduction: Atypical choroid plexus papilloma (APP, WHO grade II) were newly defined by the WHO in 2007 in addition to the previously defined choroid plexus tumors (CPT), which were choroid plexus papilloma (CPP, grade I), and choroid plexus carcinoma (CPC grade III). **Methods:** The CPT-SIOP-2000 study is an international observation and randomized treatment trial for all patients with CPT. After maximal surgical resection, all patients with APP and residual tumor, with metastases or with CPC regardless of residual tumor were treated with chemotherapy (VP16, vincristine and either carboplatinum or cyclophosphamide). Irradiation was given to patients older than 3 years of age between the second and third cycle. The other CPT patients were followed without further treatment. **Results:** Until December 2008, 23 nations registered 152 patients with CPT. In the latest interim analysis, patients with APP (n=32) had a median age of 0.7 years and were thus younger than patients with CPP (2.2 years; n=42) or CPC (2.2 years; n=34). Primary metastases were frequent in CPC (20%) and APP (16%), but also occurred in CPP (5%). Complete resection was achieved in 81% of CPP, 65% of APP, and 50% of CPC. Patients with APP had an intermediate 5-year EFS (84%±10SD) compared to CPP (91%±8SD) and CPC (28%±20 SD; $p<0.0002$). Objective early response (complete remission, partial response) after two cycles of chemotherapy among APP patients receiving further treatment was seen in 6 of 10 patients. **Conclusion:** The intermediate position of APP defined by histology was confirmed by clinical data of tumor resection and frequency of metastases but not in age at diagnosis. Further molecular studies are necessary to determine if there is a further subgroup hidden with different biology among those very young children. APP seem to respond to chemotherapy.

O039

METASTATIC CHOROID PLEXUS TUMORS - DATA OF THE CPT-SIOP-2000 STUDY

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Introduction: Choroid plexus tumors are rare, and the pattern of dissemination has not been described in details yet. **Methods:** a retrospective analysis of the CPT-SIOP-2000 database (Choroid Plexus Tumor-International Society of Pediatric Oncology - 2000) included patients randomized and treated as well as those registered only. **Results:** By June 2008 100 patients were formally registered. In 21 of those, metastases were reported (10 of 21 male, median age 2.7 years range 0.1 to 11.8). This includes five patients documented as metastatic but with a confirmation pending, 12 tumors confirmed metastatic at presentation, three tumors presenting as localized but later recurring as metastatic (secondary), and 1 presenting metastatic and again recurring metastatic. Primary location was the lateral ventricle in 20 and the 4th ventricle in 1 patent. The histology was choroid plexus papilloma 3(CPP), atypical choroid plexus papilloma 5 (APP), and choroid plexus carcinoma 11 (CPC). Primary surgery was: gross total resection 12, partial resection 6, or biopsy 2. All tumors were spread through cerebrospinal fluid. Eight patients received radiation and chemotherapy, 10 chemotherapy only, and 3 neither. Those three had complete resections of either CPP (2) or APP (1), all are alive, and one recurred. Among the other 18, the median event free survival (EFS) was 1.33 years, and the 4 year EFS 51% (+-14 SD), 4 year overall survival 72% (+-19 SD , median not reached). In all recurrences leptomeningeal spread was documented. **Conclusion:** Leptomeningeal spread is common in choroid plexus tumors, in particular when the primary location is supratentorial. The prognosis seems to be better than other metastatic brain tumors.

O040

PEDIATRIC GLIOSARCOMAS: CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS

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Purpose Gliosarcoma (GS) represents a glioblastoma with a sarcomatous component, which is presumed to be a metaplastic differentiation of the glioma cells. We studied the clinical relevance of this histological glioblastoma subentity within the pediatric population. Patients and **Methods** Patient data were obtained from the German HIT-GBM database where clinical data of more than 600 pediatric patients with centrally reviewed high grade gliomas are accumulated. By applying defined inclusion criteria (diagnosis of GS proven by central neuropathological review; patient age 0 to 21 years) 5 patients were identified. In addition, after review of English medical scientific literature 19 additional cases were found. **Results** The relative frequency of GS in the German HIT-GBM database was only 2.4%. In the whole series of 24 pediatric GS including previously reported cases the male-to-female-ratio was 1.3:1. GS was found in all pediatric age groups with a median age of 11 years but there was an unexpectedly high accumulation in infants (7 of 24 less than 3 year of age, 29 %). GS showed a strong predilection of the cerebral hemispheres (23 out of 24 cases). Symptoms of increased intracranial pressure were the leading symptoms of a short clinical history with a median duration of 1 month. Interestingly, 6 patients (25 %) were reported with a history of cranial radiotherapy prior to GS diagnosis. In 62 % of GS in our series, gross total resection was achieved. Median overall (OS) and event-free survival (EFS) of the total cohort were 13.3 and 9.8 months, respectively but infants showed superior survival. **Conclusion** GS is a very rare tumor entity in children. Literature review suggests a relatively higher incidence in infants and in patients with a previous history of radiotherapy.

O041

ROLE OF TEMOZOLOMIDE FOR THE TREATMENT OF NEWLY DIAGNOSED DIFFUSE BRAINSTEM GLIOMA IN CHILDREN- EXPERIENCE IN A SINGLE INSTITUTION

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Purpose: To assess the efficacy of temozolomide (TMZ) on diffuse brainstem glioma as concomitance with radiotherapy and as adjuvant treatment post radiotherapy in children. Patients and **Methods:** Eighteen children (median age at diagnosis, 8.3 years) with newly diagnosed brainstem tumors which were treated with TMZ at Taipei Veterans General Hospital since Jan 2004 to Dec 2008 were eligible for the study. They were divided into two groups by the different treatment modalities. RT+TMZ (radiotherapy alone followed by adjuvant TMZ) group received conventional radiation after initial diagnosis. CCRT+TMZ (concomitant chemoradiotherapy followed by adjuvant TMZ) group received concurrent chemotherapy during radiation with TMZ (75mg/M^2/day). After the completion of radiotherapy, TMZ (150mg/M^2) was administered orally once daily for five consecutive days for all enrolled patients. Treatment cycles were repeated every 28 days. We evaluate the progression-free survival in both groups of patients. **Results:** There were 10 patients in RT+TMZ group and 8 in CCRT+TMZ group. All patients experienced progression of disease. Twelve patients (75%) died in the study period and all deaths were attributed to disease progression. The median progression-free survival (PFS) was 7.4 months for the RT+TMZ group and 6.4months for CCRT+TMZ group. The 6 moths and 1year PFS in RT+TMZ group were 70% (SD, 14%) and 30% (SD, 14%) respectively and in CCRT+TMZ were 50% (SD, 17%) and 0% respectively. The log-rank test in PFS between the two groups was P=0.09. **Conclusions:** In this study, RT followed by TMZ or CCRT followed by TMZ did not alter the poor prognosis of the newly diagnosed diffuse brainstem glioma in children. In addition, CCRT followed by TMZ did not achieve a better outcome than RT followed by TMZ.

O042

ROLE OF SURGERY FOR OPTIC PATHWAY/HYPOTHALAMIC ASTROCYTOMAS IN CHILDREN

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Optic pathway/hypothalamic pilocytic astrocytomas in children are usually treated with chemotherapy following a surgical biopsy. In this report, we retrospectively considered the role of surgical intervention. In a series of 32 patients without neurofibromatosis type 1, the median age at initial treatment was 2.7 years (range, 0-12 years). Twenty-five cases were verified by histology, and seven cases were diagnosed by MRI findings. Twenty-eight patients received chemotherapy. All patients were alive at median follow-up of 65 months. Aims of surgery at the initiation of treatment were biopsy in 15 cases (1 stereotactic and 14 craniotomies) and debulking in 9 cases. The 14 open biopsies revealed pilocytic astrocytoma; however, noticeable complications occurred in six children after the biopsies. Review of preoperative MRIs showed that all had typical findings indicating pilocytic astrocytoma. The open biopsy offered no noteworthy benefit for the patients despite surgical risk and delay of chemotherapy. The extent of the nine resection surgeries was 80% or less removal, and postoperative adjuvant therapy was needed for seven of the nine patients. The remaining seven children who did not undergo surgery obtained remission with chemotherapy alone. One child underwent decompression of the optic canal that yielded amelioration of decreasing vision. After relapse in 11 patients, 19 bulk-reduction surgeries were performed. Surgical resection was not curative in any patient. In six patients, mostly older children, cystic expansion of tumor was partially resected, resulting in additional remission. In six children beyond the age of adolescent, spontaneous tumor involution was observed. In conclusion, considering the risk of open surgery and the effectiveness of chemotherapy, the role of surgical intervention is restricted to bulk-reduction surgery only when it is inevitable, especially at relapse after chemotherapy.

O043

PROGNOSITIC ANALYSIS OF 220 PATIENTS WITH INTRAMEDULLARY SPINAL CORD EPENDYMOMAS

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Objective: To assess the factors relative to the surgical prognosis of intramedullary spinal cord ependymomas. **Methods:** 220 patients harbored intramedullary spinal cord ependymomas were treated surgically in Beijing Tiantan Hospital from 2000 to 2007. We followed them on telephone and analyzed their JOA score preoperatively and postoperatively about age,gender,length of tumor and spinal cord function by Spss 11.5 software. **Results:** Age,gender and length of tumor had no significant influence on prognosis. Through multiple linear regression analysis and Logistic regression,we concluded that the postoprative JOA score highly relatived to the preoperative neurological status and other factors. **Conclusion:** Determinant factors of prognosis for intramedullary spinal cord ependymomas are preoperative neurological status;extent of removal of the lesion. **KEY WORDS:** intramedullary spinal ependymomas,surgery

O044

GRADING SYSTEM FOR DIAGNOSIS AND TREATMENT OF INTRACRANIAL NONGERMINOMATOUS MALIGNANT GERM CELL TUMORS

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Objective To discuss the clinical feature, treatment, and prognosis of intracranial nongerminomatous malignant germ cell tumors(NGMGCTs). **Methods** The records of 39 patients who were treated in Shanghai Huashan hospital between 1995 and 2007 were reviewed retrospectively. According to the classification of Matsutani in 1997, they were grouped into intermediate prognosis group and poor prognosis group based on the histology of the tumor. Clinical manifestation, diagnosis, treatment and outcome were analysis in each group. **Results** In these 39 cases, there were 15 mix germ cell tumors, 15 immature teratomas, 7 embryonal carcinomas and 2 yolk sac tumors. All patients were treated surgically. The tumor was totally removed in 29 cases, sub-totally in 5, and partially in 3. Biopsy was performed in the other 2 cases. 34 patients (87.2%) were followed up. The common 5-year survival rate was 36.8%. The 5-year actuarial overall survival rate for patients in the intermediate prognosis and poor prognosis groups were 42.6% and 0%. Chemotherapy combined radiotherapy has significant relationship with the prognosis of intermediated prognosis group (P=0.039). The 5-year survival rate of immature teratoma patients who received gamma knife surgery after surgery was 100%. It had significant difference (P=0.0049) compared to the 5-year survival rate of patients who did not receive gamma knife surgery. **Conclusions** NGMGCTs can be divided into intermediate group and poor group base on the prognosis (P=0.0003). Embryonal carcinoma can be classified to the intermediate prognosis group because of its similar prognosis with immature teratoma and mixed tumors mainly composed of germinoma or teratoma. Surgery is the first choice for NGMGCTs because treatment should be base on tumor histology. For patients in the intermediate prognosis group, combined treatment, including surgical resection, radiotherapy, chemotherapy and gamma knife surgery was effective.

O045

OPTIMAL TREATMENT STRATEGY FOR INTRACRANIAL GERM CELL TUMORS: SINGLE INSTITUTION ANALYSIS.

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Purpose: To review the optimum treatment for newly diagnosed intracranial germ cell tumors. Patients and **Methods:** A retrospective review of medical records at our department from April 1990 to March 2007 identified 108 patients, 86 males and 22 females aged from 2 months to 45 years old (median 14 years), with newly diagnosed intracranial germ cell tumors. Their diagnoses were germinoma in 83 patients, and non-germinomatous germ cell tumor in 25. **Results:** In the patients with germinoma, the 10-year overall and progression free survival rates at median follow-up period of 99 months were 86% and 74%, respectively. The recurrence occurred at 6-153 months (median 26 months) after starting the initial therapy. Patients treated with chemotherapy alone had shorter progression-free survival rate, and patients treated with chemotherapy followed by reduced-dose radiation therapy to the whole ventricle, whole brain, or craniospinal axis had significantly better progression-free survival rate compared to radiation alone or reduced-dose radiation therapy to focal field. Non-germinomatous germ cell tumors were divided into good, intermediate and poor prognosis group as proposed by Japanese Japanese Pediatric Brain Tumor Study Group. In the good and intermediate prognosis group, the 10-year overall and progression-free survival rates were 93% and 100%, respectively. In the poor prognosis group, the 3-year overall and progression-free survival rate were 56% and 29%, respectively. In both groups of non-germinomatous germ cell tumors, all of the patients, in whom radiographic lesions have disappeared after combination therapy of surgical resection, radiation therapy, and chemotherapy, were alive without recurrence. **Conclusion:** Chemotherapy followed by reduced-dose radiation therapy to the field covering the whole ventricle at least was effective in the treatment of germinoma. In the initial treatment of non-germinomatous germ cell tumor, combined treatment, including salvage surgery, is essential to achieve the complete disappearance of radiographic lesions.

O046

THE OPTIMAL RADIATION VOLUME AND DOSE FOR INTRACRANIAL GERMINOMA

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Purpose: To review the effectiveness of reduced-dose and restricted volume radiation-only therapy in treatment of intracranial germinoma (IG), and to assess the feasibility of reducing or eliminating the use of chemotherapy.**Methods and Materials:** Between January 1996 and March 2007, 51 patients with IGs underwent variable treatments at Taipei Veterans General Hospital. A retrospective analysis was done of 38 patients with either reduced radiation alone (30 Gy for 26 patients) or reduced radiation with chemotherapy (systemic vinblastine, bleomycin, etoposide, and cisplatin for 12 patients). All 38 patients received extended focal (including whole-ventricle) irradiation, and were followed to February 2008 with characterizing overall (OS) and relapse-free survival (RFS) rates. Variables associated with survival were evaluated by univariate Cox proportional-hazards regression. **Results:** Median follow-up was 62.4 months (range, 10.1-142.5 months). The total 5-year overall survival rate was 93.7%. The 5-year OS and RFS rate for patients receiving radiation only were 100% and 96.2%, respectively. For those receiving radiation plus chemotherapy were 83.3 % and 91.7% (no statistical significance). There was no any predictive factor that was significantly associated with OS or RFS rates, and chemotherapy had no significant effect on survivals but higher incidence of treatment-related toxicity. **Conclusions:** Further decrease of radiation dose to 30 Gy with whole-ventricle irradiation is sufficient to treat selected intracranial germinoma. Use of wide-field irradiation or chemotherapy should be avoided as unnecessary. We recommend exploring further radiation volume and dose reductions to eliminate the risk of radiation-induced treatment complications. Systemic chemotherapy is only recommended for patients at high risk of recurrence or metastasis. Reduction of radiation doses to 30 Gy may be feasible, even without chemotherapy. **Key Words:** Germinoma, whole ventricle irradiation, radiotherapy, complication, craniospinal irradiation.

O047

GERM CELL TUMORS IN THE BASAL GANGLIA: CLINICAL OUTCOMES AND DIAGNOSTIC UTILITY OF POSITRON EMISSION TOMOGRAPHY (PET)

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Introduction Germ cell tumor (GCT) in the basal ganglia is a curious neoplasm. Atypical clinical presentation and peculiar radiological findings are common. In the present study, we analyzed the clinical outcomes of basal ganglia GCTs. Furthermore, the diagnostic utility of positron emission tomography (PET) was analyzed. **Materials and Methods** The clinical data of 21 consecutive patients from one institution were retrospectively analyzed. Nineteen patients were male and two were female. The median age at diagnosis was 13 years (range 7-19 years). Progressive hemiparesis was the most common symptom, followed by bulbar symptoms, psychiatric problems, symptoms related to the increased intracranial pressure (IICP), and hyperkinetic movements. [F18]-fluorodeoxyglucose (FDG) PET was performed in 12 patients. [C11]-methionine PET was performed in 5 patients (2 for primary tumors and 3 for recurred metastatic tumors). **Results** Nineteen tumor were germinomas and two were mixed GCTs. The median period of diagnostic delay was 7 months (1-31 months). [18F]-FDG PET showed a hypometabolic lesion in 11 patients and a hypermetabolic lesion in only one patient with a mixed GCT. In contrast, [C11]-methionine PET showed a hypermetabolic lesion in all patients. The two patients with a mixed GCT expired within one year from the diagnosis. The mean follow-up period of the 19 patients with a germinoma was 46 months (1-106 months): four patients (21%) had tumor progression and three patients (16%) expired at the last follow-up. The patients with poor outcomes had a significantly prolonged period of diagnostic delay ($p=0.003$). The hyperkinetic movement disappeared after treatment in all patients. The hemiparesis slightly improved in five patients through rehabilitation, but was unchanged or even deteriorated in 14 patients. **Conclusions** Timely diagnosis of basal ganglia GCTs could affect the clinical outcomes. [11C]-methionine PET may be helpful in the early diagnosis.

O048

LATE EFFECTS OF TREATMENT FOR PEDIATRIC BRAIN TUMOR PATIENTS

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Fortunately, survival rates for children diagnosed with malignant brain tumors have improved with advances in surgery, radiation therapy, and chemotherapy. As a result, understanding the long-term impact of therapy including the neurocognitive and psychological effects of cancer treatment are important as most children who often present with various deficits. Significant sequelae, including intellectual decline, as well as variable attention-concentration, slow processing speed and motor deficits have been observed as a result of treatment, with specific risk factors including young age at the time of treatment, higher dose and larger brain volume involved in radiotherapy, longer follow-up time since therapy, and treatment with methotrexate. In an effort to preserve intellectual functioning and quality of life, many current treatment studies have been utilizing reduced doses and volumes of radiotherapy, while others are employing postoperative chemotherapy to delay or avoid radiation therapy in these children. Two prominent multi-center intervention studies in the U.S. involve the use of Methylphenidate (Ritalin) medication and the other utilizes a Cognitive Remediation Training Program in order to address symptoms such as attention, memory and academic achievement difficulties following treatment for pediatric cancer and have demonstrated modest success.

The emotional effects of cancer upon a pediatric patient vary depending on the diagnosis, the location of the tumor and the treatment type, and on the patient's family dynamics and preexisting psychological conditions. The vast majority of studies to date indicate that survivors of pediatric cancer are generally at no greater risk for long-term emotional sequelae than one would expect to see in a healthy population. It is important to note, however, that these studies often do not include brain tumor patients, mainly because of the physical effects that brain tumors and their treatments have on the central nervous system (CNS) and the apparent difficulty in separating organic and psychological causes of discrepancies in patient QoL. Of those studies that do include brain tumor patients, approximately half conclude that these patients are at no greater risk than are the healthy controls, whereas the other half concluded that they are at greater risk. However, studies have found that pediatric brain tumor patients consistently score lower in the domains of psychosocial health and of physical health than do other, non-CNS tumor patients. Thus, while information about brain tumor patients' QoL continues to emerge, there does appear to be an increased risk for deficit in certain domains of physical and psychosocial functioning. It will be important to provide counseling and support before and during the transition to adulthood with experiences such as employment, marriage, and parenthood.

O049

CARING FOR THE CAREGIVER OF A BRAIN TUMOR PATIENT

Elana Farace

USA

I will discuss meeting the needs of the family member as a way of better caring at home for patients with brain tumors. We will go over interventions to improve caregiving at home as well.

O050

LATE ENDOCRINE EFFECTS IN CHILDHOOD BRAIN TUMORS

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National Center for Child Health and Development

Endocrine complications are the most frequent and clinically relevant among the late effects in childhood brain tumors. Hypothalamo-pituitary hormonal axis can be impaired by the neighboring brain tumor itself or by therapeutic modalities, especially radiation and surgical treatments. In many cases endocrinopathy can be predicted by the risk factors such as site and nature of the tumor and dosage of irradiation in the hypothalamic area. Clinicians should also be aware that it may develop several years or even a decade after the end of oncologic treatments.

Endocrinopathy in childhood and adolescence often presents growth disturbance and/or abnormal pubertal development (i.e. precocious, delayed or absence of puberty). Therefore, statural and pubertal monitoring as well as regular measurements of thyrotropin, thyroxin and morning cortisol is essential during the long-term follow-up of the survivors at risk for endocrine complications.

Treatments for endocrine disorders are usually effective and often helpful in improving quality of life (QOL). Children and adolescents, however, need induction of therapy at their pin-point timings corresponding to their specific developmental stages in order to achieve better final results. In this context early detection of endocrine disorders through the adequate monitoring and then involvement of pediatric endocrinologists at a suitable stage are mandatory.

O051

PEDIATRIC BRAIN TUMOR: CURRENT STATUS OF PATIENT'S CARE AND PROBLEMS IN JAPAN

Yutaka Sawamura

Hokkaido University

Survival rates of children with brain tumor have significantly improved over the years due to developments in diagnostic techniques, neurosurgery, chemotherapy, radiotherapy, and supportive care. However, neoplasms occurring in the central nervous system remain an important cause of cancer-related deaths in children. This lecture presents the current problem in the medical care system for children with brain tumor in Japan and introduces some modern multi-disciplinary programs for treatment of difficult tumors.

In our country, due to a lack of registration system, there is no exact data concerning the incidence of pediatric brain tumor. At the time of initial diagnosis, the child is seen at a local community hospital where the knowledge of brain tumors is very small. Unfortunately, often the child does not get sent to the major treatment center that specializes in childhood brain tumors and the outcome for them is not sufficient. It has been emphasized that we do need to establish specific center hospitals and educational program to encourage young pediatric neurooncologists in our country.

Prognosis is still highly dependent on clinical characteristics, such as the age of the patient, histological type, stage, and localization of tumor. This uniqueness makes the treatment strategy complex in individual patient. However, increased knowledge about the long-term functional outcome of children with brain tumor and late-effects of treatment is being obtained and has been useful to further improve outcome for patients. The process to establish the current treatment protocol in Japan will be introduced being focused on intracranial germ cell tumors.

O052

COMMENTARY

Mark W. Kieran

Dana-Faber Cancer Institute and Childen's Hospital, Boston Associate Professor, Harvard Medical School

O053

ADULT BRAIN TUMORS

Jun A. Takahashi

Kitano Hospital

Primary brain tumors include various histological types. For most types of tumors in most locations except deep-seated tumors such as brain stem gliomas, surgical resection is recommended. And their removal should be as complete as possible with preservation of neurological function. Total resection of intaraaxial infiltrating tumors by surgery alone is generally impossible. Additional options of radiation therapy and/or chemotherapy vary according to histology and degrees of resection.

Patients with incurable or unresectable tumors are considered as candidates for clinical trials.

O054

COMMENTARY

W.K. Alfred Yung

M.D. Anderson Cancer Center

O055

PEDIATRIC BRAIN TUMORS – FROM RESEARCH TO HOPE

Mike Traynor

President, Pediatric Brain Tumor Foundation, www.pbtfus.org

The Pediatric Brain Tumor Foundation is honored to be here today. For the past 26 years, we have supported the search for the cause of and cure for childhood brain tumors. We want to find ways to improve those treatments, and we hope to do that with your help.

A Mother's Words

In the words of one mother, "We cling to hope, and hope lies in research." That is why the PBTF is going to enormous lengths to fund research to save the lives of these precious children.

The Numbers

I won't burden you with too many numbers, but here are some important ones to remember about brain tumors: This year in the United States alone, the Central Brain Tumor Registry of the U.S. tells us, 3,750 children will be diagnosed with a primary brain tumor. That means that TODAY, while we're at this meeting, 10 families are getting the devastating news that their baby has a brain tumor. Even worse, 1,360 of the children diagnosed this year will die an early death. Forty percent of kids with a brain tumor will be dead within five years of their diagnosis.

A Difficult Journey

The journey these kids face is daunting. To begin with, brain tumors are complicated to diagnose. The symptoms often mimic that of other childhood diseases, with nausea, headaches, and hearing and vision problems.

These tumors are sometimes inoperable, and pathological diagnosis is sometimes difficult, especially with 126 different types of tumors in existence.

And of course, once the tumor is diagnosed, the treatment options aren't great. As you know, even if a brain tumor is benign, its treatment is not. Surgery is invasive and can damage vital areas of the brain that control neurological functions. Few drugs will cross the blood-brain barrier. Those that do can weaken the immune system and leave children susceptible to a host of other infections and possible hearing loss. Radiation to a developing brain can lead to long-term cognitive challenges and physical disabilities.

The long-term effects on children and their families are devastating. We have only yet begun to see the consequences of cancer treatment as these children age.

There is a fine line between a healthy life and one filled with daunting medical challenges. This is the line that children diagnosed with a brain tumor walk each day of their lives. The path followed by young brain tumor patients is filled with countless challenges that are nearly overwhelming for them in comparison to physically healthy children.

Furthermore, if they survive the rigors of this all-consuming disease, and the devastation of their treatment, they then embark upon a long and often immensely frustrating journey to adulthood. These children can experience an ongoing decline in mental and physical capabilities, which impacts their self-esteem, their ability to retain learned information, and their ability to engage in physical activities. Often they face other cancers brought on by their treatment. The things that come as second nature to other children can be monumental obstacles in their daily lives.

The emotional and financial toll on the families of brain tumor patients is also devastating. Jobs and homes are lost. Bankruptcies are not uncommon. Children require special long-term care by parents, but what happens when parents die? Who then cares for the patient? Marriages sometimes break up under the strain. Siblings who feel ignored may have behavioral problems. Grieving takes place during and after the illness. Even if a child's brain tumor is not fatal, there is loss with living.

Research Challenges

All of these children, living and dead, are MY heroes. They are the heart of the Pediatric Brain Tumor Foundation's efforts. Until a cure is found, we will continue our search for new non-invasive and more effective treatments. We are working to overcome considerable research challenges:

- Few centers of excellence
- Limited understanding of the disease
- Lack of tissue samples
- Widely varying response to treatment
- Insufficient funding for research
- Difficult to attract qualified clinicians and researchers
- More oncologists need to be trained in this specialty

Funding Challenges

There are also many funding challenges. To begin with, there is a lack of funding for childhood diseases. A long-term commitment is required for funding to have impact. And few organizations are wholly dedicated to childhood brain tumor research and family patient issues.

- In addition, low population numbers make it unattractive to pharmaceutical companies for research funding and new drug development. Only one new drug has been developed for the treatment of pediatric brain tumors in the past 30 years.
- It's also difficult to achieve research collaborations, something that we are working very hard to do.
- The PBTF is one of the largest funding sources for childhood brain tumor research, but we can't do it alone. The federal government's help is needed to get pharmaceutical companies involved in research funding and drug development, not just in the U.S., but all over the world.

Reasons for Hope

However, the work we are doing in the PBTF Institute labs is turning research into hope.

- In 1984 when we began our work long-term effects were not an issue because most children diagnosed with a brain tumor died. Today many brain tumor patients are living longer.
- We have vastly improved radiation delivery methods, meaning that fewer kids suffer the awful effects of whole-brain radiation.

- The U.S. government has made a stronger commitment to advocates in most major peer reviews over the past few years, thereby ensuring greater care and outcomes for the children.
- Our advocacy work in Washington, D.C., and around the world has led to increased visibility for the issue of childhood brain tumors.
- Thanks to the efforts of the PBTF and other interested parties, there are new research programs underway at universities where pediatric brain tumor research was never done before—we know because we're receiving grant applications from more and more institutions each year.
- We have driven an increase in collaboration among researchers at the international level through our Institutes, and we're encouraged by the possibility of new biological treatments for brain tumors.
- The PBTF has raised more than $50 million in this fight so far. But we have a long way to go before we are finished.

PBTF Research Funding
We fund cutting-edge medical research at dozens of the world's leading institutions, from coast to coast in the U.S., and in Canada, Australia, Germany and the United Kingdom.

PBTF Institute Program
We believe that research collaborations are the answer to the fast-forwarding of brain tumor research. Researchers at the Pediatric Brain Tumor Foundation Institutes at Duke University, the University of California, San Francisco, and the Hospital for Sick Children in Canada are working together to share their findings as well as tissues and data.

In May 2008 we announced an additional $6 million grant to our research institute at Duke University. Funding for Duke and our two other institutes now exceeds $14 million. We are greatly encouraged by the way the doctors and researchers at these institutions are working together.

Fostering Global Collaboration
We're already seeing the fruits of that collaboration. We held our second annual PBTFI research conference last spring, and the excitement of discovery was uplifting. Their level of enthusiasm is the highest we've seen in 26 years of grant funding. These researchers, many of whom are here for this meeting in Yokohama, will get together again this October to share their successes.

And that same month, the PBTF will sponsor the first-ever International Pediatric Basic and Translational Research Invitational Conference. This type of scientific collaboration is a bright new source of hope for every child with a brain tumor, from Chitose to Chicago to Chelsea and on around the world.

We also see increased interest in pediatric brain tumor research that we've never seen before, fostered by groups such as the International Brain Tumor Alliance who are here today to help move the understanding of brain tumors, including pediatrics, to all corners of the globe.

Many, many basic and clinical researchers are working on new discoveries, including: Mitch Berger and his team at UCSF and Darell Bigner's group at Duke, and many others, in the United States. Research is also happening in other countries, led by Jim Rutka at Sick Kids Hospital in Toronto, Dr. Sawamura, Dr. Matsutani, Dr. Nishikawa and their colleagues here in Japan; Stefaan van Gool in Brussels; David Ashley in Australia; David Walker in Nottingham, England; Manfred Westphal in Germany; and many, many more. This tells us that we have much to be encouraged about regarding the future outcomes of childhood brain tumors.

Partners in Hope
We also support researchers by funding data collection and a variety of professional meetings, including:

- The Central Brain Tumor Registry of the United States, for which we provided the founding grant
- The World Federation of Neuro-Oncology
- The Society of Neuro-Oncology, including the founding grant for the prestigious Journal of Neuro-Oncology, which is the official neuro-oncology journal of Japan, the U.S. and Europe. We also sponsor the Society of Neuro-Oncology Foundation, fund their Basic Research Award for Excellence, and provide grants to support their annual meetings.
- Brain Tumor Epidemiology Consortium meetings
- International Pediatric Neuro-Oncology Symposium
- International Symposium on Brain Tumor Research & Therapy
- Peter Steck Memorial Research Award & Lecture
- Childhood Neurology Society

PBTF/Japan partnerships
Our partnership with Japan is also strong. We have sponsored medical conferences here over the years, and we also gave the Children's Cancer Association of Japan a grant to bring a social worker to the U.S. for cross-cultural training.
PBTF Family Support

In addition to research, the Pediatric Brain Tumor Foundation believes that it's critical to offer emotional and educational support to families in their time of need. Our programs include educational resources, patient services, college scholarships, advocacy efforts, and the Ride for Kids® program.
We strongly encourage you to share information about our services. We primarily serve the United States, but our materials are also printed in Spanish and we offer resources to people worldwide.

Online Services
Our website, www.pbtfus.org, gives families a greater number of resources and easier access to information. The site also includes new survivor outreach information. Our family support program includes a social worker who is available to assist patient families by phone or email.

Here in Japan, Dr. Sawamura at Hokkaido University runs a Japanese language website with information for patient families. You can access that page from the link listed here. The Children's Cancer Association of Japan is also an excellent resource for patient families.

Advocacy in Action
The Pediatric Brain Tumor Foundation also believes that raising awareness of this disease creates hope. As an active member of the brain tumor advocacy community, the PBTF is a:

- founding member of the Alliance for Childhood Cancer
- patient advocate on the National Cancer Institute's (NCI) Specialized Program of Research Excellence (SPORE) for brain tumors

- representative on the Patient Advocates Research Team (PART) Council for the NCI's SPORE in cancer
- patient advocate, National Institute of Neurological Disorders and Stroke (NINDS)

We also make Congressional visits to educate U.S. lawmakers on childhood cancer issues.
We invite you to join us in advocating for quality care in each of your countries for pediatric and adolescent brain tumor patients, development of new pediatric oncology drugs, long-term follow-up care for survivors, and representation of patients' needs and viewpoints when research efforts are translated into clinical trials.

Fundraising Efforts
Research is incredibly costly and requires million of dollars to fund; we hope you will join in the effort to raise the funds needed in your respective countries for pediatric brain tumor discoveries.
One of our fundraising efforts is our Ride for Kids motorcyclists' charity program. It is the backbone of our medical research and family support program funding. In 1984, the first year of our work, we raised $4,000. Last year PBTF Ride for Kids programs in 37 cities resulted in more than $5.2 million in donations to help cure the kids, and we're on track to raise even more with 39 events in 2009. This program is also an important part of our family support efforts, because it brings together the community of brain tumor survivors and their families. Our Celebration of Life program following each ride is just that: a celebration of the lives and accomplishments of these children.
In addition to Ride for Kids, we raise awareness and funds through:

- Direct mail, which helps us appeal to individuals who support similar charitable causes.
- A radiothon program, which lets us partner with radio stations to broadcast patient stories and solicit donations from the public.

We also have thousands of people around the world who raise funds for us in many original ways. Some hold walkathons, others run marathons. We've even had schoolchildren send us their pennies. Every cent makes a difference in our fight against childhood brain tumors.

Faces of Hope
One of the most visible signs of hope is that brain tumor survivorship is increasing. For instance, when we began raising funds in 1984, we did not need scholarships for brain tumor survivors. Now more children are living longer, and making plans to attend college.

However, a brain tumor places an enormous financial burden on a family. The PBTF helps make higher education possible by offering scholarships. Here are a few of the "faces of hope" that we are helping send to college.

To date we have awarded 397 scholarships to brain tumor survivors, and the program is growing dramatically with the addition of the Tim & Tom Gullikson Family Support Fund. How's that for hope?

Here is a perfect example. Michelle Higa was our first scholarship recipient to graduate from college. She is now working on her master's degree in psychology. Like so many young adults who had a pediatric brain tumor, Michelle wants to help children going through medical crises.

Celebrating life is what the Pediatric Brain Tumor Foundation is all about. We celebrate the lives of those children who are no longer with us, as well as the lives of the young people who continue to suffer, by working harder each day to find a cure for this dread disease. Thank you for your time today, and for everything you are doing to help us in our fight. Please visit our website, www.pbtfus.org, to learn more about how you can help us turn research into hope.

O056

THE EUROPEAN BRAIN TUMOUR PATIENT AND CAREGIVER COMMUNITY – ACHIEVEMENTS, CHALLENGES AND THE PURSUIT OF HOPE

Denis Strangman

Chair, International Brain Tumour Alliance (IBTA), www.theibta.org

There are many challenges facing the brain tumour patient.

Brain tumours attack the very essence of who we are. Brain tumours affect the physical, emotional and cognitive capabilities of a person, thus impacting on quality and length of life, employment and personal relationships. They strike people of any age, from tiny babies to the elderly, male or female, in any geographic locality. Based on current knowledge, brain tumours cannot be prevented or realistically screened for. Their causes are, as yet, largely unknown.

Beyond the patient's own personal situation, and across Europe and beyond, there is a desperate lack of funding for research into more effective therapies for brain tumours. Although some progress in treatment has been made, there can be inequity in accessing promising new therapies. There are sometimes misdiagnoses and late diagnoses. There are not enough specialists and specialist centres. There is a lack of consistency in registries from country to country.

For the caregiver of someone with a brain tumour, the challenges are different but also extremely daunting. A woman caring for a relative with a brain tumour said: "It can be hard to put someone else first all the time – to let someone go but be there for them still. It's like standing on the shore watching [him] out at sea. Sometimes the waves bring him back closer to me and sometimes the tide takes him away again."

In this difficult setting, brain tumour support, awareness-raising, advocacy and fund-raising are crucial.

The European brain tumour patient and caregiver group community is rising to these challenges and achieving progress through various activities. In some European countries – though by no means all – brain tumour foundations and groups, where they exist, are responding to a desperate need by energetically raising money for research. They offer support and information and, very importantly, advocate with determination for a better, more equitable deal for patients and caregivers.

Through its two primary activities – the promotion of the International Brain Tumour Awareness Week (1-7 November 2009) and the Walk Around the World for Brain Tumours (1 January – 7 November 2009) - the International Brain Tumour Alliance (IBTA) unites patient groups in Europe (and other parts of the world) in a global and very public effort to raise funds and awareness of this illness. More than 30 European patient organisations relevant to brain tumours supported these two projects in 2008. Around the world, over 150 patient and professional groups and organisations were supporters.

Through the achievements of such initiatives, across Europe and beyond, the situation for brain tumour patients and their caregivers is improving, giving hope to those whose lives are touched by this devastating disease.

O057

THE REALITY OF BRAIN TUMORS: CURRENT STATUS AND FUTURE PERSPECTIVE IN ASIAN COUNTRIES

Akiko Higuchi, MSW

Pediatric Oncology Social Worker
Children's Cancer Association of Japan

"Why my child?" is the first reaction any parent will have when they were told of their child's illness. In 1968, our foundation, Children's Cancer Association of Japan was first established in Japan to support families with children afflicted with childhood cancer. Since then, the social workers at CCAJ have received countless phone consultations. Fears, anxieties, and uncertainties expressed by parents are constant and have not changed since its inception. "Is the hospital we are going trustworthy?" "Aren't there alternative treatments?" "We are worried about our financial situation." "What is the cure rate of this illness?" "How could we explain the disease to our child?" "Who can look after the siblings?" "What will happen to his/her studies while in treatment?" "We have to make a decision on the child's treatment options and we don't know which is best." "We want to meet others who have the same illness." Those are but some of the information they want.

In recent years, the number of consultation regarding childhood brain tumors has been alarmingly increasing and the contents of questions varied and urgent. Childhood leukemia which has been the main concern in the medical field has seen not only the remarkable improvement in the cure rate, but also due to the acceptance in the concept of total care involving professionals, it has become possible for the leukemia patients to receive a variety of social support from the time the child is admitted to a hospital.

On the other hand, the dissemination of information and support system has not been well developed for childhood brain tumors despite the childhood brain and spinal cancers have the second highest morbidity rate among childhood cancers. The reasons are their numerous varieties, existence of different treatments and prognosis depending on the location and the age of patients, poor prognosis, and complexity of communication due to treatments involving multiple fields of medicine.

Even though the methods of controlling the disease have much improved due to progress of research and treatment methods, often patients are exposed to late-effects, have to face drastic change in their life style, and overcome huge obstacles trying to be socially independent. In addition to above, the proliferation of conflicting information given on internet and lack of appropriate information, and shock of diagnosis and huge problems that the families face in treatment process are the main reasons we see the increase in the consulting cases.

Children's Cancer Association of Japan was founded in 1968 by parents whose children died of childhood cancer. The members of CCAJ are striving to help families and children afflicted with cancer alleviate difficulties and worries. We also help improve their quality of life by coordinating support from medical fields and other supporting institutions and individuals. CCAJ has now 19 associate organizations nationwide and membership of over 3,000 and will celebrate its 41st anniversary this year.

The main activities are following:

1) Funding for medical research projects for doctors, nurses, and other institutions on the subjects on treatment and/or care of childhood cancers.
2) Consultations by social workers, assisting different groups such as different cancer sub-groups, parents who lost their children to cancer, cancer survivors, siblings of cancer patients, coordinating and educating volunteers and planning workshops.
3) Supporting families financially for treatments, and management of lodging facilities.
4) Dissemination of information by publishing periodicals, newsletters, informational booklets. We also hold publicity activities to advocate the disease.

Even if the medical environment is well developed and support system established, it is important that interaction and sharing of information among families to continue. Also vitally important is the cooperation of medical professionals and the patients' families. Groups such as Japan Brain Tumor Alliance and Japan Pediatric Brain Tumor Network involving all brain tumor patients have been established and expanding their activities.

Our mission is to spread our activities internationally together with above mentioned groups, and not only strive for the patients and the families to be able to receive quality treatment but work hard to improve our supporting role including enrichment of their daily lives after the treatment.

O058

MOLECULAR GENETIC SIGNATURES THAT DEFINES SUBTYPES OF HUMAN GLIOMAS

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We performed a comprehensive genetic profiling in a large series of astrocytic and oligodendroglial tumors to identify specific molecular signatures that could help define biologically relevant subtypes of human gliomas. We studied 402 tumors (38 pilocytic astrocytomas (PA), 22 diffuse astrocytomas (A), 62 anaplastic astrocytomas (AA), 183 glioblastomas (173 primary GB (pGB), 10 secondary GB (sGB)), 34 oligodendrogliomas (O), 20 anaplastic oligodendrogliomas (AO), 20 oligoastrocytomas (OA), 23 anaplastic oligoastrocytomas (AOA)). All tumors were examined by 1Mb array-CGH. TP53, CDKN2A/p14ARF, RB1, PTEN and IDH1 were investigated for mutation. Total 1p/19q loss were predominantly found among O and AOs but rare in astrocytic tumors. TP53 mutations were common among A, AA and sGB but less common in OA, AOA and pGB and rare among O and AO. TP53 and total 1p/19q deletions were mutually exclusive. IDH1 mutations were frequently found among all oligodendroglial tumors, A, AA and sGBs but rare in pGBs and not found in PAs. IDH1 mutations were strongly correlated with either TP53 mutation or total 1p/19q loss. RB1 pathway alterations were exclusively found among grade III or IV tumors, and almost always associated with p53 pathway alterations. PTEN mutations or EGFR amplifications were exclusively found among AAs and GBs. The data suggest that IDH1 mutation is the earliest change in both oligodendroglial tumors and adult astrocytic tumors with the exception of pGBs. IDH1 mutations followed by total 1p/19q loss may characterize oligodendrogliomas while IDH1 mutations followed by TP53 mutations may characterize astrocytomas. RB1 pathway alterations are associated with malignant progression in all types. pGB develop through distinct pathways by acquiring concurrent alterations of the RB1 and p53 pathway, with or without PTEN mutations or EGFR amplification but not IDH1. Thus, we demonstrate that sequential acquisition of particular genetic abnormalities may define distinct subtypes of gliomas.

O059

INTEGRATION OF GENOMIC ALTERATIONS AND EXPRESSION PROFILING IN GLIOBLASTOMA MULTIFORME

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Purpose: Glioblastomas (GBM) are highly malignant and heterogeneous gliomas with very poor prognosis. The biological and molecular characterization of these tumors is still challenging and impacts their therapeutic management. Previous genomic surveys have revealed the highly rearranged nature of GBM genome and transcriptome. However, the impacts of tumor DNA aberrations on gene expression remain unclear. **Methods:** We investigated copy number alterations (CNA) and gene expression to identify causal genetic events in GBM. High-resolution maps of somatic chromosomal alterations were obtained for 20 GBM. Gene expression profiling was carried out on the same tumor samples, and compared to those obtained on non-neoplastic brain samples. Concordance between CNA and gene expression was identified by two complementary approaches (correlated or targeted probes). The resulting GBM signature was validated with an independent microarray data set of 81 GBM and 23 normal brains. **Results:** Loci targeted for high-priority minimal common regions (MCR) of recurrent CNA were defined and combined with gene expression profiles performed on the same tumor samples. Genes with concordant changes in CNA and expression levels were defined as over- / under-expressed genes located in amplified / deleted regions, or as MCR-genes with expression correlated to the corresponding genomic state. After validation, we found that the expression of 318 genes was significantly affected by CNA. Associated enriched GO process annotations were related to cell cycle disorder, cellular adhesion and angiogenesis. The gene signature included well-known GBM genes such as EGFR, PDGFA, and p16INK4 but also novel candidate genes. Two tumor suppressor genes PCDH9 and STARD13, involved in tumor invasiveness and resistance to etoposide, were validated by qPCR in an independent set of 57 glioblastoma. **Conclusions:** This study shows the power of combining genomic alterations and gene expression to identify robust transcriptome signature and putative tumor biomarkers in GBM.

O060

GENOME-WIDE ASSOCIATION STUDY OF ADULT GLIOBLASTOMA RISK.

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Glioblastoma, the most common type of adult malignant brain tumor, has a very low survival rate. Risk factors associated with glioblastoma other than ionizing radiation and rare genetic syndromes are not well-established. Here we present the results of a genome-wide association study (GWAS) with positive replications of several top single nucleotide polymorphisms (SNPs). Initial screening for top SNPs was conducted using several data sources: 525 glioblastoma cases and 602 population-based controls from the San Francisco Adult Glioma Study, 3,390 controls from Illumina iControlDB, and 70 glioblastoma cases from The Cancer Genome Atlas. Case-control comparisons of 306,154 autosomal SNPs yielded several SNPs with $p<10^{-6}$, including 7 SNPs in 9p21 (*CDKN2B*) (p-values: $6*10^{-9}$ to $7*10^{-7}$), 3 SNPs in 6p21 (p values: $6*10^{-8}$ to $8*10^{-7}$), 1 intronic SNP in *TERT* ($p=6*10^{-10}$), and 1 SNP in 13q34 ($p=4*10^{-7}$). Three SNPs [one linked ($r^2>0.8$) with SNPs in the 9p21 region, another linked ($r^2>0.8$) with SNPs in the 6p21 region, and one was the same SNP in 13q34] were available for replication using Mayo Clinic data for 125 glioblastoma patients and 196 hospital controls and 2,937 Wellcome Trust controls. The GWAS associations in 9p21 ($p=0.01$) and 13q24 ($p=0.004$) were replicated. The current study suggests that the regions around *CDKN2B* are important for the development of glioblastoma; *CDKN2B* is located in a region in 9p21 that is frequently deleted in glioblastoma. The very significant result for the 1 SNP in *TERT* must await replication by other studies, though a recent GWAS of lung cancer reported a significant association with the same *TERT* SNP.

*Note: JS Chang, M Wrensch, RB Jenkins, and RF Yeh contributed equally to this work. P Yang and JK Wiencke are the senior authors from the Mayo Clinic and University of California, San Francisco, respectively.

O061

GENETIC DIVERSITY ASSOCIATED WITH SURVIVAL IN MALIGNANT GLIOMAS IDENTIFIED BY LINKAGE DISEQUILIBRIUM-BASED ANALYSIS OF HAPLOTYPE BLOCK REGIONS OF DNA REPAIR GENES

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The influence of inherited genetics on outcome of therapy in malignant brain tumors remains poorly understood. In this study, using a linkage disequilibrium (LD)-based approach, we examined the relationship between genetic diversity in 45 DNA repair genes and survival in 301 adults with anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM) treated on temozolomide and carmustine-based protocols. We genotyped a subset of 1,267 tagging SNPs within or near 45 genes in high LD with a region of 20 kb flanking each gene, on a customized Affymetrix platform. Associations were performed after testing for Hardy-Weinberg conformity and gender mismatches. The results identified tSNPs in and around EXO1, DDB2, LIG1, MGMT, MSH2, MSH4, PAN3, RAD52, TDG, XRCC1, and XRCC5 to be highly associated with survival. Although, several gene variants were associative in both AA and GBM, a subset of SNPs were associative in only one category. The identification of highly polymorphic regions of DNA repair genes in association with outcome of temozolomide-/carmustine therapy in malignant gliomas is consistent with the role of DNA repair in mechanisms of tumor resistance to these agents. Resequencing is ongoing to establish the specific causative SNPs and to examine the biology underlying their association with treatment outcome in malignant gliomas. Supported by grants RO1 CA127872, RO1 CA 112519, P50CA108786 and P30-CA14236 from the NIH, USA and the Pediatric Brain Tumor Foundation of the US.

O062

DIFFERENTIAL GENE-EXPRESSION IN RECURRENT GLIOBLASTOMA MULTIFORME

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Purpose: Identification of GBM subgroups might improve an aimed individualized therapeutically approach. Consequently, in our study we looked at patients with early versus late tumor recurrences in order to identify molecular markers, which might be associated with those different growth patterns. These markers, in turn, could be new therapeutic aims. **Methods:** Study population comprised ten pairs each of primary and recurrent tumors occurring within 180 days after initial diagnosis (short-term recurrences, STR) and later than 300 days after initial diagnosis (long-term recurrences, LTR), respectively. Exons 5-8 of the p53 gene and the entire coding region of PTEN were investigated by sequence analysis. EGFR amplification was determined. Loss of heterozygosity (LOH) analysis of markers known to be involved in glioma pathogenesis was performed. A chip-based expression analysis was performed in 6 samples (3 STR, and 3 LTR) using Affymetrix, GeneChip Human genome U133 Plus 2.0. Two candidate genes were further evaluated by real-time PCR based mRNA expression analysis. **Results:** p53 mutations are less common in STR than in LTR, while genetic variants of the PTEN gene are more frequent in STR. In STR, p53 and PTEN variants occur in combination, while LTR harbour either p53 or PTEN variants. Clustering after Chip analysis revealed clear differentiation between STR and LTR. Construction of a 6-gene comprising predictor is possible. A set of 53 differentially expressed genes was identified. GAS6 and SNTG turned out to be promising new candidates for determination of early recurrence. **Conclusion:** STR and LTR can be distinguished by p53 and PTEN mutation status and by gene expression profile. Out of a set of 53 differentially expressed genes, GAS6 and SNTG appeared be suitable markers for early recurrence and potential therapeutic approaches. Of course, our study sample is rather small, and data need further confirmation.

O063

MULTI-ARM COMBINATORIAL STUDY OF SIGNAL TRANSDUCTION MODULATORS USING A SEQUENTIAL ACCRUAL DESIGN – A REPORT OF NABTC 0502

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Background: Unlike select examples in oncology such as imatinib for CML, single- agent signal transduction modulators have been ineffective in most cancers including glioblastoma. Combinations of agents based on established signaling pathways may be more effective. However, performing individual phase I/II trials for each combination is inefficient. We developed a 3-arm phase I/II trial using a sequential accrual design combining sorafenib with erlotinib, temsirolimus or tipifarnib. **Methods:** The North American Brain Tumor Consortium (NABTC) conducted a phase I studies of sorafenib (VEGFR/PDGFR/Raf inhibitor) in combination with erlotinib (EGFR inhibitor), temsirolimus (mTOR inhibitor), or tipifarnib (farnesyltransferase inhibitor) in recurrent GBM. Eligibility criteria included histologically proven GBM, radiologic progression, ≥ 18 yrs old, KPS ≥ 60, adequate bone marrow reserve and organ function. There was no limit on the number of prior therapies. No enzyme-inducing antiepileptic drugs were allowed. Dose-finding used a standard 3 + 3 design with the MTD was defined as the dose with DLTs in 1/6 or fewer patients. Serum pharmacokinetic (PK) studies were performed. Accrual was sequential, decreasing study pauses for maturation of toxicity data. **Results:** Overall 49 patients were enrolled. Based on 17 patients, the MTD was sorafenib 400 mg BID and erlotinib 100 mg qD. Based on 13 patients, the MTD was temsirolimus 25 mg qweek and sorafenib 400 mg BID. The MTD was sorafenib 200 mg BID and tipifarnib 100 mg qD x 21 days based on 19 patients, but the dose of tipifarnib is below therapeutic levels. PK studies showed no drug-drug interactions for sorafenib with tipifarnib or temsirolimus. However, there was no accumulation of erlotinib, suggesting a drug-drug interaction with sorafenib altering erlotinib metabolism or clearance.

Conclusions: This study demonstrated that the sequential accrual design accelerates study completion and decreases logistical problems of phase I studies in multicenter trials. Phase II doses were successfully determined for sorafenib with temsirolimus and with erlotinib, permitting enrollment onto the phase II component. The new phase I study using an alternating week schedule of tipifarnib will be performed to permit a daily dose more likely to reach therapeutic concentration.

O064

PROGRESSION-FREE SURVIVAL: AN IMPORTANT ENDPOINT IN NEURO-ONCOLOGY CLINICAL TRIALS

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Many endpoints have been used in the context of clinical trials in neuro-oncology including objective response, overall survival, median time to tumor progression, progression-free survival and other landmark endpoints, such as PFS-6 months or PFS-12 months. Endpoint determination is critical to assess the efficacy of drugs or treatment strategies in both phase-2 and phase 3 trials. There are pros and cons with each of these endpoints, and the challenge remains as to whether one or another should be used in a given situation, and whether there is acceptance across the neuro-oncology community, regulatory agencies or industry sponsors. Given the costs of conducting trials, and the impact of endpoints on patient accrual and expectations, protocol design has become more and more relevant, particularly with the advent of novel agents and strategies that may influence our interpretation of MR based imaging which is critical for endpoints that use response or progression as major determinants of futility or success of treatment. We will present examples of the use of progression-free survival as one potential endpoint in both newly diagnosed and recurrent disease, and the implication of landmark PFS as a potential valid endpoint for phase-2 trials.

O065

MULTICENTER PHASE II TRIAL OF TALAMPANEL WITH STANDARD RADIATION AND TEMOZOLOMIDE IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA: REPORT OF TWO YEAR SURVIVAL DATA

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Background: Recent data suggests that the glutamatergic system is important in the proliferation and migration of glioblastoma cells. Talampanel is a well tolerated, oral AMPA receptor blocker that could be beneficial when added to conventional glioblastoma therapy. **Methods:** The primary purpose of this safety and activity (phase II) trial was to estimate overall survival in adults with newly diagnosed glioblastoma treated with talampanel in addition to standard radiation and temozolomide (RT+TMZ). A secondary purpose was to evaluate talampanel toxicity in this setting. Talampanel was initiated with RT+TMZ and discontinued for toxicity or disease progression. **Results:** Survival results were compared to historical controls. Seventy-two patients were enrolled from December 2005 to July 2006. Their median age was 60 years (range 37-85), median KPS was 90 (70-100), and 77% had a debulking procedure. With a minimum of 18 months follow-up, 42 patients (58%) have died yielding a median survival of 18.3 months (95% CI = 14.6 to 22.5 months). When the 60 patients who were 18-70 years old on this trial were compared with the EORTC (RT+TMZ) data, the median survival (20.3 vs 14.6 months) and percent surviving at 24 months (41.7% vs 26.5%, p=0.02) appeared superior. Data on MGMT methylation and post-progression treatment with VEGF targeted therapies for this population will be available for presentation. **Conclusion:** Talampanel was well tolerated and did not appear to increase the known hematologic or non-hematologic toxicities of TMZ. Talampanel can be added to RT+TMZ without significant added toxicity. These encouraging survival results in this study suggest that blocking AMPA receptors may be a useful strategy in glioblastoma.

O066

ONGOING CLINICAL TRIALS AND THE FUTURE DIRECTION OF GLIOMA TREATMENT

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Because of the proposed sensitivity to chemotherapy of oligodendroglial tumors both the RTOG and EORTC had investigated if these tumors benefit from adjuvant PCV chemotherapy. These studies, EORTC study 26951 and RTOG 9402, both showed that the addition of PCV chemotherapy (consisting of procarbazine, CCNU and vincristine) to 59.4 Gy radiotherapy does increase progression free survival without improving overall survival in anaplastic oligodendroglioma and anaplastic oligoastrocytoma. A major finding of both studies is the large difference in prognosis of patients with and without combined 1p/19q loss. Based on these differences in survival and the clear different outcome in anaplastic oligodendroglioma with 1p/19q loss, EORTC and the collaborative groups felt that it was no longer rational to treat these patients according to histology without taking the genotype of these tumors into account. For studies in anaplastic gliomas it was therefore proposed to classify into anaplastic glioma without 1p/19q loss and anaplastic oligodendroglial tumors with 1p and 19q co-deletion. Another challenge is the definition of a proper endpoint for these trials. Taken a long preservation of neurological functions even at progression, overall survival seems to be the most relevant outcome parameter. The outline and initiatives in grade III gliomas (EORTC 26053/22054 – CATNON plus the co- deleted trial) are presented.

Standard therapy for glioblastoma is surgical resection aimed to be as complete as possible, respecting neurological function followed by chemoirradiation with temozolomide. TMZ given as concomitant and adjuvant therapy to RT has shown to increase progression-free survival (PFS) (rate at 6 months: 53.9 % vs. 36.4 %) and median survival (14.6 vs. 12.1 months) compared to adjuvant treatment with RT therapy only (EORTC 26981/22981 NCIC CE.3 trial. Still, many patients do not respond to therapy. The resistance of cells against DNA damage caused by nitrosoureas and temozolomide is at least in part mediated by the DNA-repair enzyme O6-methylguanin-DNA-methyltransferase (MGMT). Epigenetic silencing of the MGMT gene by promoter methylation compromises this DNA repair and has been associated with longer survival in (glioblastoma) patients who are treated with alkylating or methylating agents. An analysis of the EORTC 26981/22981 NCIC CE.3 trial showed, that indeed patients with glioblastoma containing a hypermethylated MGMT promoter benefited from TMZ (overall survival (OS) rate at 24 months: 46% vs. 23 %), whereas those who did not have a methylated MGMT promoter did have a significantly worse survival and less benefit from the addition of temozolomide to RT (OS rate at 24 months: 14% vs. <2 %). This raises the question if the small benefit from chemoirradiation observed in this group outweighs the toxicity and costs of the temozolomide treatment, and calls for the development of more effective drug regimens for this specific group of patients. Although there may be small numbers of patients with an unmethylated MGMT promoter that do benefit from combined chemo-irradiation, for the entire subgroup of these molecularly defined GBM patients the overall benefit is questionable.

Most interestingly, the phase II trial with the integrin inhibitor cilengitide also demonstrated a marked benefit mainly in the patients with glioblastoma containing a methylated MGMT promoter. Consequently, the current Merck/EORTC phase III trial is designated to delineate the role for cilengitide in glioblastoma with methylated MGMT. Even earlier, Eli Lilly took the approach to examine the protein kinase C beta inhibitor, enzastaurin, together with radiotherapy but without TMZ in patients with glioblastoma containing an unmethylated MGMT promoter.

This raises the general question whether treatment in glioblastoma trials should not only be stratified according to MGMT but entry into those trials limited by MGMT status. This would call for different approaches of GBM patients, depending on the MGMT promoter gene status. The primary question to address in GBM with unmethylated MGMT promoter gene is the identification of drugs that provide more survival benefit as compared to TMZ. The current EORTC trial initiatives are presented.

O067

THE RESULT OF A CLINICAL TRIAL FOR MALIGNANT GLIOMAS BY JCOG-BRAIN TUMOR STUDY GROUP (JCOG 0305)

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Purpose: Japan Clinical Oncology Group (JCOG)-Brain Tumor Study Group conducted a multiinstitutional randomized controlled trial on malignant gliomas entitled, a randomized controlled phase II study of chemoradiotherapy using ACNU versus procarbazine and ACNU for astrocytoma grade 3 and 4, with the support of the Health and Labour Sciences Research Grants of the Ministry of Health, Labour, and Welfare in order to establish a standard therapy for malignant gliomas in Japan. **Method:** The patients with newly diagnosed supratentorial astrocytoma grade 3 or 4 were enrolled and randomized into two groups. The patients in Group A were treated with ACNU (80mg/m^2 iv) during the postoperative radiotherapy (RT, 60Gy local), while those in Group B received procarbazine (80mg/m^2 for 10 days per os) preceding administration of ACNU. Each regimen was continued every 8 weeks for 2 years if it was tolerable for the patients and their disease did not progress. The primary endpoint was the overall survival rate and the secondary endpoints were the response rate on the MRI and the frequency of the adverse events. Procarbazine is expected to reduce O6-methylguanine-DNA methyltransferase (MGMT) and enhance the anti-cancer activity of nitrosoureas. The protocol was activated in April 2004 and 111 patients were registered by the end of August 2006 from 19 collaborating neurosurgical institutes of JCOG-BTSG. **Results:** The overall survival of the patients treated with ACNU+RT was 16.2 months and that of procarbazine+ACNU+RT was 18.7months, while PFS of both groups were 6 months. CTCAE grade3/4 were observed in 40-60% of the patients. **Conclusion:** ACNU-based chemoradiotherapy was an effective but toxic treatment.

O068

CURRENT CLINICAL TRIALS OF GLIOMA THERAPY AND SITUATIONS OF NEUROONCOLOGY PRACTICE IN KOREA

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There has been no qualified sponsor-investigator clinical trial program and the standard therapies have been the all we could do for the treatment of malignant glioma patients in the Korean Brain Tumor Society. We have just started to join two international clinical trials since 2008. Here, the past and current status of neuro-oncology field in Korea as well as Eastern-Northern Asian countries will be introduced, and clinical outcome of concurrent radiotherapy and temozolomide chemotherapy for 100 patients of four university hospitals of Korea (Advisory Board of S-P Korea) will be presented.

O069

HISTOGRAM ANALYSIS OF PERFUSION MR IMAGING DATA FOR THE ASSESSMENT OF TUMOR RESPONSE DURING GLIOMA THERAPY

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Purpose: A recently developed histogram analysis of relative cerebral blood volume (rCBV) from the entire tumor has been reported to offer excellent inter-observer agreement for quantitative analysis and demonstrate the heterogenous morphologic features of glioma vascularity. We aimed to determine whether histogram analysis can be adopted in the assessment of tumor response during glioma therapy. **Methods:** We retrospectively studied 51 dynamic susceptibility contrast 3-T MR imaging data of 29 patients (mean age 50.5 years, range 18-76) with histologically confirmed gliomas (9 low grade, 20 high grade). rCBV maps were created and normalized to unaffected white matter. Histogram width (HW), peak height position (PHP), and maximum value (MV) of the entire tumor were measured from normalized histogram distribution. **Results:** The values (mean ± S.D.) of HW, PHP, and MV were 4.64 ± 2.03, 4.58 ± 2.63, and 6.29 ± 2.79 for the preoperative imaging of high grade gliomas (n=8), and 3.83 ± 1.96, 2.66 ± 1.66, and 4.73 ± 1.96 for the final imaging which showed definite radiological tumor progression or confirmed tumor recurrence by biopsy (n=8). Thirty-two imaging data obtained during the median imaging follow-up of 3.7 months were divided into 2 groups (progression vs. stable/radiation necrosis) according to the follow-up result and 3 parameters were compared. All 3 parameters were positively correlated with tumor progression (HW, 3.05 ± 2.18 vs. 1.02 ± 0.50; PHP, 2.39 ± 1.71 vs. 0.94 ± 0.28; MV, 4.13 ± 2.83 vs. 1.56 ± 0.52) and MV was the most predictive with multivariate analysis. **Conclusion:** Our results suggest that histogram analysis of rCBV can be a more objective and useful diagnostic tool to determine tumor response during the treatment of gliomas, compared with Region of interest method for maximum rCBV, which can be highly operator dependent.

O070

DYNAMIC PERFUSION MR IMAGES CAN PREDICT THE PSEUDOPROGRESSION MIMICKING TRUE PROGRESSION IN THE TREATMENT OF NEWLY DIAGNOSED GLIOBLASTOMA

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Introduction: Tumor progression of glioblastomas entirely depends upon the radiologically contrast-enhancement on MR images. Disease progression can be difficult to interpret because it looks like pseudoprogression. The aim of this study was to study the real incidence of pseudoprogression during/after concomitant chemo-radiotherapy (CCRT) for newly diagnosed glioblastomas and the predictability of dynamic susceptibility-weighted contrast-enhanced perfusion MR scans for the pseudoprogression/ true progression. **Methods:** This present study was a single arm, prospective cohort study designed to evaluate the incidence and outcome of pseudoprogression during CCRT followed by adjuvant temozolomide (TMZ) treatment for newly diagnosed glioblastoma. **Results:** Between Jul. 2004 and Feb. 2008, seventy-six consecutive patients (median age 50 years) who underwent CCRT followed by adjuvant TMZ treatment were enrolled in this study. MGMT promoter was methylated in 26 patients (34%) and unmethylated in 50 patients (66%). Enlargement of lesions, evidenced at the MRI scan in 59 of 76 patient (77.6%), was subsequently classified as pseudoprogression in 31 patients (52.5%) and true progression in 28 patients (47.5%). Of these 31 patients, 18 patients developed pseudoprogression within the period of CCRT (hyperacute phase) and 13 patients during the adjuvant TMZ treatment (early phase). Lower value less than 1.75 on the perfusion images could be predictive of pseudoprogression with the sensitivity of 91% and the specificity of 82%. **Conclusions:** The current study supports high incidence of pseudoprogression during the treatment for newly diagnosed glioblastomas. Perfusion MR imaging can be used to predict the possibility of pseudoprogression in the treatment of glioblastomas.

O071

THE ANTI-TUMOR EFFECT OF CONVECTION-ENHANCED DELIVERY OF MICELLE-AM80 WITH SYSTEMIC ADMINISTRATION OF TEMOZOLOMIDE.

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Background. Retinoic acid derivatives exert anti-tumor efficacy by inhibiting cell proliferation, inducing cell differentiation, apoptosis, and growth arrest. All-trans retinoic acid (ATRA), one of retinoic acid derivates, has been extensively used for the treatment of acute promyelocytic leukemia (APL). Am80, a novel synthetic retinoic acid known to be more potent than ATRA, is allowed to use only for ATRA resistant APL patients. **Purpose.** We evaluated the antitumor efficacy of CED of micelle-Am80 against the rodent intracranial U87MG xenograft model in conjunction with systemic TMZ administration. **Methods.** Cytotoxicity of Am80 for U87MG cell line were examined in vitro by MTS assay. Cells were incubated with 50, 100, 200, and 500μM of Am80 for 2, 4, and 6 days. The expression level of phospho-Akt and phospho-MAPK in the cells treated by Am-80 were evaluated by western blotting. Toxicity of micelle-Am80 after CED was evaluated after infusion into the rat brain striatum. The anti-tumor efficacy was also evaluated using rodent intracranial U87MG xenograft model. **Results.** Am80 at 50μM revealed no cytotoxicity against U87MG cell. However, cell viability decreased as concentration increases from 100μM to 500μM. Enhanced cytotoxicity was observed when Am80 was combined with TMZ. Western blotting revealed decreased expression of phospho-Akt and phospho-MAPK in cells treated with Am80. When cells were treated with combination of Am80 and TMZ, further decrease of these phosphorylated proteins were observed. In vivo toxicity study revealed no toxicity with CED of Am80 in rodent striatum. Survival of U87MG intracranial xenografted rodents treated with CED of micelle-Am80 combined with systemic administration of TMZ was significantly longer than those treated with CED of Am80 or systemic TMZ as well as control rats (P&lt65308;0.01). **Conclusions.** CED of micelle-Am80 synergistically enhanced the antitumor efficacy of systemic TMZ treatment.

O072

AN IMPLANTABLE MAGNETIC BREATHER PUMP FOR METRONOMIC CONVECTION ENHANCED DELIVERY OF BIOLOGICAL AGENTS IN MALIGNANT GLIOMAS

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Convection enhanced delivery has been used with limited success in the treatment of malignant gliomas. Although direct delivery of biological agents to malignant glioma patients has been performed safely with good volume of distribution, increased survival has not been demonstrated. One reason for this lack of success is in the limitation of current convection enhanced delivery techniques, as multiple externalized catheters are inserted into the tumor for limited time periods (ie 5-7) days and removed; repeat infusions require another surgery for catheter placement. For the past two years, we have been designing and performing proof of principle experiments on an implantable magnetic breather pump system for maligant gliomas. The system has five components: 1) an intratumoral delivery catheter (for unresectable tumors) or an intracranial pouch (for tumor cavity in resected tumors), 2) a reservoir for tapping the catheter or intracranial pouch, 3) a connecting catheter to link the reservoir, 4) an internalized pump with canisters containing biological agents of choice, capable of delivering drugs at microliters/hour, 5) a lab-on-a-chip to sample tumor fluid to measure specific cytokines (ie vascular endothelial growth factor) or drug levels to monitor treatment progress. The magnetic breather pump will be implanted much like a shunt, with the delivery pump secured at the chest wall. The canisters in the delivery pump may be accessed via subcutaneous ports to “refill” the drugs, and can be wirelessly reprogrammed to deliver at specific time intervals and different cycles using Medical Implant Communication Service (MICS) interface. We are currently developing a “pathfinder” with all five components connected to demonstrate proof of principle in-vitro. If the “pathfinder” is successful in-vitro, it will be reduced to an implantable system, and tested in-vivo in animal models. Successful completion of animal studies will result in human Phase I studies.

O073

INTRACEREBRAL ADMINISTRATION OF CPG OLIGONUCLEOTIDE FOR PATIENTS WITH RECURRENT GLIOBLASTOMA.A PHASE II STUDY.

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Background/Purpose Immunostimulating oligodeoxynucleotides with CpG motifs (CpG-ODN) have shown a promising efficacy in cancer models. Intra-tumoural infusions of CpG-ODN in glioblastoma patients were well tolerated at doses up to 20 mg, . In a phase I clinical trial. A phase II trial was designed to study the efficacy of a local treatment by CpG-ODN in patients with recurrent glioblastomas (GBM). **Patients and Methods** Patients with recurrent GBM occurring at least three months after radiotherapy and previously treated with chemotherapy received 20mg CpG- ODN (CpG-28) by convection-enhanced delivery. The primary endpoint was the percentage of the patient without tumour progression at 6 months after inclusion. Secondary endpoints were tolerance, survival, and radiological response. **Results** Thirty-four patients were enrolled in two centres between November 2004 and March 2006. Thirty-one patients received the treatment. The 6-months progression free survival (PFS) was 19%. One partial response and 3 minor responses were seen. The median overall survival was 28 weeks. Eight patients (24%) were alive after 1 year and 5 patients (15%) were alive 2 years after inclusion. Treatment was usually well tolerated. As previously reported, the most common toxicities were lymphopenia, mild fever , seizures, and transient neurological worsening. **Conclusion** Despite a few cases of radiological responses, CpG-28 showed a modest activity on the 6-months PFS in this population. However, the presence of long term survivors suggest that this treatment might have been beneficial in some patients, whose molecular or clinical characteristics remain to be defined.

O074

BIODISTRIBUTION AND IMAGING STUDIES ON TRANSFERRIN TARGETING PEG LIPOSOME ENCAPSULATING BOTH BSH AND IODINE CONTRAST AGENT BY CONVECTION ENHANCED DELIVERY ON RAT GLIOMA FOR EXPERIMENTAL BORON NEUTRON CAPTURE THERAPY

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Objective: To augment therapeutic efficiency of boron neutron capture therapy, we used transferrin (TF)-conjugated polyethylene-glycol (PEG) (TF-PEG) liposome encapsulating sodium borocaptate (BSH) and iodine contrast agent, Iomeprol with intratumoral convection enhanced delivery (CED) in rat glioma model. **Materials and Methods:** Boron-10 (^{10}B) concentration of F98 rat glioma cells was investigated in vitro using TF-PEG liposome and PEG liposome as control. For in vivo biodistribution studies, ^{10}B concentrations in intracerebrally transplanted F98 tumor, blood and normal brain were investigated using these boron delivery systems with CED, and computed tomography (CT) scan was carried out at the selected time after CED. **Results:** ^{10}B concentrations of F98 glioma cells 6 h after exposure to PEG liposome and TF-PEG liposome were 16.1 and 51.9 ng ^{10}B/^{10}6 cells respectively. ^{10}B concentrations in F98 glioma tissue 24 h after CED was 22.5 and 82.2μg/g, by PEG liposome and TF-PEG liposome, respectively with lower ^{10}B concentrations in the blood and normal brain of tumor-bearing rats. The CT image showed the vivid and stable enhanced image of transplanted tumor by Iomeprol even 48 h after CED by TF-PEG liposome. On the contrary, enhanced image had been already washed out 24 h after CED by PEG liposome. **Conclusion:** The combination of TF-PEG liposome encapsulating BSH and Iomeprol and intratumoral CED enables not only a precise and potent targeting of boron delivery on the tumor tissue but also following the trace of boron delivery administered intratumorally by CT imaging.

O075

INTRAOPERATIVE PHOTODYNAMIC DIAGNOSIS AND THERAPY FOR MALIGNANT GLIOMA USING SECOND-GENERATION PHOTOSENSITIZER TALAPORFIN SODIUM

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A new photosensitizer, Talaporfin sodium, was reported to be effective for both photodynamic diagnosis(PDD) and photodynamic therapy(PDT) of several cancers. We employed Talaporfin sodium for the intraoperative PDD and PDT for glioblastoma patients. 22 consecutive patients, 27 times operation with glioblastoma (16 primary and 6 recurrent, aged 23-82 years) received intravenous doses of Talaporfin(40mg/m2) 24 hours prior to craniotomy. Intraoperatively, tumor fluorescence was visualized using modified operating diode laser (664nm) microscope. Fluorescence-guided resection of tumor tissue was performed, and additional PDT (150mW/cm2, 27J/cm2) was performed to 11 cases on invading tissues in eloquent area. A clear fluorescence signal was demonstrated in the tumor bulk and peritumoral zone, and the satisfactory resection of contrast-enhancing tumor was confirmed and achieved 19.6 months of median survival time in the primary glioblastoma. Mild liver dysfuncion was occurred in 11.6% of cases, and skin photosensitivity was occurred in 1 case. The clinical experience in this study indicates the efficacy and safety of Talaporfin-induced PDD and PDT for glioblastoma patients.

O076

MUTATIONAL PROFILING OF HIGH GRADE GLIOMAS REVEALS INACTIVATING MUTATIONS IN IDH1

This Abstract nominated the Hoshino Award.
Please refer to P057 on page 149.

O077

KCN1, A NOVEL SMALL MOLECULE HIF INHIBITOR PREVENTS GLIOMA GROWTH THROUGH BINDING OF PLECTIN-1 AND INHIBITION OF THE HSP90 PATHWAY

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Micro-regional hypoxia is a well-known characteristic of glioblastoma that appears as the tumor mass outgrows the existing vascular supply. Hypoxic areas of solid tumors are resistant to traditional chemo- and radio-therapies, highlighting the need to develop new therapies for their targeting. A common property of these cells is the expression of HIF-1, a key transcription factor that coordinates adaptive responses to hypoxia, providing cancer cells the means to survive and proliferate under hypoxic conditions. To identify specific small molecule inhibitors of HIF-1, we screened a combinatorial library of 10,000 natural product-like chemical compounds. We sh–ow that KCN1, a lead inhibitor identified in this screen, potently inhibits HIF-1 activity in various cancer cell lines (IC50≈4$_{m}$M), while exerting minimal effects on the levels of HIF-1$_{b}$, other short-lived proteins, or control proteins. To identify the molecular mechanism of action of this novel inhibitor, we compared the changes in global gene expression caused by KCN1 to those caused by a variety of previously characterized drugs using the Connectivity Map database. The gene expression signature of KCN1 was found to be similar to those produced by known inhibitors of Hsp90. Biochemical analyses confirmed that KCN1 modulates Hsp90 function by inhibiting the association of the chaperone with a model client protein. However, in contrast to known Hsp90 inhibitors such as geldanamycin, KCN1 does not inhibit the enzymatic activity of Hsp90 via direct binding to the ATPase pocket. Rather, the small molecule alters the availability of Hsp90 to its clients, including HIF-1$_{a}$, by a novel mechanism of action. We found that KCN1 augments the interaction of Hsp90 with a cytoskeletal protein called plectin-1. This changes the bioavailability and subcellular distribution of the chaperone. Detailed experimental evidence supporting this novel mechanism will be presented. In an effort to translate KCN1 to the clinic we evaluated its toxicity and anti-tumor efficacy in an animal model. Systemic administration of KCN1 in nu/nu mice harboring sc human LN229 glioblastoma xenografts evidenced strong anti-tumor effects in the absence of noticeable toxic effects. These data suggest that KCN1 is a promising new agent for the treatment of malignant gliomas in a clinical trial.

O078

DIRECT TYROSINE PHOSPHORYLATION OF GSTP1 BY EGFR INDUCES JNK ACTIVATION IN GLIOBLASTOMA CELLS

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In malignant gliomas, the c-jun N-terminal kinase (JNK) pathway regulates tumor growth and resistance to apoptosis, and correlates with histologic grade and epidermal growth factor receptor (EGFR) tyrosine kinase expression. We recently reported that glutathione S-transferase P1 (GSTP1), a major metabolizing and stress response signaling protein that is frequently overexpressed in brain tumors, is a downstream target of EGFR and undergoes EGFR-dependent tyrosine phosphorylation, in vitro and in vivo, resulting in an enhancement of its enzymatic function and increased drug resistance. Intracellularly, the dimeric GSTP1 exists in a reversible equilibrium with its monomeric form, the predominant form under most conditions. Monomeric GSTP1 binds to JNK and acts as an endogeneous JNK inhibitor. In this study, we investigated the impact of the EGFR-dependent tyrosine phosphorylation of GSTP1 ion the activation of JNK in glioma cells. We showed that the EGFR-phosphorylation of GSTP1 shifts the GSTP1 dimer-monomer equilibrium toward the monomeric state. Kinase analyses showed that the c-Jun phosphorylation by JNK was enhanced by phosphorylated-GSTP1 and suppressed by unphosphorylated-GSTP1. EGFR activation resulted in dissociation of GSTP1 from the GSTP1-JNK1 complex in a cell-free system and in glioma cells. Mutation of the phospho-acceptor Tyr198 to phenylalanine in the GSTP1 protein and over-expression of the Tyr198Phe mutant GSTP1 protein decreased JNK activity and c-Jun phosphorylation in EGFR-overexpressing glioma cells. Together these results indicate that in malignant glioma cells the tyrosine phosphorylation of GSTP1 by EGFR shifts GSTP1 from the dimeric to the monomeric form leading to dissociation of GSTP1 from the GSTP1-JNK complex and reactivation of JNK, which is independent of JNK upstream signaling. This regulation of JNK signaling function by the EGFR-GSTP1 crosstalk, defines a novel signaling network with a potential to regulate the biology and therapeutic outcome in malignant gliomas. *Supported by NIH grants RO1 CA127872, RO1 CA 112519, P50CA108786 and P30-CA14236.*

O079

QUANTITATIVE EXPRESSION ANALYSIS REVEALED SIGNIFICANT CORRELATION OF EGFR VARIANT III (vIII) AND NOTCH EXPRESSION IN GLIOBLASTOMA

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EGFRvIII is the exon 2-7 deletion mutant and commonly identified in glioblastoma patients. This leads to the ligand-independent tyrosine kinase activity that activates persistent downstream phosphatidylinositol 3-kinase pathway and promotes tumor growth, which makes EGFRvIII a therapeutic target. However, functional role of EGFRvIII in the stem cell biology, another therapeutic target, has not been evaluated.In this study, we aim to quantitate the expression level of EGFRvIII and stem cell marker genes, and identify the stem cell marker which might have a functional association with EGFRvIII. First, using recently development real time RT-PCR assay for detecting EGFRvIII, we evaluated EGFRvIII expression in 105 GBM samples. We could detect and quantitate EGFRvIII expression in 22 samples (20%) with variable expression level. Next, to evaluate the expression of stem cell marker gene, we selected 21 EGFRvIII positive samples and 23 negative samples, and performed SYBR based real time RT-PCR assay. The stem cell marker gene included in this study are follows; CD133, Nestin, BMI-1, MELK, Notch1, Notch2, Notch3 and Notch4. We also examined the expression level of other growth factors such as EGFR, EGFR2, EGFR3, EGFR4, PDGFRA and PDGFRB. We evaluated the statistically association of each stem cell marker gene expression with EGFRvIII. Of the 14 genes, the result showed that EGFR ($p<0.01$), Notch2 ($p=0.03$), Notch3 ($p=0.02$) and Notch4 ($p=0.01$).were significantly highly expressed in the EGFRvIII positive cases. This suggests that downstream pathway of Notch 2-4 may be functionally associated with EGFRvIII activation.

O080

EGFRvIII PREFERNTIALLY ACTIVATES STAT5 AND GAB1, CAN BE ACTIVATED BY FORCED DIMERIZATION AND LOCATES TO THE NUCLEUS

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Aberrant EGFR signaling is a major contributing force to glioma progression and treatment resistance. The most prevalent mutation, EGFRvIII, promotes growth and survival of cancer cells. More recently, new point mutations in the extracellular domain of EGFR have been identified by The Cancer Genome Atlas. We are investigating the signaling of these abnormal receptors to identify novel druggable targets. We have analyzed the EGFRvIII specific signal using shotgun phosphoproteomics based on recovery of tyrosine-phosphopeptides and mass spectrometry. Glioma cell lines expressing EGFRvIII, a kinase inactive form of EGFRvIII and wild-type EGFR (LN428 PTEN +; LNZ308 PTEN-) were compared by this approach. Spectral intensity and count was used in a label-free quantification, and revealed 150 and 180 phosphoproteins respectively in the two cell lines used. Several phosphorylations showed a significantly greater level in EGFRvIII cells when compared to wild-type EGFR. These include c-Met, Stat5 and Gab1. The signaling of EGFRvIII is ligand-independent and low intensity. Whether it dimerizes has remained controversial. Furthermore, the low intensity signal makes it challenging to study. We have created a chimeric EGFRvIII that can be dimerized experimentally, using a variant FKBP12 domain and cognate small molecule, a process termed chemically induced dimerization (CID). CID increases EGFRvIII signaling several fold in intensity, without leading to increased internalization or changes in the signaling nodes activated downstream, and is allowing us to investigate the nature of the EGFRvIII signal in greater depth than before. In itself, the observation that forced dimerization increases the signal of EGFRvIII is strong evidence that it ordinarily does not dimerize to a significant degree. Another possible mechanism behind EGFRvIIIs impact on glioma biology is differential cellular localization and we have found that it localizes to the nucleus in an activity dependent manner, and requires Y1173 and Y845.

O081

ASSOCIATION OF POLYMORPHIC VARIANTS IN EGFR AND LRIG2 AND MENINGIOMA RISK

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BACKGROUND: Both genetic and epigenetic mechanisms contribute to meningioma development by altering gene expression and protein function. The epidermal growth factor receptor (EGFR) is dysregulated or overexpressed in brain tumors and its expression is directly correlated with tumor malignancy and unfavorable prognosis. The leucine-rich repeats and immunoglobulin-like domains (LRIG) family proteins are membrane bound proteins that have been proven to inhibit EGFR signaling through interaction with ubiquitinilation. The human LRIG2 is prominently expressed by myelinating cells and cytoplasmic expression of LRIG2 is associated with poor survival of oligodendroglioma patients. The aim of this study was to test the hypothesis that polymorphic variations in the EGFR and LRIG2 genes influences risk of meningioma. **METHODS:** 376 meningioma cases, and 1103 controls recruited in the Nordic Interphone study were genotyped. 89 SNPs in the EGFR gene and 6 SNPs in the LRIG2 gene were selected to capture most of the polymorphic variants in these genes. **RESULTS:** The EGFR gene SNP (rs759171) showed a higher minor allele frequency in meningioma (3%) compared with controls (1%). The highest risk of meningioma was associated with homozygosity for the EGFR variant (OR = 3.10 CI, 95% 1.33, 7.25). When analyzing the LRIG2 gene SNP (rs1216801), the minor allele frequency was higher in meningioma (16%) compared with controls (12%). A significant association between risk of meningioma and homozygosity for the LRIG2 variant (OR = 1.50 CI, 1.05, 2.15) was also evident. **CONCLUSIONS:** As far as we know this is the first study were meningioma risk has been analyzed in association with polymorphic variants of the EGFR and LRIG2 genes. Functional studies in this area are important to assess whether polymorphic variants are also associated with meningioma tumor phenotype.

O082

A NOVEL P53 BYSTANDER EFFECT: PARACRINE INDUCTION OF APOPTOSIS IN TUMOR CELLS THROUGH THE INDUCTION OF GALECTIN-3 SECRETION

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P53 tumor suppressor expression is lost in a variety of brain tumors. P53 is a transcription factor that regulates the expression of a large number of proteins, some of which are critical effectors of its tumor suppressive activities. In the present study we demonstrate the existence of a novel mechanism whereby the tumor suppressor p53 can control the growth of malignant gliomas and other solid tumors. We provide evidence that activation of p53 by gamma-irradiation, ultraviolet or chemotherapy in wtp53 cells can induce cell death specifically in bystander cancer cells (but not normal cells) through p53-controlled release of a death-promoting factor. To identify this factor we performed proteomic analyses (2GE-MS and ICAT) on supernatant of glioma cells with inducible p53 (tet-on system) and identified a number of candidate secreted proteins. Using antibody neutralization and siRNA approaches we demonstrated that galectin-3 (Gal-3), a 31 kDa β-galactoside-binding pro-apoptotic factor was the mediator of this bystander effect. To identify the mechanism, we first showed that Gal-3 was not a direct target of wt-p53; rather its secretion is facilitated by p53 transcriptional activation of TSAP6, a key mediator of the non-traditional secretory pathway. We further demonstrated the biological importance of p53 controlled Gal-3 secretion by showing that it inhibits anchorage independent tumor cell growth *in vitro* and can strongly reduce malignant glioma formation *in vivo*. We next investigated the mechanism of death and confirmed its specificity for tumor cells. We first showed that Gal3 induces a caspase 9-dependent apoptotic pathway in over 20 different tumor cell lines of different origins investigated so far, while normal cells were unaffected. Second, we demonstrated that Gal3 induced apoptosis in tumor cells could be inhibited by depletion of beta1 integrin using siRNA. Further investigations are ongoing to unravel the mechanism underlying the tumor cell specificity. In conclusion, our data demonstrate that p53 can exert cell extrinsic control over tumor growth through a novel mechanism leading to increased secretion of galectin-3. Gal3 in turn induces apoptosis in adjacent tumor cells, suggesting that it can be directly exploited for tumor therapy. -- This work is supported by the Goldhirsh Foundation. --

O083

P53: AN ANTAGONIST OF EGFR-MEDIATED METABOLIC ACTIVITY IN HUMAN MALIGNANT GLIOMA CELLS

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Activated epidermal growth factor receptor (EGFR) signaling is one of the most prominent molecular alterations in malignant gliomas and sustains many of their key oncogenic capabilities. However, activation of EGFR signaling also increases energy demands by enhancing protein synthesis and nutrient consumption. Our recent finding that EGFR inhibition confers protection from hypoxia-induced cell death is congruent with this. Other recent work has demonstrated that p53 has an important role in the processing of starvation signals and that the p53-dependent molecular mediators of the Warburg effect, synthesis of cytochrome c oxidase 2 (SCO2) and TP53-induced glycolysis and apoptosis regulator (TIGAR), regulate glucose consumption and mitochondrial function. Notably, EGFR amplification and p53 mutations are almost mutually exclusive events in glioblastoma. We therefore hypothesized that the presence of wild-type p53 in glioma cells with activated EGFR is necessary in order to limit metabolic demands induced by deregulated signal transduction processes in the presence of hypoxia and nutrient depletion. We here report that shRNA-mediated gene suppression of p53 as well as the mutant temperature-sensitive dominant-negative p53VAL135ALA increase glucose consumption and lactate production in p53 wild-type human malignant glioma cells and enhance hypoxia-induced cell death. These findings suggest that glioma cells may benefit from retaining p53 wild-type status by reducing their vulnerability towards tumor hypoxia and offer an explanation for the dichotomy of EGFR amplification and p53 mutations in glioblastoma.

O084

SPONTANEOUS TRANSFORMATION OF P53 NULL SUBVENTRICULAR ZONE CELLS IN VIVO, AND USE IN ORTHOTOPIC THERAPEUTIC TESTING

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PURPOSE: To determine whether p53 deficient subventricular zone (SVZ) cells have the potential to undergo spontaneous transformation in vivo, and whether transformed cells can be used in therapy response experiments. **METHODS:** Athymic nu/nu mice were injected with either luciferase modified p53 null SVZ cells, or the same cells modified with human telomerase reverse transcriptase (hTERT) and human papilloma virus E6/E7. Cells were monitored for proliferation by bioluminescence imaging (BLI), and resultant tumors were harvested for use in orthotopic allograft therapy response experiments. **RESULTS:** BLI revealed steady cell growth in all mice receiving intracranial (IC) injection with hTERT + E6/E7 modified cells in contrast to six mice showing stable (n = 3) or lack of (n = 3) luminescence following injection of p53 null cells. In addition to the IC injections, one mouse each received subcutaneous (SC) injection with each type of cell. p53 null + hTERT + E6/E7 cells showed progressive SC growth, producing a tumor that has been resected and whose cells have been used for tumor propagation, both in SC and IC locations. SC injected p53 null cells showed no indication of growth for 100 days, but thereafter showed rapid growth, producing a large tumor that has been used as a source of cells for intracranial injection in a series of 20 mice, 10 of which were treated with temozolomide. Temozolomide treatment significantly extended the survival of mice with IC tumor ($p<0.0001$). **CONCLUSIONS:** Our study makes the following points regarding the use of p53 null CNS cells from genetically modified mice: 1) Cells can be used to assess tumorigencity effects of transferred genes, such as hTERT and E6/E7; 2) Cells have the ability to spontaneously transform in vivo; and 3) Spontaneously transformed cells can be used in an orthotopic engraftment model approach for studying therapeutic response.

O085

ADP-RIBOSYLATION FACTOR 6 REGULATES GLIOMA CELL INVASION THROUGH THE IQ-DOMAINGTPASE-ACTIVATING PROTEIN 1-RAC1-MEDIATED PATHWAY

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A common pathobiological feature of malignant gliomas is the insidious infiltration of single tumor cells into the brain parenchyma, rendering these deadly tumors virtually incurable with available therapies. The ADP-ribosylation factor 6 (ARF6), which belongs to ARF family of small GTP-binding proteins with multiple roles in fundamental biological processes, has recently been shown to play an important role in tumor cell invasion. In gliomas, exogenous expression of EFA6A, a guanidine exchange factor (GEF) for ARF6 in glioma cells enhanced cell motility and invasiveness in vitro. However, whether ARF6 exerts a direct impact on glioma cell invasion is largely unknown. In this study, we report that ARF6, a Ras superfamily small GTPase, is abundantly expressed in invasive human glioma cells. In vitro, cellular depletion of ARF6 by siRNA decreased Rac1 activation, impaired HGF- and serum-stimulated glioma cell migration. In vivo and ex vivo, stable knockdown of ARF6 by siRNA markedly decreased the invasive capacity of invasive glioma in the brain. Ectopic expression of ARF6 by glioma cells promoted cell migration through activation of Rac1. Upon stimulation, IQGAP1, a key regulator of cell adhesion and migration was recruited to the membrane of the leading edges of migrating cells together with ARF6, allowing forward protrusion. Cellular depletion of ARF6 by siRNA abrogated the recruitment of IQGAP1 into cell membrane and attenuated the formation of the protrusions at the invasion fronts. Finally, using co-immunoprecipitation assays, we found that ARF6 was associated with Rac1 and IQGAP1 in glioma cells upon HGF stimulation. Knockdown of IQGAP1 by siRNA significantly inhibited the ARF6-promoted Rac1 activation and cell migration. In conclusion, these data suggest that ARF6 signaling is pivotal for glioma cell invasion in the brain and IQGAP1 is required for ARF6-mediated Rac1 activation and glioma cell invasion.

O086

ROLES OF SPHINGOSINE-1-PHOSPHATE RECEPTOR IN GLIOMA CELL PROLIFERATION AND PATIENTS SURVIVAL

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Background: Sphingosine-1-phosphate (S1P) is a bioactive lipid that signals through a family of five G protein-coupled receptors, termed $S1P_{1-5}$, and regulates cellular proliferation, migration, and survival. We investigated expression and role of S1P receptors in glioma.

Methods: We analyzed the expression and production of S1P receptors in 58 human glioma specimens by quantitative RT-PCR, Western blotting and immunohistochemistry. The relevance of S1P receptors expression levels to survival in glioblastoma was examined by Kaplan-Meier method. We examined the mechanism of S1P receptors pertaining to glioma cell proliferation and invasion by experiments manipulating gene expression in glioma cell lines.

Results: Expression of $S1P_1$ receptor was significantly lower in glioblastoma than those in normal brain ($P<0.01$) and diffuse astrocytoma ($P<0.05$). Immunoblot demonstrated that normal brain expressed more $S1P_1$ receptor protein than glioblastoma. $S1P_1$ receptor was immunolocalized predominantly to the astrocytes in normal brain, but faint staining was observed in glioblastoma. Downregulation of $S1P_1$ receptor expression correlated with poor survival of patients with glioblastoma ($P<0.05$). $S1P_1$ receptor small interfering RNA promoted cell proliferation in high expressor glioma cell lines (T98G, G112). Cell proliferation was promoted by pertussis toxin, an inactivator of Gi/o type of G proteins, to which $S1P_1$ receptor is coupled exclusively. Forced expression of $S1P_1$ receptor in low expressor cell lines (U87, U251) resulted in decreased cell growth both *in vitro* and *in vivo*. In migration and invasion assays, no significant change was observed by manipulating $S1P_1$ receptor gene. Furthermore, we found a significant association between the expression of S1P1 receptor and early growth response-1, a transcriptional factor which has a tumor suppressor function through PTEN in glioblastoma ($P<0.05$).

Conclusions: These data show that downregulation of $S1P_1$ receptor expression enhances the malignancy of glioblastoma by increasing cell proliferation and correlates with shorter survival of patients with glioblastoma.

O087

EPHA3 REGULATES CANCER PROGENITOR CELL SELF-RENEWAL AND PROLIFERATION IN GLIOMA NEUROSPHERE CULTURES

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Purpose: Eph receptors constitute the largest sub-family of receptor tyrosine kinases and interact with membrane-bound ligands termed ephrins. Eph and ephrins have many vital functions including cell adhesion, migration and axon guidance. Eph and ephrins have been found to be aberrantly expressed in many malignancies including brain tumour. The purpose of this study was to investigate Eph receptor function in glioma brain tumour stem cell neurosphere cultures. **Methods:** Gene expression was investigated by Q-PCR and immunoblot analysis in high grade glioma surgical specimens and cell lines. Targeted reduction of Eph expression was performed using an inducible shRNA system. Eph receptor function was also inhibited using soluble ephrin-Fc protein and the EphA3 mAb IIIA4. **Results:** In this study we investigated Eph and ephrin family expression in a screen of 37 high grade glioma tumour specimens. Results highlighted elevated expression of EphA3 and the high affinity ligand ephrin A5 in a significant proportion (70%) of samples. To further investigate EphA3 function the receptor was down regulated using shRNA in an EphA3+ glioma neurosphere cell line. Inducible down regulation of the EphA3 receptor resulted in initiation of neuronal and glial cell differentiation following activation of the ERK/MAPK pathway. A reduction in total stem/progenitor cell proliferation was also observed when treated with EphA3 shRNA (46%) or soluble ephrin A5-Fc (33%) to inhibit EphA3 function. Furthermore, treatment with the EphA3 mAb, IIIA4, inhibited cell proliferation (>50%) and induced rapid internalisation of the receptor, cell spreading and increased adhesion. The reduction in cell numbers was not associated with apoptosis or cell cycle arrest. CFSE division tracking identified slower cell divisions in populations in which EphA3 signalling was attenuated. **Conclusions:** We propose EphA3, in part, regulates cancer stem cell self renewal and cell division rate in glioma and could prove a potential therapeutic target.

O088

THE ALTERNATIVE TRKAIII SPLICE VARIANT, EXPRESSED BY NEUROBLASTOMAS AND GLIOBLASTOMAS, TARGETS THE CENTROSOME AND PROMOTES GENETIC INSTABILITY

Cancelled

O089

PRESENCE OF AN ALTERNATIVE LENGTHENING OF TELOMERE (ALT) MECHANISM AS A FAVORABLE PROGNOSTIC MARKER IN PATIENTS WITH GLIOBLASTOMA

This Abstract nominated the Hoshino Award.

Please refer to P059 on page 150.

O090

PROGNOSTIC SIGNIFICANCE OF MICRORNA-196 IN GLIOBLASTOMA

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MicroRNAs (miRNA) are short non-coding RNAs associated with diverse biological processes. They are also involved in tumorigenesis and function as both tumor suppressors and oncogenes. Large scale miRNA expression in glioblastomas, however, has not been quantitatively estimated to date. Here we determined global miRNA expression profiles of 12 malignant gliomas (8 glioblasotmas (GBM) and 4 anaplastic astrocytomas (AA)) using TaqMan Human MicroRNA Array v1.0 (Applied Biosystems) by real-time PCR method. In total, the expression of 365 mature human miRNAs was examined. Expression of the 16 miRNAs was significantly altered the two histological subtypes. Of them, the most significantly regulated miRNA was miR-196a ($p=0.0038$). In addition, miR-196b (family member of 196a) and miR-21, which was previously reported to be up-regulated in GBM, were also included. This result was validated in another panel of 105 gliomas (74 GBMs, 18 AAs and 13 diffused astrocytomas) with individual Taqman microRNA assay (Applied Biosystems) by real time PCR method. Furthermore, we examined the relationship between expression levels of these miRNAs and overall survival in 39 primary GBM patients. GBM patients with high miR-196 expression level showed significantly poor survival by Kaplan-Mieir method ($p=0.0073$). Multivariate analysis demonstrated that high level of miR-196 was an independent and significant predictor of overall survival in GBM patients ($p=0.036$, $HR=2.81$). In conclusion, our results suggest that miR-196 overexpression could be relevant for the malignant progression of glioma. Consequently, miR-196 is of significant prognostic value for predicating survival in GBM patients.

O091

DEVELOPMENT OF A NOVEL MOUSE MODEL OF GLIOBLASTOMA MULTIFORME USING LENTIVIRAL VECTORS

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Animal models of cancer have been very instructional in understanding the genesis of the tumors and very useful to test the new strategy of treatment. Among the brain tumors, glioblastoma multiforme (GBM) is the most malignant, and there is still need to generate the animal model for this tumor that can recapitulate the abnormal molecular events causing the formation of human GBMs. We have recently developed a novel method to induce GBMs in adult immunocompetent mice by injecting Cre-loxP controlled lentiviral vectors expressing oncogenes in a small number of cells. By injecting Cre-loxP controlled lentiviral vectors into the specific region of the adult GFAP-Cre mice that express Cre recombinase specifically in GFAP expressing cells, we established cell type and region specific expression of oncogenes. Activated form of H-Ras and AKT were induced in fewer than 60 $GFAP^+$ cells in hippocampus, subventricular zone or cortex of GFAP-Cre/$p53^{+/-}$ mice. Over 70% of the mice developed tumors when transduced in subventricular zone, and 100% of the mice developed tumors when transduced in hippocampus. However, tumors were rarely found when transduced in cortex. These tumors from hippocampus and subventricular zone showed the characteristics of human GBMs including pleomorphism, hypervascularity, necrosis and pseudopalisading. Transplantation of less than 100 brain tumor cells into the naive recipient mouse brain lead to the formation of GBM-like tumors. When these tumor cells were cultured in the stem cell medium, they formed neurospheres and contained $CD133^+$ cells. Moreover these tumor cells can differentiate into neurons and astrocytes in response to the addition of fetal bovine serum to the stem cell medium. These results indicate that our novel mouse model can be used for a wide variety of brain tumor research including the brain tumor stem cell investigation, and might be useful for the deeper understanding of GBMs.

O092

PHENOTYPIC MODULATION OF EXPERIMENTAL GLIOMAS: PROGRESSION FROM GLIOMATOSIS CEREBRI-LIKE LESIONS TO GLIOBLASTOMA

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Glioblastoma animal models still remain a big challenge in basic and translational research as the most commonly used cell line-based models do not recapitulate the invasive growth of human glioblastomas in patients. Recently, we have established a human glioblastoma xenograft model in nude rats that is highly invasive. Here, we characterize this model with an emphasis on neuropathological and radiological features. Immunodeficient nude rats were xenografted with biopsy spheroids derived from 29 patients diagnosed with primary brain tumours. Amongst these, 25 were primary glioblastoma multiforme (GBM), three were grade II gliomas and one was a grade III oligoastrocytoma. We monitored the animals with MRI and 11 of the resulting tumours were subsequently passaged in vivo for up to 19 generations while tumour take rates and survival data were recorded. The tumour take rate for rats xenografted with primary GBM biopsies was 77% and remained close to 100% at subsequent in vivo passages, whereas only one out of four lower grade tumours engrafted. Average time from transplantation to the onset of symptoms was 125 days. Culture time of the spheroids correlated inversely with tumour take rates, but not with survival. After 4-5 in vivo passages the tumours changed to a complete glioblastoma phenotype becoming more vascular, with increased cell proliferation, less apoptosis but more necrotic areas. By extended passages, the tumours gradually became less invasive and appeared more circumscribed (after 8-9 passages). In conclusion, the model provides reproducible high take rates and survival times if parameters related to tumour burden and the operative procedure are standardized. Furthermore, passaging in vivo modulates these tumours phenotypically, resulting in different combinations of angiogenic and invasive growth patterns. We therefore propose that the presented model provides a powerful tool for dissecting aspects related to brain tumour growth and progression.

O093

ISOLATION AND CHARACTERIZATION OF INTERLEUKIN 13 RECEPTOR ALPHA2-SPECIFIC HEPTAPEPTIDES

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Purpose. We have been developing specific delivery vectors to Glioblastoma Multiforme (GBM). Interleukin 13 receptor alpha 2 (IL-13Rα2), a cell membrane receptor, has been found to be over-expressed in more than 75 percent of GBM patients and absent in normal brain. Recently, we have begun a search for small peptides binding specifically IL-13Rα2. **Methods.** To isolate the peptides, we used the phage-display library with peptides presented on the surface of filamentous M13 phages. We screened Ph.D-C7C (New England Biolabs)library, with a diversity of 1.2 billions. The phage library was panned using GBM cell lines which over-express IL-13Rα2 and corresponding cell lines, which do not express the receptor. Phages were eluted from the IL-13Rα2 positive cells and subjected to additional binding/amplification cycles. **Results.** We have isolated 3 different peptide phage clones. ELISA experiments confirmed that these 3 peptide phage clones bound to the recombinant IL-13Rα-Fc receptor chimera protein and not to an irrelevant IgG-Fc control or bovine serum albumin protein. Moreover, ELISA experiments including the other IL-13 receptor protein, IL-13Rα1, demonstrated that one of the peptides is truly specific for IL-13Rα2. Furthermore, a 1000 fold excess of the IL-13 ligand did not block the peptide's binding to the IL-13Rα2. Also, cell-binding phage titer experiments were carried out on selected cell lines. We found that 200 times more peptide phages bound to the IL13Rα2 positive cell lines compared to the cell lines which do not over-express the receptor. **Conclusions.** We have identified specific heptapeptides that bind to IL-13Rα2. The peptides are binding to the receptor at the site different from that used by the natural ligand, IL-13. These peptides will be further developed for diagnostic, imaging and molecular therapeutic interventions in GBM.

O094

AUTOLOGOUS SH3-DOMAIN GRB2-LIKE 1 (SH3GL1) ANTIBODY AS A NOVEL SERUM MARKER SPECIFIC TO LOW-GRADE GLIOMA

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Glioma is the most common primary malignant central nervous system (CNS) tumor in adults and remains deadly disease despite of the development of the surgical procedure, radiotherapy and chemotherapy in the last decade. We used the serological identification of antigens by recombinant cDNA expression cloning (SEREX) to determine the novel serum markers in glioma. The phage expression library, constructed using mRNA derived from the U-87 MG glioblastoma cell-line, was screened using sera from 48 patients with glioma and 31 independent genes were isolated. The enzyme-linked immunosorbent assay (ELISA) using recombinant proteins indicated that the levels of serum antibodies to SH3-domain GRB2-like 1 (SH3GL1) were significantly higher in patients with low-grade glioma than in healthy volunteers ($P = 0.0045$) or in patients with high-grade glioma ($P = 0.0243$). The independent validation test, using other sera from the patients, presented similar results. ELISA in the purified recombinant proteins of deletion mutants of SH3GL1 showed that approximately 10-20 amino acids in the C-terminal were indispensable as the epitope site. In immunohistochemical staining, overexpression of SH3GL1 proteins in cytoplasmic region of glioma cells according to its histological grade, but not in glial cells, was observed. Consequently, the levels of anti-SH3GL1 autoantibodies can be a novel low-grade glioma-specific serum marker.

O095

HIGH SOLUBLE P-SELECTIN AND LOW PLATELET COUNT AS THROMBOSIS RISK MARKERS IN GLIOMA PATIENTS

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Background Glioma patients are at high risk for venous thromboembolism (VTE). However, predictive laboratory parameters have not been identified. High platelet count (PLC) and increased soluble P-selectin (sPsel) were reported as risk factors in cancer patients. We investigated sPsel and PLC as risk markers in glioma patients. **Methods** The Cancer and Thrombosis Study (CATS) is a prospective observational study, whose endpoint is the occurrence of objectively confirmed VTE. sPsel was measured in the third week after neurosurgical intervention using a human sPsel Immunoassay (R&D Systems, Minneapolis, USA). Multivariable Cox regression analysis was applied to calculate hazard ratios (HR) for VTE including PLC, sPsel, age, sex and extent of surgery. **Results** 140 patients with newly diagnosed high grade glioma were analysed (52 women; median age 54.5 years [interquartile range (IQR): 42.8-5.1]) during a median observation time of 309 (range: 3-1664) days. Twenty patients developed VTE (6 women, 14 men; thereof 2 events were fatal pulmonary embolisms. The cumulative probability of VTE was 10% at six and 15% at twelve months. sPsel levels (ng/mL) were higher in patients with VTE compared to those without (median=51.8, IQR: 36.9-66.0 versus median=38.8, IQR: 30.7-52.1, p=0.011). PLC (G/l) was significantly lower in patients with (median=214, IQR: 166-248) than in those without VTE (median=255, IQR: 200-327; p=0.011). In multivariable regression analysis high sPsel (75th percentile: 55.1ng/mL) and low PLC (25th percentile: 198G/L) were significant risk markers of VTE (HR=3.4, 95% CI 1.3-9.0, and HR=3.3, 95% CI 1.2-8.8, respectively). **Conclusion** Our study revealed two strong predictive risk markers for VTE in glioma patients. Elevated sPsel is associated with a three-fold increased risk of thrombosis. In contrast to patients with other solid tumours, low PLC is associated with increased thrombosis risk.

O096

CLINICAL OUTCOME AND PROGNOSTIC FACTORS OF GLIOMATOSIS CEREBRI

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The aim of this study was to investigate clinicoradiographic aspects together with pathologic features of an institutional series of gliomatosis cerebri (GC) and to analyze potential prognostic factors. Between 1997 and 2004, fifty patients underwent radiotherapy with diagnosis of GC based on radiographic feature and confirmed with biopsy. A retrospective analysis was conducted on these patients including the entire medical records, radiographic data and pathologic features. Survival outcome and potential prognostic factors such as the patient age, sex, performance status, the location of major involvement, grade of contrast enhancement, performance of decompressive surgery, and pathologic subtype and grade were analyzed. The median progression free survival and overall survival lengths were 15.7 and 25.9 months, respectively. Poor prognostic indicators included higher pathologic grade, disease with contrast enhancement, lesions involving deep gray matter beyond the hemisphere or involving cerebellum and brainstem, and poor patient performance status. Given a wide variety on clinicoradiographic feature and histopathologic characteristics in GC, the prognosis should be fairly predicted in every individual patient to direct an optimal management.

O097

CASE-CONTROL STUDY OF LONG TERM SURVIVORS OF PRIMARY GLIOBLASTOMA

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BACKGROUND: Survival of patients with glioblastoma (GBM) remains poor. However, a small percentage of primary GBM patients live ≥ 3 years. Relatively little is known about the patterns of care and outcomes of these patients. **METHODS:** Nested case-control study of long term survivors (cases) compared to standard survivors (controls) of GBM. Pathology was confirmed by a single neuropathologist who reviewed all cases and controls included in the study. Each long term survivor (LTS) was matched to standard survivors (SS) by age and postoperative Karnofsky Performance Status (KPS). Clinical characteristics, treatment regimens and outcomes were reviewed. Logistic regression models were used to assess potential associations between baseline factors and the probably of long term survival. **RESULTS:** Matching yielded 27 cases (LTS) with 81 controls (SS). Baseline factors were as follows: female LTS 40.1%, female SS 34.6%, male LTS 59.3%, male SS 65.4%, LTS with hemispheric tumors 96.3%, SS with hemispheric tumors 93.8%, LTS with callosal tumors 3.7%, SS with callosal tumors 2.5%, LTS with multifocal GBM 0, SS with multifocal GBM 3.7%, LTS with subtotal resection (STR) 51.9%, SS with STR 59.3%, LTS with gross total resection (GTR) 29.6%, SS with GTR 23.4%, LTS with biopsy 18.5%, SS with biopsy 17.3%, percent of LTS receiving a vascular endothelial growth factor (VEGF) inhibitor 26.1%, percent of SS receiving a VEGF inhibitor 23.4%. There were no significant associations between the baseline factors considered and long term survival. Median overall survival for LTS was 4.76 years (range 3.15-10.54 years) and for SS was 1.30 years (range 0.54-2.99 years). **CONCLUSIONS:** Analysis yielded no significant associations between gender, tumor location, extent of resection or treatment with a VEGF inhibitor and the probability of long term survival. Other factors, such as molecular or genetic differences, may help account for differences in survival and are being assessed.

O098

EFFECTS OF PREGNANCY ON NATURAL HISTORY OF WHO GRADE II GLIOMAS. A QUANTITATIVE ANALYSIS OF THE TUMOR GROWTH RATES.

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BACKGROUND WHO grade II gliomas (GIIG) are progressive tumors characterized by a continuous growth before malignant transformation. During the initial period, their radiological mean diameter increases at an average rate of 4mm/year and the Velocity of Diametric Expansion (VDE) has been shown to be predictive of prognosis. The recent optimization of the therapeutic management of GIIG has allowed an improvement of their prognosis and an increase of the number of women considering a pregnancy. The study goal was to determine whether pregnancy could modify the natural history of GIIG, using a quantitative approach of the VDE.**METHODS** We asked the French Glioma Study Group (REG) and the ANOCEF group to collect all cases of pregnancy in the adult female population of GIIG for whom MRI longitudinal follow-up was available. Repeated measurements of the mean tumor diameter were performed before, during and after pregnancy. Then, VDE were fitted by linear regression. **RESULT** S7 pregnancies in 7 females harboring a GIIG were reviewed (1992-2007). Six oligodendrogliomas and 1 mixed glioma were discovered before and during pregnancy in 4 and 3 cases, respectively. The median VDE was at 4.7mm/year before or after pregnancy. During pregnancy, the VDE increased markedly in 5 cases (median VDE 8.5mm/year) and remained stable in 2 cases. In one of the 5 patients negative group, radiotherapy was performed shortly after delivery and can be responsible of the VDE decrease. **CONCLUSION AND PERSPECTIVES** The present results, using a quantitative approach of VDE measurements, suggest a possible negative interaction of pregnancy on the natural history of GIIG. We propose to inform the patient about the possible risks. For each envisaged pregnancy, the benefit-to-risk ratio should be carefully weighted. If a pregnancy is decided, we advise to perform a close neurological and MRI follow-up. Besides, cases collection continues.

O099

WHOLE-BRAIN RADIOTHERAPY VERSUS OBSERVATION AFTER RADIOSURGERY OR SURGERY OF 1-3 BRAIN METASTASES - RESULTS OF THE EORTC 22952-26001 PHASE III STUDY

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Purpose: The EORTC designed a phase III trial to define the role of adjuvant whole brain irradiation (WBRT) after local treatment (surgery or radiosurgery) of 1-3 brain metastases from solid tumors. **Methods:** Patients eligible for radiosurgery had metastases ≤2.5-3.0 cm in diameter and in case of surgery a complete resection was mandatory. Only patients with absent or stable systemic disease or with asymptomatic synchronous primary tumors, and with WHO PS 0-2 were allowed. Patients were randomized to receive either adjuvant WBRT (30Gy in 10 fractions) or observation (OBS). Primary endpoint was time to WHO PS deterioration to ≥3. Secondary endpoints were time to intracranial progression, frequency of neurologic death and overall survival. Analysis is by intent-to-treat (Logrank, two-sidedα=0.05). **Results:** Since 1996 to 2007, 359 patients were recruited. Median time to WHO PS ≥3 was 9.8 months (95% CI 8.0-11.7) in the OBS arm and 9.8 months (95% CI 7.8-12.6) in the WBRT arm (p>0.5). Overall survival was 10.9 months (95% CI 9.2-14.6) in both arms (p>0.5). Cumulative incidence of intracranial progression at 6 and 24 months was 39.7% (95% CI 32.5-46.8) and 54.0% (95% CI 46.7-61.3) of the OBS pts., but only 15.2% (95% CI 9.9-20.5) and 31.4% (95% CI 24.5-38.2) of the WBRT pts (p<0.0001). Intracranial progression was a cause of the death in 77/179 pts (43%) of the OBS group and in only 45/180 pts (25%) of the WBRT group. **Conclusions:** After radiosurgery or surgery of a limited number of brain metastases, adjuvant whole brain radiotherapy reduces the frequency of intracranial relapses and neurologic deaths but does not prolong the time period of functional independence and overall survial time.

O100

THE IMPACT OF CYST FORMATION AND TUMOR HEMORRHAGE ON LOCAL RECURRENCE IN BRAIN METASTASIS

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Objective The purpose of this study is to examine the impact of cyst formation and tumor hemorrhage on the local recurrence after surgery for single brain metastasis. **Materials and methods** Between July 2001 and February 2007, 90 patients went through surgery for single brain metastasis at our institution. 63 patients (70%) were male, and 27 (30%) were female. Mean age was 55 (+12) years. The primary sites were lung in 58 (64%), colon in 11 (12%), breast in 6 (7%), kidney in 5 (6%), and liver in 5 (6%). Hemorrhagic brain metastasis was noted in 11 patients. Among non-hemorrhagic 79 patients, 12 patients had large cystic brain metastasis which was determined by diameter of the tumor cyst (greater than 3 cm). Postoperative RT was given in 4 patients (32%) in cystic group and 21 (30%) in solid group (p=0.45), and was given in 24 (30%) in non-hemorrhagic group and in 4 (36%) in hemorrhagic group (p=0.73) **Results** Among 11 hemorrhagic brain metastases, local recurrence was developed only in one patient. Among 79 non-hemorrhagic brain metastasis, 32 (40%) showed local recurrence. 12 month progression free survival was 90% in hemorrhagic group, and 46% in non-hemorrhagic group (p=0.04). 7 patients (58%) showed local recurrence among 12 large cystic brain metastases, and 25 (37%) in the other group. 6 month progression free survival in large cystic group was 57% and 75% in the other group. 12 month progression free survival was 0% in large cystic group and 52% in the other group (p=0.02). **Conclusion** It was found from the results that hemorrhagic metastasis rarely recurred; however large cystic metastasis frequently recurred after surgery regardless of adjuvant therapy. Therefore, it seems reasonable to conclude that postoperative RT and close follow-up should be necessary in large cystic metastasis.

O101

NEOPLASTIC MENINGITIS - IS MRI AS SENSITIVE AS CSF CYTOLOGY?

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Background: Although CSF cytology and MRI are standard methods for the diagnosis of neoplastic meningitis (NM), this complication of neoplastic disease still remains to be difficult to detect in some cases. We therefore re-evaluated the sensitivity of gadolinium(GD)-enhanced MRI and cerebrospinal-fluid (CSF)-cytology for the diagnosis of LM differentially for solid and haematological malignancies and for normal or elevated cell counts. **Methods:** we identified retrospectively 101 cases of NM diagnosed in our CSF laboratory since 1990 with complete data of both MRI and CSF-cytology. 34 had haematological, 67 solid neoplasms. CSF-cell counts were increased in 63 and normal in 35 patients. **Results:** for haematological neoplasms, MRI was positive in 53%. CSF cytology was positive in 97%. In solid tumours, we found MRI-sensitivity of 0.81 and cytological sensitivity of 0.76. With normal CSF-cell-counts, MRI was positive in 63%, (0,57 haematological, 0,75 solid malignancies), CSF-cytology 78%, (0,9 in haematological, 0,64 in solid neoplasms). In cases of increased cell-counts, MRI-sensitivity was 0.75 (0,52 for haematological, 0,87 for solid malignancies), and sensitivity of CSF-cytology was 0.89 (1,0 for haematological and 0,82 for solid neoplasms).23 patients were treated with intrathecal MTX or Ara-C, 16 patients with liposomal Ara-C. 62 patients were not treated intrathecally. **Conclusions:** we confirmed here the high overall sensitivity of MRI for the diagnosis of neoplastic meningitis. The best sensitivity, however, was seen in solid tumours and elevated cell counts. In haematological malignancies, a markedly lower sensitivity of MRI was seen. Of note, we consider the very high sensitivity of cytology in haematological malignancies to be artificial due to methodological reasons of this retrospective study. We conclude that MRI is a very sensitive method to detect NM especially in solid tumours and elevated cell counts. With normal cell counts and haematological neoplasms, CSF-cytology remains to be superior to radiological methods.

O102

IMMUNOTHERAPY WITH CPG-ODN IN NEOPLASTIC MENINGITIS(FOR PATIENTS WITH NEOPLASTIC): PHASE I TRIAL.

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Phosphorothioate oligodeoxynucleotides containing CpG motifs (CpG-ODNs) are immunostimulating agents with antitumor effects in animal models. Neoplastic meningitis is a devastating disease, with no efficient therapeutic available. A phase 1 trial was conducted in patients with neoplastic meningitis to define the safety profile of subcutaneous injections, combined or not with intrathecal administration, of a CpG ODN.Methods Cohorts of three patients were treated for 5 weeks with escalating doses of CpG-28 (level 1: 0.1mg/kg/week subcutaneously; level 2: 0.3mg/kg/week subcutaneously; level 3: 0.3mg/kg subcutaneously associated with 3 mg intrathecally every other week). The diagnostic of neoplastic meningitis was based on CSF cytology or clinical symptoms with meningeal enhancement on MRI. The primary endpoint was tolerance. Secondary endpoints were time until neurological progression and survival.ResultsNine patients (3 per level) were treated between march 2007 and may 2008. Primary cancer was malignant glioma, breast cancer ,melanocytoma , ependymoma and small cell carcinoma. Median age was 51 years and median KPS was 70%. In patients who were treated with subcutaneous injections only, no significant improvement in clinical or radiological symptoms were seen. In the three patients who were treated with both subcutaneous and intrathecal administrations, clinical improvement was observed. However these patients received concomitant treatment with spinal radiotherapy or systemic chemotherapy which might have impacted the outcome.Adverse effects possibly or probably related to the studied drug were moderate and consisted in grade 2 lymphopenia, anemia and neutropenia, local erythema at injections sites, fever, seizure and back pain.The median time until neurological progression was 9 weeks. The median survival was 17 weeks . One patient is still alive. Conclusion CpG-28 was well tolerated at doses up to 0.3mg/kg subcutaneously and 3 mg intrathecally. Main side effects were limited to local erythema, lymphopenia and fever. Escalating doses of CpG-28 for intra-thecal administration is on-going.

O103

ESTABLISHING MYELOABLATIVE CHEMOTHERAPY PROTOCOLS IN LOW-RESOURCE COUNTRIES: THE PAKISTAN EXPERIENCE OF THE CURE2CHILDREN FOUNDATION.

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Many children diagnosed with brain tumors in lower-income countries may not have access to appropriate neurosurgical and/or radiotherapy standards.

The potential of high-dose chemotherapy with autologous stem cell rescue in the context of childhood brain tumors to improve treatment efficacy while reducing long-term toxicities and costs has not been fully explored and might be usefully applied in middle-income countries to compensate for inadequate neurosurgery and/or radiotherapy.

Since August 2008, the Cure2Children Foundation has supported both financially and professionally the development of two centers in Pakistan for stem cell transplantation applied to the cure of thalassemia major, a very prevalent (50.000 cases in Pakistan) and curable deadly genetic disease. The methodology employed consists of matched-related allogeneic bone marrow transplantation after administration of myeloablative chemotherapy (thiotepa 10 mg/kg, busulfan 14 mg/kg and cyclophosphamide 200 mg/kg). Management standards for central venous access, severe pancytopenia, immunesuppression, hospital infection control, and other relevant issues have been addressed by local training as well as with web-based data management and videoconferencing. A total of seven transplants have been performed so far, three in an established center (National Institute of Blood Diseases, Karachi) and four in the two newly developed services mentioned above. No transplant-related deaths or untoward morbidities have occurred at present, detailed clinical and cost analysis will be presented.

We believe that this experience might be relevant to the possible implementation of autologous transplantation for the cure of some brain tumors of childhood in lower-income regions.

O104

MANAGEMENT OF BRAIN TUMORS IN THE PHILIPPINES

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The Philippines is a very diverse country because of the large socioeconomic gaps. Thus we have centers with very advanced technology and medical expertise at par with international standards for treating brain tumors but are inaccessible to a large majority of people because of the high cost.

For the low income groups, free medical expertise as well as minimal hospital fees are available in tertiary care government hospitals, such that benign brain tumors can be treated. However, there are only a handful of these, hence the long waiting time for imaging studies, elective surgeries and whole brain radiotherapy. For tumors requiring adjuvant treatment with chemotherapy and other types of radiotherapy, the high cost of medicines and lack of radiotherapy equipment in government hospitals coupled with minimal insurance coverage limits care for these patients.

To standardize care, several brain tumor centers offering multi-disciplinary treatment have been set up. Regular brain tumor board meetings are also held in various centers. A year ago, the Philippine Society for Neuro-oncology was started to enhance brain tumor education and management .

Each doctor seeing brain tumor patients tries to help by doing surgeries or chemotherapy in government hospitals where they can be done for free or at one-third of the cost, by referring patients for assistance to receive medicines at a reduced cost from either government agencies or other funding agencies. Despite this, the majority of low income patients will not get the care they need, not because of a lack of medicines nor a lack of medical expertise but mainly because of the lack of money to pay for diagnostic tools, chemotherapy or radiotherapy.

Long term solutions are needed such as increasing imaging and radiotherapy facilities in government hospitals, lowering the cost of chemotherapeutic drugs or improving accessibility through various foundations. We can continue to improve the quality of care by continuing education programs and by involvement in clinical trials to increase patient options.

O105

THE CHALLENGES OF BUILDING AND SUSTAINING A PEDIATRIC NEURO-ONCOLOGY PROGRAM IN A DEVELOPING COUNTRY

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BACKGROUND

Establishing a pediatric neuro-oncology service can be challenging given the complexity of brain tumors in children. Creating such a service requires the involvement of different disciplines such as neurosurgery, neuropathology, and radiation oncology. These challenges are tougher and more multifaceted in a developing country than a developed one.

STEPS NEEDED TO BUILD AND SUSTAIN A NEURO-ONCOLOGY SERVICE

The basic factors required to build and sustain a successful pediatric neuro-oncology service are common to developed and developing countries. These include establishing a dedicated multidisciplinary team, empowering nursing, providing palliative care, and adhering to approved disease-specific protocols and guidelines.

For developing countries, twinning initiatives with developed countries have been shown to enhance and speed the progress of newly established services in leukemia and lymphoma, but their contribution remains to be tapped for brain tumors. Integrating telemedicine into a twinning program can improve the quality of care of patients, especially if implemented in a prospective fashion.

OBSTACLES ALONG THE WAY

It is a major challenge to coordinate different disciplines to work in conjunction and approve standard therapeutic approaches in developing countries. Another major obstacle is the inherent attitude toward nursing in many developing countries. Although twinning and telemedicine can positively influence a newly developed neuro-oncology service, it still can provoke sensitive issues to local teams in developing countries. To avoid unconstructive impact of telemedicine and twinning the mentoring team should be perceptive of such issues. This can be achieved though frank and continuous communication.

CONCLUSION

There is an urgent need to improve the care for children with brain tumors in developing countries. Immediate steps need to be taken at the local and international levels and individual plans developed for each program and country.

O106

CHILDHOOD BRAIN TUMORS INCIDENCE AND CARE IN THE KYRGYZ REPUBLIC

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BACKGROUND
There were some important obstacles regards pediatric brain tumors registrations and cares in the Kyrgyz republic.

STATEMENT ON THE PURPOSE OF THE STUDY: study childhood brain cancer incidence, age, sex, and ethnic differences.

METHODS USED. Childhood cancer registration in Kyrgyzstan is carried out by network of population-based regional registries, National Center of Statistic since 1983. Collected date from forms submitted along morphological findings and death certificates. The population figures and cancer incidence rates were provided in for age (0-4, 5-9, 10-14), ethnic groups, for each sex and calendar years. Counted crude, age-standardized rates (ASR) per 1 000 000. Estimated population relative risk in the urban and rural areas.

SUMMARY OF RESULTS AND STATISTICAL ASSESSMENTS. There were 270 registered with new diagnoses of brain cancer in children (1983-2007): 150 (ASR 7.5) boys, and 120 (ASR 6.9) girls. Male/female proportion is 1.25. Total ASR annual childhood cancer incidence was 74.8. The most frequent diagnostic groups were leukaemia's (30.3%, ASR 20.8), non-Hodgkin lymphomas (9.9%, ASR 7.3). Non-registered patients – 17%.
Analyses of geographical variations showed highest incidence of brain tumours in urban (Bishkek, Chui) than rural mountains regions (Osh, Naryn, Issyk-kul), (RR=2.2, CI 95% 1.57-8.99). Incidence rate was significantly higher in Russians (ASR 10.8), compared with native Kyrgyz's (5.7) and Uzbeks (8.5).
Only one pediatric oncology department (30 hospital beds) is in Bishkek (capital) on whole republic. Surgical treatment performed at the National (adult) hospital without radiation or chemotherapy. About 60% of childhood population (South part - Osh, Djalalabat, and Batken areas) haven't any possibility to receive any treatment, even chemotherapy or adequate initial brain surgery.

CONCLUSIONS. Childhood brain cancer incidence in Kyrgyzstan is low and similar to those reported from some Asian developing countries. The setting (radiotherapy) at the National cancer center is unsatisfactory both with regard to trained physicians, medical supplies and supportive care. Too many patients are seen in an inadequately staffed and equipped department. High risk patients with brain tumors are at a definite loss, especially since supportive care is basically absent. There is an urgent need to improve the care for children with brain tumors.

O107

MANAGEMENT OF CANCER PAIN IN DEVELOPING COUNTRIES

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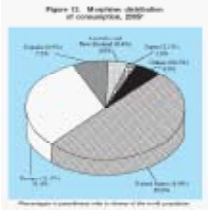
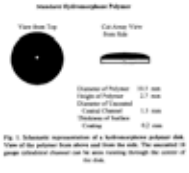
Cancer pain is often undertreated even in developed countries with abundant resources and easy access to oral, parenteral, and transdermal opioids. In developing countries, where opioids are generally not available, the challenges to providing adequate analgesia to patients with cancer are often insurmountable. In spite of concerted efforts by the World Health Organization and other organizations to make oral opioids available and to educate physicians and government officials, little progress has been made in relieving pain in cancer patients in this important area. Major obstacles include: 1) a limited budget for pharmaceutical agents, 2) preference for agents that prevent (ie vaccines) or cure (ie antibiotics) disease, 3) a focus on children and young adults who have many years to live, and 4) concerns about compliance (as immediate release opioids must be taken every three to four hours), storage (especially in poor households), and drug diversion. The documented association between drug diversion, addiction, violence, and AIDS in the West is worrisome to public health officials in developing nations who face many other important health issues.

Nevertheless, the management of cancer pain is of high priority given the rapidly increasing incidence of cancer in the developing world and the fact that approximately 70% of patients with cancer experience severe pain during the course of their illness. As a result, novel approaches that address fundamental concerns regarding opioid availability in these countries are needed. One such approach employs an inexpensive, non-biodegradable, polymeric implant designed to provide continuous hydromorphone to the subcutaneous tissue for one to three months. This subcutaneous implant is constructed of materials that are all approved by the FDA, can be implanted by a physician extender, and releases at a continuous rate without an initial "burst". The materials and manufacturing are designed to provide an inexpensive product suitable for use in developing nations. It has the potential to reduce concerns about patient compliance, drug storage, and opioid diversion while making opioids available to patients in rural areas and reducing the number of follow-up visits necessary for medication refills,.





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***Disclosure: Dr. Grossman holds several patents on the implantable hydromorphone polymer and is a principal in Axxia Pharmaceuticals**

O108

DESCRIPTIVE EPIDEMIOLOGY OF CEREBRAL GLIOMAS IN ALBANIA; COMPARATIVE ANALYSIS DURING THE PERIOD OF 1993-2004

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Purpose:
Descriptive epidemiology of cerebral gliomas has recently been the subject of several studies, reporting an increase in brain tumor rates (both incidence and mortality). Epidemiological data from low and middle income (LAMI) countries, and especially Albanian data, have not been reported. The purpose of this study is to make a descriptive analysis of cerebral gliomas in Albania between 1993 and 2004.

Methods:
The cancer database of information on pathologically proven intracerebral gliomas identified in our department between 1993 and 2004 was reviewed. The incidence rates by histological subtype, age and gender were calculated and compared in three time intervals divided by two &quotmilestones&quot, the introduction of CT scan in 1997 and of MRI in 2001. Variation of the incidence between 1993 and 2004 was also analyzed.

Results:
The highest cumulated incidence was observed in the high grade astrocytoma group (1.1/100/000/year) followed by the low grade glioma group (astrocytoma and oligodendroglioma and mixed tumors) (0.9/100.000/year). A significant increasing trend of 75% per year and per time interval was observed in the incidence of astrocytic tumors in all age groups demonstrating the important impact of radiological improvement. The incidence rates of high grade astrocytoma and low grade glioma displayed a Gaussian distribution with mean age at 50-60 years and 31-40 years respectively.

Conclusions:
This study represents an interesting analysis of data belonging to three different periods: a) 1993-1997, which represents the early post-communist period when CT and MRI were lacking, b) 1997-2000, when the CT scan was used, and c) 2001-2004 when the MRI was also used. As a result of these two important radiological developments, our data show a relative and absolute (as stated in observations made in other countries) increase in the incidence rate of cerebral glioma.

O109

AGE AS PREDICTIVE FACTOR IN GLIOBLASTOMAS: POPULATION-BASED STUDY

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We have evaluated 715 glioblastoma patients who were diagnosed in the Canton of Zurich, Switzerland between 1980 and 1994, to provide information on how patients were treated at the population level. Despite a general policy during the study period of treatment by surgical intervention aimed at maximum tumour removal followed by radiotherapy, there was a marked tendency toward limited intervention and lack of treatment with advancing patient age. Of those younger than 65 years, 82% were treated either with surgery followed by radiotherapy, surgery alone or radiotherapy alone, versus 47% of patients 65 years or older. Only 25% of patients older than 75 years underwent surgery and/or radiotherapy, while the remaining patients were given best supportive care (BSC). The mean age of patients was 54.5 years for those treated with surgery and radiotherapy, 58.3 years for surgery alone, 62.2 years for radiotherapy alone and 69.2 years for BSC. Among patients who were treated with surgery plus radiotherapy and those treated with radiotherapy alone, younger patients (<60 years) had a significantly higher survival rate than older patients (≥60 years). In contrast, no significant difference in survival was observed between younger and older patients treated with surgery alone or receiving BSC, suggesting that lower survival rates in elderly patients with glioblastoma may be at least in part due to a lesser response to radiotherapy.

O110

TUMOR TISSUE IDENTIFICATION IN THE PSEUDOCAPSULE OF PITUITARY ADENOMA: SHOULD THE PSEUDOCAPSULE BE REMOVED FOR TOTAL RESECTION OF PITUITARY ADENOMA?

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OBJECTIVE: The pseudocapsule can be found in the transition zone between the adenoma and surrounding normal pituitary tissue. We investigated the precise histology of the pseudocapsule and evaluated the remission rate, pituitary function and recurrence rate after intensive resection of the pseudocapsule. **METHODS:** In 616 patients with pituitary adenomas (Hardy type I to III) over a period of 14 years, we have introduced intensive resection of the pseudocapsule to achieve complete tumor removal. A combined pituitary function test and radiological study were performed before surgery, one year after surgery and at subsequent 1.5 year intervals. **RESULTS:** Pseudocapsules were identified in 343 (55.7%) patients and the distinct pseudocapsules were observed in 180 (52.5%) patients. In the remaining 163 patients, the pseudocapsules were incompletely developed. Tumor infiltration was present in the pseudocapsule in 71 (43.6%) patients. The presence of a pseudocapsule was more frequent in prolactin (PRL)-secreting tumors (70.9%) than in growth hormone (GH)-secreting (55.0%) and adrenocorticotropin (ACTH)-secreting (40.0%) tumors. In the 243 patients of the total resection group, surgical remission rate was 99.1% in clinically non-functional tumors (CNPTs), 88% in GH-secreting, 70.6% in PRL-secreting, and 100% in ACTH-secreting tumors. The surgical remission rate was 86.2% in the presence of a pseudocapsule and 94.3% in the absence of a pseudocapsule. Preoperative hypopituitarism improved in 140 patients (57.6%), persisted in 47 patients (19.3%), and was aggravated in 33 patients (13.6%). The tumor recurrence rate was 0.8% in the total resection group and was 42.1% in the subtotal resection group. **CONCLUSION:** We have shown that tumor tissue is a frequently present within the pseudocapsule, suggesting that any tumor remnant in the pseudocapsule could be a source of recurrence and an obstacle to achieving complete remission. These results indicate that intensive resection of the pseudocapsule could result in higher remission rate without deteriorating pituitary function.

O111

MANAGEMENT OF PITUITARY ADENOMA WITH STEREOTACTIC RADIOTHERAPY AT PRINCESS MARGARET HOSPITAL.

This Abstract nominated the Hoshino Award.
Please refer to P067 on page 153.

O112

LONG TERM FOLLOW UP OF PATIENT WITH ACROMEGALY AFTER LINAC RADIOSURGERY

This Abstract nominated the Hoshino Award.
Please refer to P060 on page 151.

O113

SEVERAL TIPS TO AVOID COMPLICATIONS OF INTRACYSTIC CHEMOTHERAPY WITH BLEOMYCIN FOR CRANIOPHARYNGIOMA IN CHILDREN

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Objectives: We have reported the effectiveness of intracystic chemotherapy with bleomycin for cystic craniopharyngiomas. This result has been confirmed by the following reports. However, relevant complications, including cerebral ischemia, visual and hypothalamic damages, associated with local bleomycin therapy have been also reported. We have, so far, experienced no such significant complications with our methods. Here we report the way to avoid such complications.

Methods and results: Intracystic belomycin administration was performed on 14 children since 1988 in our institutes. When radiological examinations indicated cystic craniopharyngiomas, we removed the cyst wall partially to confirm the pathological diagnosis and then placed the Ommaya reservoir tube into the tumor cavity. It is important to place all the side holes of tube within the cyst cavity and to tighten its entrance so as to avoid leakage of infused bleomycin. Bleomycin was administrated 2 weeks postoperatively via the Ommaya reservoir connected to the tube. Cystography should be performed prior to bleomycin administration. A smaller dose (5 mg or less) per injection or infrequent (every other day) injections would lessen the complications. In addition, the concentration of bleomycin in the cyst is most important. If the dilution of bleomycin is not enough, the drug may leak through the wall of the cyst. We suggest an appropriate concentration of bleomycin solution is 1 mg/ml or less and the timing of injection is immediately after the aspiration of cystic fluid. In 9 children the cysts have almost disappeared and the children have achieved a good school life. Four children are also achieving a good life condition after additional stereotactic radiosurgery. No severe complications were obeserved.

Conclusion: We recommend several tips for appropriate usage of bleomycin to avoid complications and to achieve a good result.

Keywords: craniopharyngioma, bleomycin, complication

O114

INTRACYSTIC BLEOMYCIN THERAPY FOR CRANIOPHARYNGIOMA IN CHILDREN.

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BACKGROUND: Craniopharyngioma is a histologically benign tumor that originates from epithelial nests or from areas of squamous metaplasia. The incidence of craniopharyngioma is 2-10% in pediatric primary brain tumors. Surgical removal and radiation therapy are associated with significant risk for morbidity in the pediatric population with craniopharyngioma. Intracystic bleomycin therapy (IB) has been proposed as a treatment for predominantly cystic craniopharyngioma. The aim of the study was to review the effectiveness of intracystic bleomycin therapy in our craniopharygioma children. **METHODS.** Pediatric craniopharyngioma patients treated with IB at Taipei Veteran General Hospital had been enrolled to participate the retrospective review from 1999 to 2008. Brain MRI or CT was performed every 3 months to evaluate the objective response of intracystic bleomycin injection. **RESULTS.** There are 7 boys and 2 girls received the intracystic bleomycin therapy. The median age is 7.8 years (range from 3.3 to 11.8 years). The median follow-up duration was 44 months (range from 9 to 79 months). For the total 9 patients, 8 achieved good response to IB therapy, and 1 achieved progression. The patient with progression showed no response to IBT and tumor relapsed quickly after 2 months of treatment. The median progression-free-survival was 29 months (range from 2 to 79 months.). **CONCLUSIONS.** Our preliminary results showed intracystic administration of bleomycin is a valid and effective therapy for certain children with craniopharyngioma. IBT may delay the need for aggressive surgery or radiation therapy for several years. However, further prospective randomized studies are needed to evaluate the feasibility and effectiveness of this treatment.

O115

INTRATUMORAL BLEOMYCIN INJECTION AS A PRIMARY THERAPY FOR CYSTIC CRANIOPHARYNGIOMA

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While total resection is a desirable goal for the treatment of craniopharyngioma, it is not always accomplished, and is associated with permanent endocrinological and visual deficits. This knowledge has led us to perform intratumoral bleomycin injection as a primary therapy for cystic craniopharyngioma. Five patients (4 adult and 1 child) in whom cystic craniopharyngioma was recently diagnosed were treated primarily with a schedule of 3 mg of intracystic bleomycin three times a week for 5 weeks. One patient was excluded due to bleomycin leakage in reservoir permeability test. Remainders didn't require subsequent resection and radiotherapy. Median follow-up period was 36 months.There was a reduction in cyst size greater than 90% with the preservation of visual and endocrinologic function.Intracystic bleomycin administration could be a feasible option for the treatment of predominantly cystic craniopharyngioma in terms of tumor control and tolerability.

O116

DIFFERENTIAL IMAGING CHARACTERS OF THE TUMORS INVOLVING THE CAVERNOUS SINUS

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Purpose: Preoperation diagnosis of 10-20% cavernous sinus tumors remains unclear. Tumor rarity is one factor, but common lesions with atypical manifestations maybe another. This review aims at illustrating these untypical manifestations, so as to improve the accuracy of imaging diagnosis of common tumors within the cavernous sinus. **Methods:** From 2000-2008, a total of 445 cavernous sinus tumors were surgically resected in a single neurosurgical center. Meningioma (38.7%), schwannoma (17.8%), bone derived tumor (13.0%), cavernoma (11.2%), dermoid or epidermoid tumor (6.7%), pituitary adenoma (4.7%), metastatic tumor (3.6%) accounted for the most common types of cavernous sinus tumors. We retrospectively reviewed 56 patients who were either indefinitely diagnosed or misdiagnosed before operation. Their imaging characters were carefully reviewed, including the tumor location, brightness and contrast properties of CT and MRI results, enhancement patterns, presence of tumor compression or incorporation of the ICA, the positional relationship of the tumor to the dural layers of the cavernous sinus walls, the extra-sinus invasion or derivation, manifestions on MRS, SPECT and PET. The operation records were also closely examined, regarding blood supply, hardness, and most importantly, the positional relationship of the tumor and neurovascular structures within the cavernous sinus. Both intra-operative and pathological findings were also thoroughly compared with preoperational imaging characteristics. **Results:** The size of these tumors was usually extremely large or small in comparison with their typical counterparts. Growth patterns of the tumor were of potential value in indicating the tumor properties in larger tumors while location was indicative in smaller ones. Intra-lesional patterns on MR imaging were suggestive of certain pathological changes under certain circumstances. **Conclusion:** Detailed insight of the imaging characteristics may add more knowledge to the differential diagnosis of tumors within the cavernous sinus.

O117

RESULTS OF THE QUEEN SQUARE MENINGIOMA STUDY: EPIDEMIOLOGY, EPILEPTIC CHARACTERIZATION, PRESENTING SYMPTOMS, TREATMENT, CLINICAL OUTCOME AND RECURRENCE.

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Introduction: Despite meningiomas being the most common benign intracranial tumour little is known about their association with epilepsy and outcome. The aim of this study was to define their epidemiology, identify their relation with epilepsy, and the factors predisposing to recurrence as well as prediction of outcome. **Material and methods:** The case notes of 1101 patients treated in the National Hospital for Neurology and Neurosurgery, London, UK 1975 to 2000 were analyzed. Data collected included age, sex, clinical symptoms, GCS, tumour location, type of pre and postoperative epilepsy, Simpson grade, treatment, recurrence and outcome using Karnofsky score. **Results:** Mean follow up: 36months. Females: 65%, males: 35%. Age at presentation ranged from seven to 97 (mean 56) years. Meningiomas were more likely to present with visual symptoms (25.28%), headaches (21.19%), seizures (17.20%) and paresthesia (10.97%). Presenting symptoms and tumour location were statistically linked (p&lt:0.0001). Symptoms and recurrence were not strongly related (p&lt:0.475) as opposite to symptoms and postoperative Karnofsky score (P&lt:0.0001).Simpson resection grade (I &II are less likely to recur), and pre and postoperative epilepsy are related to tumour recurrence. Preoperative epilepsy is linked to tumour location (p&lt:0.013) and postoperative epilepsy, age and histopathology results were linked to recurrence (p&lt:0.0001)as well as tumour location (p&lt:0.006)and laterality (left tumours recur more than right-sided) (p&lt:0.006) . The presence of psammoma bodies was related to a benign histological diagnosis (p&lt:0.004). Postoperative radiotherapy reduced recurrence rate (p&lt:0.0001). Recurrence peaks on the 24 months and 10th year from the first treatment date. **Conclusions:** Assessment of the identified factors may aid in outcome prediction and preoperative counselling and decisions related to patients treatment.

O118

KI67 PROLIFERATION INDEX DOES NOT PREDICT SURVIVAL IN PATIENTS WITH NON-BENIGN MENINGIOMAS: ANALYSIS OF 86 CASES

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Background: Non–benign (atypical and malignant) meningiomas are rare tumors and only few studies on larger series have been preformed so far. Because of their tendency to recur and thus poorer prognosis, they constitute a separate biological and clinical entity compared to benign meningiomas. **Methods:** 92 patients with non–benign meningiomas operated on in years 1990–2002 at the University Medical Centre Ljubljana were analyzed in our retrospective study. Adopting the newest (2007) WHO classification criteria, 86 tumors were microscopically reclassified as primary atypical or malignant meningiomas. A histological, immunohistochemical and statistical analysis was undertaken to determine the correlation of several clinical, radiological, histological, and immunohistochemical factors with survival. **Results:** Mean and median overall survival times were 6.9 and 6.3 years, respectively. Overall 5– and 10–year survival rates were 74.7% and 54.3%, respectively. Univariate analysis of 86 non–benign meningioma patients confirmed female sex (p=0.035) and age under 56 years (p=0.004) were significantly related to prolonged survival. Among histological factors, microinvasion into brain parenchyma (p=0.011), dense cellularity (p= 0.030), prominent nucleoli (p=0.020), and sarcomatous appearance (p=0.019) all correlated with decreased survival. There was no difference in survival between patients with low (equal or less than 5%) or high (greater than 5%) proliferation index (p=0.329). **Conclusion:** In patients with totally excised atypical and malignant meningiomas, presence or absence of microinvasion into brain parenchyma is a better predictor of survival than Ki67 proliferation index.

O119

ANALYSIS OF 56 MENINGIOMA CASES CLASSIFIED AS WHO GRADE III

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Background: According to the WHO classification 2007, there are three subtypes of WHO grade III as anaplastic (malignant), rhabdoid and papillary meningiomas. Here we analyze 56 meningioma cases classified as WHO grade III in our hospital from 2004 to 2007, when 2,310 meningioma cases were operated (male 697, female 1610). **Methods:** 47 cases are anaplastic meningiomas, 3 cases are rhabdoid meningiomas and 6 are papillary meningiomas. 21 patients of them are recurrent cases who had operations before. Follow up period is at least one year. **Results:** The patient age ranges from 10yrs to 79yrs with mean age 51.1yrs, with a male to female ratio of about 3:2. The distribution position of these tumors are as follows: 21 at convex of brain hemisphere, 15 into sagittal sinus, 11 at skull base, 4 at tentorium, 3 in lateral ventricles, 1 at spinal cord and 1 at sellar region. Resection extent of Simpson grade is I 34, II 16, III 5, and IV 1. Rhabdoid meningiomas had rather worse prognosis than papillary meningiomas. Mean survival time of anaplastic meningiomas is 52.9months (range from 13months to 8years) in 25 patients, 12 patients died at follow-up time with mean survival time 54.3months (range from 1 month to 10 years) and 9 patients lost contacts. Half of patients underwent radiotherapy after operation, and half of them had not shown recurrence. Only 6 patients had chemotherapy without much effect. **Conclusions:** Quick progression, high rate of recurrence and mortality are characters of malignant meningiomas, though each subtype has a little different prognosis. Treatment for malignant meningiomas is still relied on the extent of resection and very difficult. Radiotherapy has poor outcome and risk for malignant transformation. So it is very urgent to look for appropriate combined treatment for this type of meningiomas.

O120

CHORDOMA AND CHONDROSARCOMA OF THE SKULL BASE: COMPARATIVE ANALYSIS OF OUTCOMES IN 30 PATIENTS

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To review the skull base chordomas and chondrosarcomas and analyze comparative outcomes between these tumors. Between 1991 and 2005, thirty consecutive patients with pathological diagnosis of chordoma (n=19) or chondrosarcoma (n=11) of the skull base were managed by multimodal treatment combining one or more surgical approaches with conventional radiotherapy and/or GKRS. A retrospective analysis was conducted on these patients. Follow-up data were complemented by a most recent telephone interview with the patients or their family members. Age of the patients ranged from 3 to 69 years (median, 36 years). Seventeen patients were female and 13 male. Average length of follow-up was 56 months (range, 2-172 months). A total of 43 surgical approaches (31 for chordoma vs. 12 for chondrosarcoma) were performed at the initial or recurrent setting. Adjuvant radiotherapy and GKRS were performed on 21 and 6 patients, respectively. Four deaths occurred during the follow-up, two of which resulted from progression or recurrence of chordoma, one from pulmonary embolism, and another one from the sepsis unrelated primarily to the tumor. Recurrence of chordoma was observed in 5 patients out of 13 (38%) and 6 out of 10 (60%), each within 3 and 5 years after the initial treatment, whereas only 1 recurrence of the chondrosarcoma was observed in 2 years after the surgery with no radiation. Among 15 survivors with the chordoma, 4 patients were suffering from severe disability with progressive disease. In contrast, most patients with the chondrosarcoma harbored stable disease and less disabling symptoms except one instance of recurrence.Outcomes in the functional status as well as survival were much worse in chordomas than those in chondrosarcomas. The authors recommend a maximum safe resection, possibly to the extent of total resection at the initial setting, combined with complementary treatment, especially GKRS for the management of the skull base chordomas.

O121

TREATMENT WITH HIGH MARGINAL DOSE IS MANDATORY TO ACHIEVE LONG-TERM CONTROL OF SKULL BASE CHORDOMAS AND CHONDROSARCOMAS BY MEANS OF STEREOTACTIC RADIOSURGERY

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PURPOSE: The purpose of this study was to evaluate the effect of stereotactic radiosurgery (SRS) for chordomas and chondrosarcomas of the skull base and to determine the optimal marginal dose to control these tumors. **METHODS:** Fourteen patients with histologically confirmed chordomas or chondrosarcomas underwent 16 sessions of SRS using gamma knife. The mean tumor volume was 12 cm3 and the marginal doses ranged from 10 to 20 Gy (mean, 15 Gy). Lower marginal doses of 12 Gy on average (range, 10-12.5 Gy) was applied for 4 patients since they underwent prior fractionated radiotherapy, and partial treatment for which parts of tumors were excluded from planned target volume (PTV) because of their proximity to critical normal structures such as the optic pathway and the brainstem was applied for 5 patients. The whole tumor volume was covered with higher marginal doses of 18 Gy on average (range, 16-20 Gy) for 6 patients. The mean follow-up period after SRS was 55 months. **RESULTS:** Progression free survival (PFS) rates at 1, 2, and 5 years after SRS were 93%, 51%, and 34%, respectively. Five-year PFS rate for patients whose tumors were completely covered by PTV was 53 months and tended to be higher than that of 17 months for partially treated patients ($p = 0.11$). Five-year PFS rates for patients underwent SRS with higher and lower marginal doses were 60% and 14%, respectively, which were significantly different ($p = 0.023$). Tumor progression after partial irradiation mainly occurred from sites where delivered doses were reduced. **CONCLUSIONS:** Sufficient marginal dose at least 18 Gy appears crucial to obtain tumor control in case of chordomas. Proper combination with surgical resection to detach tumors from critical structures and to reduce tumor volume is necessary to completely deliver sufficient marginal doses to lesions at the time of SRS.

O122

RESULTS OF PROTON BEAM THERAPY FOR CHORDOMA AND CHONDROSARCOMA OF THE SKULL BASE: SHIZUOKA CANCER CENTRE EXPERIENCE

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Purpose: To assess the results of proton beam therapy (PBT) in the treatment of skull base chordomas and chondrosarcomas..**Methods:** Between April 2004 and August 2008, 11 patients (median age, 40years) with chordomas (n=6) and chondrosarcomas (n=5) underwent proton therapy at Shizuoka Cancer Centre. The median tumour dose was 67 GyE. Late toxicity was assessed using the NCI-CTCAE grading system. The median follow-up time was 29.1 months (range, 3-55.8 months). **Results:** Actuarial 2-year local control rates were 80% for chordoma and 100% for chondrosarcoma. No regional failure or distant metastasis was observed, except for one patient with dedifferentiated chordoma. Four patients had pharyngitis, otitis media, and skin trouble of grade 1 to 2 as acute adverse events. No patients presented with post-therapeutic brainstem or optic pathways necrosis or dysfunction. **Conclusion:** The results compare favorably to other irradiation series. Observed toxicity was acceptable. These preliminary results are encouraging, but should be confirmed with a long-term follow-up.

O123

CDK INHIBITOR BLOCKS DNA REPAIR FOLLOWING G2 CHECKPOINT ACTIVATION IN HUMAN GLIOMA CELLS TREATED WITH TEMOZOLOMIDE

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Temozolomide (TMZ) induces prolonged G2-M arrest of glioma cells, and then non-apoptotic cell death associated with senescence-like phenomenon (in p53-wild type cells) or mitotic catastrophe (in p53-non-functional cells). To clarify the mechanism of TMZ resistance and to develop more effective TMZ-based regimen, we established U87MG-derived TMZ-resistant (TR) clones by serial treatment with TMZ. We found that TR clones underwent only transient or minimal G2 arrest following TMZ treatment even though G2 checkpoint was clearly activated as in their parental cells and that G2 checkpoint inhibitor re-sensitized these clones to TMZ. Since it has been suggested that G2 checkpoint system might suppress the linkage between DNA damage and cell death, we tested the effect of a cdk inhibitor flavopiridol which could target cdc2 (cdk1), a key protein in G2 checkpoint pathway, on TMZ-treated cells. Flavopiridol (< 10 nM) potentiated the cytotoxicity of TMZ, and suppressed the expression of key proteins at G2-M transition including polo-like kinase 1, aurora kinases and Pin1. FACS analysis revealed flavopiridol induced accumulation of TMZ-treated cells, but not untreated cells, exclusively at G2 (4N DNA content). This "complete" G2 arrest was associated not only with increased expression of γ-H2AX, a DNA double strand break marker, but also with increased release of cytochrome C in cytoplasm, which suggests that flavopiridol promoted apoptotic cell death signal in TMZ-treated glioma cells. Flavopiridol also enhanced cytotoxity of TMZ to the cells with over-activated Akt which has been previously shown to promote TMZ resistance, and re-sensitized TR clones to TMZ. Our results suggest that TMZ resistance could be promoted by enhanced DNA repair activity in G2-M transition following G2 checkpoint activation, and that cdk inhibitor enhanced TMZ cytotoxicity by suppression of this activity. We conclude that cdk1-targeted compound might be useful as chemosensitization agent for gliomas

O124

TEMOZOLOMIDE-INDUCED DIFFERENTIAL EXPRESSION OF DNA REPAIR GENES IN MALIGNANT GLIOMA CELLS

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Temozolomide (TMZ), an active alkylating agent in treating malignant gliomas, kills tumor cells with ill-defined molecular mechanism. To understand TMZ genotoxicity, we analyzed temporal regulation of DNA repair genes with levels of DNA damage, checkpoint activation and cellular status of O6-methylguanine-DNA methyltransferase (MGMT). During the first post-drug treatment cell cycle (PTC), rapid DNA double strand breaks (DSBs) were detected (1h) in MGMT-deficient cells, followed by ATM phosphorylation, p53 accumulation, transient G1/S arrest and significantly reduction of S-phase progression. Concurrently, global transcription repression was discerned, followed by 2 to 5-fold up-regulation of 9 nucleotide/base excision repair (BER/NER) and 5 DSB repair genes. During the second PTC, prominent G2/M arrest was accompanied by more than 5-fold up-regulation of 29 genes, which were members of multiple DNA repair pathways. In MGMT-proficient cells, significantly lower levels of DSBs (less than 30% of MGMT-deficient cells) and lack of NER gene up-regulation were identified in the first 6h when substantial level of MGMT was observed. Association of NER and rapid DSB formation was further demonstrated by overlapping nuclear staining of the major NER endonuclease, XPG and the DSB marker, g-H2AX proteins. In conclusion, our data suggest that distinct transcriptional regulation of DNA repair genes contributes to the different TMZ genotoxicity at the first two PTCs. To our knowledge, this is the first study analyzing temporal regulation of global DNA repair and corresponding genotoxicity in different MGMT context for SN1-type alkylating agent. Our findings will also have significant implications for improving O6-alkylguanine based cancer chemotherapy.

O125

MGMT PROMOTER GENE METHYLATION AS DETERMINED WITH MULTIPLEX LIGATION-DEPENDANT PROBE AMPLIFICATION (MLPA) IS PROGNOSTIC FOR SURVIVAL AFTER RADIOTHERAPY BUT NOT PREDICTIVE FOR OUTCOME TO CHEMOTHERAPY IN OLIGODENDROGLIAL TUMORS

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Introduction. Anaplastic oligodendroglial tumors (AOD) are chemosensitive tumors. Two large randomized trials not see improved survival in patients treated with adjuvant pivotal trial on chemo-irradiation with temozolomide suggested that benefit from the addition of temozolomide to radiotherapy was basically confined to patients with a methylated MGMT gene promoter and thus no alkyltransferase expression. . In the present study we assessed the impact of MGMT promoter methylation on progression free survival (PFS) and overall survival (OS) using MLPA in the prospective randomized EORTC study 26951 on adjuvant PCV on 368 patients with AOD. The control arm received only radiotherapy (RT), the experimental arm RT plus PCV.

Material and methods. MGMT methylation was assessed using a methylation-specific MLPA (MRC-Holland) based on methylation sensitive restriction analysis. To estimate the fraction of methylated MGMT promoter DNA, normalized values obtained with each MGMT probe of digested DNA samples (in which only methylated DNA will remain undigested and produce a PCR product) were divided by normalized values of corresponding undigested DNAs. For the MGMT gene promoter CpG methylation in three regions was assessed, from which an average score was calculated; a ratio above 0.25 is considered indicative of methylation. Statistical analysis was done using this cut-off and with the MGMT average score expressed as a continuous variable, both for PFS and OS.

Results. In 152 of the 165 patients with sufficient material available for MGMT promoter analysis a result was obtained. Thirty-nine of these tumors showed 1p/19q co-deletion. In 121 of the 152 cases (80%) an MLPA average consistent with methylation was observed, which included 38 of the 39 1p/19q co-deleted samples (97%, $p < 0.001$). MGMT promoter methylation was found to be equally prognostic in both the RT and the RT/PCV treated patients, for both PFS and OS. In multivariate analysis using MLPA average as a continuous variable, MGMT promoter methylation and 1p/19q were independent prognostic factors. The PFS hazard ratio reduction of MGMT promoter methylation was 0.303 [95% confidence interval (CI) 0.135-0.677], for 1p/19q co-deletion 0.423 [95% CI: 0.232-0.768]. The cut-off as proposed for MS-MPLA was not optimal to separate patients in two groups of different prognosis.

Conclusion. 1p/19q co-deletion was strongly associated with MGMT gene promoter methylation. In this homogeneously treated group of AOD patients, the independent prognostic favourable effect of MGMT promoter methylation on both PFS and OS was equally strong in the RT group as compared to the RT/PCV group. There is currently no mechanistic explanation for the improved PFS in MGMT gene promoter methylated tumors after RT only, and other possible explanations need to be investigated.

O126

A PHASE I, OPEN LABEL, MULTI-CENTER STUDY TO ASSESS THE SAFETY AND TOLERABILITY OF CEDIRANIB [RECENTIN™] IN COMBINATION WITH LOMUSTINE CHEMOTHERAPY FOR PATIENTS WITH RECURRENT GLIOBLASTOMA

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Purpose: The objective of this study was to assess the safety and tolerability of cediranib (Recentin™), an oral, pan-VEGF receptor tyrosine kinase inhibitor, in combination with oral lomustine and to determine a dose for further studies with this combination in recurrent glioblastoma patients.

Methods: Patients at least 18 years old with pathological confirmation of recurrent glioblastoma were eligible. Patients could not have received previous anti-VEGF therapy and could have had no more than 2 prior chemotherapy regimens. Patients completing the first cycle (6 weeks) or having a dose limiting toxicity (DLT) before 6 weeks were evaluable. A total of 12 patients were enrolled into the study. Six patients were enrolled in the first cohort at a dose of cediranib 30mg in combination with lomustine 130 mg/m^2. Another 6 patients were enrolled in the second cohort at a dose of cediranib 30mg in combination with lomustine 110 mg/m^2.

Results: There were 2/6 DLTs reported in the first cohort (one grade 3 fatigue, one hypertensive crisis due to untreated hypertension). Five of 6 patients developed grade 3 or 4 hematological toxicities attributed to lomustine. The lomustine dose was reduced to 110 mg/m2 for the second cohort. There was 1/6 DLT (grade 3 fatigue) in the second cohort.

Conclusions: The results of this phase I study demonstrate that the combination of cediranib 30mg with lomustine 110 mg/m^2 is safe and tolerable. An international, multicenter, randomized phase III trial has been initiated.

O127

MULTIARM COMBINATORIAL STUDY OF SIGNAL TRANSDUCTION MODULATORS USING A SEQUENTIAL ACCRUAL DESIGN: A REPORT OF THE NORTH AMERICAN BRAIN TUMOR CONSORTIUM (NABTC) 0502

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Background: Single agent signal transduction modulators generally have been ineffective in most cancers including glioblastoma. Therefore, inhibiting multiple signaling pathways may be a more effective. However, performing individual phase I/II trials for each combination is inefficient. This study used a sequential accrual design to test three combinations of sorafenib, with erlotinib, temsirolimus or tipifarnib. **Methods:** The NABTC conducted phase I studies of sorafenib (VEGFR/PDGFR/Raf inhibitor) in combination with erlotinib (EGFR inhibitor), temsirolimus (mTOR inhibitor), or tipifarnib (farnesyltransferase inhibitor) in recurrent GBM. Accrual was sequential, decreasing study pauses for maturation of toxicity data. Eligibility: histologically proven GBM, radiologic progression, >18 yrs old, KPS >60, adequate bone marrow and organ function. No limit on the number of prior therapies. No enzyme-inducing antiepileptic drugs were allowed. Dose-finding used a standard 3 + 3 design. MTD was defined as the dose with DLTs in 1/6 or fewer patients. Serum pharmacokinetic (PK) studies were performed. **Results:** Overall 49 patients were enrolled onto phase I. The MTD was sorafenib 400mg BID and erlotinib 100mg qD. The MTD was temsirolimus 25mg qweek and sorafenib 400mg BID. The MTD was sorafenib 200mg BID and tipifarnib 100mg qD x 21 days, but the tipifarnib is below therapeutic levels. PK studies showed no drug-drug interactions for sorafenib with tipifarnib or temsirolimus. However, there was no accumulation of erlotinib, suggesting that sorafenib alters erlotinib metabolism or clearance. **Conclusions:** This study demonstrated that sorafenib can be combined with other targeted agents in and a sequential accrual design decreases logistical problems of phase I studies in multicenter trials. Phase II doses were successfully determined for sorafenib with temsirolimus and with erlotinib, permitting enrollment onto the phase II component. The new phase I study using an alternating week schedule of tipifarnib will be performed to permit a dose more likely to reach therapeutic concentrations.

O128

PHASE II STUDY OF BEVACIZUMAB PLUS ETOPOSIDE AMONG RECURRENT MALIGNANT GLIOMA PATIENTS: FINAL STUDY RESULTS

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Background: Recurrent malignant glioma (MG) patients treated with bevacizumab (BV), a neutralizing monoclonal antibody to vascular endothelial growth factor (VEGF) with or without chemotherapy, have noted significant therapeutic benefit. In this study, we evaluate the efficacy of BV plus etoposide (E), a topoisomerase inhibitor, among recurrent MG patients. **Methods:** Recurrent patients with no more than three prior episodes of recurrence are eligible, while those with prior BV treatment or prior intracranial hemorrhage are excluded. The primary outcome measure is 6 month progression-free survival (6-PFS). BV is dosed at 10 mg/kg intravenously every other week. Etoposide is orally administered daily (50 mg/m2) for days 1-21 of each 28-day cycle. **Results:** Fifty-nine patients (GBM, n=27; grade 3 MG, n=32) with a median of 2 prior progressions have enrolled. With a median follow-up of 45 weeks, median overall survival (OS) for GBM and grade 3 MG patients were 46 and 47 weeks, while the 6-PFS is 44% and 40.6%, respectively. The most common toxicities were neutropenia (41%), fatigue (22%), and infection (20%) and were grade 2 in most cases. One patient developed grade 1 intracranial hemorrhage and 1 patient had a grade 4 GI perforation. **Conclusions:** Combination of bevacizumab and etoposide is well tolerated in recurrent MG patients and is associated with encouraging anti-tumor benefit. Accrual is complete and an update of further treatment and follow-up will be presented.

O129

BEVACIZUMAB AND NITROSOUREA IN PATIENTS WITH RECURRENT MALIGNANT GLIOMA: A PHASE II MULTICENTER ITALIAN STUDY

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Purpose of study: Bevacizumab (BV) has shown a promising activity in recurrent malignant gliomas (MG) in combination with irinotecan. Few data are available on the combination of bevacizumab and nitrosoureas. **Methods:** In this ongoing phase II study patients with MG recurrent after surgery, radiation therapy and temozolomide are eligible. The treatment consists of an induction phase with BV at 10 mg/kg intravenously on day 1 and 15 and fotemustine (FTM) (a nitrosourea with elevated lipophilic properties) at 75 mg/m^2 intravenously on day 1 and 8, followed after a 3-week interval by a maintenance phase with BV at 10 mg/kg i.v and FTM 75 mg/m^2 i.v every 3 weeks until tumor progression or unacceptable toxicity. Patients undergo clinical and MRI assessment. The co-primary endpoints are objective response rate (ORR) and progression-free survival at 6 months (PFS6), with secondary endpoints of safety time to tumor progression (TTP) and overall survival. **Results:** From April 2008 to December 2008, 34 patients were enrolled, and 31 (22 glioblastomas and 9 anaplastic gliomas) are evaluable for response. Overall response rate (2 CR and 9 PR) was 35% (glioblastomas 33%, anaplastic gliomas 41.5%). Steroids were reduced in 50% of patients. Sixteen of 31 patients progressed with a TTP of 2.6 months (1-8.5). Progression was local in 10/16, local + leptomeningeal spread in 3/16 and gliomatosis in 3/16. Fifteen patients are free of tumor progression (from 2 to 8 months). Toxicities included grade III-IV neutropenia in 3 patients, grade III-IV piastrinopenia in 5 and grade III thrombosis in 2. Seventeen patients developed mild to moderate fatigue, 6 arterial hypertension and 3 grade I intratumoral haemorrhage. **Conclusions:** Combination of bevacizumab and fotemustine in recurrent malignant gliomas is safe and promising. Updated results, monitoring of CBV with perfusion MRI and correlations between MGMT promoter methylation and response/outcome will be presented.

O130

BEVACIZUMAB PLUS ERLOTINIB IN RECURRENT HIGH-GRADE GLIOMA: A PHASE II TRIAL

This Abstract nominated the Hoshino Award.
Please refer to P052 on page 147.

O131

PHENOTYPIC AND PHYSIOLOGIC EFFECTS OF BEVACIZUMAB IN A HIGHLY INFILTRATIVE AND ANGIOGENIC HUMAN GLIOBLASTOMA MODEL

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We have established highly invasive non-angiogenic and less invasive highly angiogenic GBM xenografts by passaging human glioblastoma biopsies in nude rats. The animal model shows that GBM progression is characterized by two distinct tumor phenotypes; one stem-like phenotype that relies on pro-invasive programmes and one less infiltrative angiogenesis dependent phenotype that shows loss of stem cell markers. Recent clinical information indicates that bevacizumab treatment has a dramatic effect on the contrast enhancing tumor compartment whereas the invasive part of the tumour is affected to a lesser extent. At present it is unclear if the remaining tumour cells represent a particular stem cell like subpopulation within the GBM.To address this question we assessed the treatment response of GBM xenografts to bevacizumab. In particular we asked the following: 1. To what extent does anti-angiogenic treatment effectively reduce tumor volume by decreasing proliferation and/or inducing tumor cell death, as opposed to simply inducing a normalization of blood vessels. 2. To what extent does it induce the survival and proliferation of the cancer stem-like cell leading to increased tumor invasiveness and the development of secondary tumor foci. 3. Alternatively, does it trigger a conversion of the tumor cell metabolism? Finally we address whether the phenotypic changes induced by anti-angiogenic treatment are sustained after re-implantation into the nude rat brain. Animals were treated with bevacizumab 10 mg/kg i.v. once weekly. Before sacrifice tumours were evaluated by T1 and T2 weighted MRI, and dynamic contrast enhancement MRI (DCE-MRI). Magnetic resonance spectroscopy (MRS) was applied to assess the metabolic tumor profile after treatment. Immunohistochemical analysis showed a normalization of vascular elements after treatment, these observations were correlated with the MRI observations and with the histological phenotype. In addition, tumor cell proliferation and apoptosis, and the behavior of the invasive, cancer stem-like cell population was quantified.

O132

RESPONSE ASSESSMENT IN NEURO-ONCOLOGY

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Accurate determination of response and progression is crucial in the evaluation of new treatments for brain tumor and in the care of individual patients. Macdonald,s criteria (Macdonald DR, et al. J Clin Oncol 1990; 8: 1277-80) are based on measurement of cross-sectional area of enhancing tumor, taking into account changes in steroid dose and neurological status. These criteria have been widely used in clinical trials of brain tumor therapy. Limitations include difficulty in measuring tumors with complex shapes and components (i.e., cystic or post-operative tumors) or indistinct borders, non-tumor factors that can produce imaging changes (i.e., enhancement due to post-operative inflammation, infarction, hemorrhage, infection, epilepsy), reaction to local therapies (convection-enhanced delivery, chemotherapy wafers, radiosurgery, etc.), post-treatment changes that may mimic tumor (i.e., pseudoprogression, radiation necrosis), and lack of applicability to non-enhancing tumors (i.e., low-grade gliomas). Anti-angiogenic therapies, which may reduce MRI enhancement by restoring the blood-brain barrier, while non-enhancing T2/FLAIR hyperintense tumor may continue to enlarge, highlight the difficulty of assessing response to novel treatments. The RECIST criteria (Therasse P, et al. J Natl Cancer Inst 2000; 92: 205-16) use uni-dimensional tumor measurements and have all the limitations of Macdonald,s criteria.

The Response Assessment in Neuro-Oncology (RANO) group is an ongoing unofficial international multi-disciplinary consensus-building effort to develop new response criteria to address these issues. Three working committees (high-grade glioma, low-grade glioma, surgical therapies) are outlining strengths and limitations of current response criteria, evaluating new imaging modalities, exploring clinical, neuro-cognitive and quality of life endpoints, and will propose standardized response guidelines. The input of clinicians, investigators, industry, regulatory agencies, and funding bodies will be obtained. Preliminary guidelines will be presented at ASCO 2009 in Orlando. This WFNO presentation will summarize the current status of the RANO effort.

O133

CREATION OF A CHEMOTHERAPY SIMULATOR TO PREDICT THE CONCENTRATIONS OF SYSTEMICALLY ADMINISTERED CHEMOTHERAPY WITHIN BRAIN TUMOR TISSUE

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Backgound: While neurosurgeons and radiation oncologists caring for patients with brain tumors carefully pre-plan interventions using image based guidance systems and simulators, oncologists choose systemic administration with no quantitative estimates of drug distribution. Clinicians generally assume that small lipid soluble agents penetrate an intact blood brain barrier and water soluble agents enter brain tumors in a manner similar to available radiographic contrast agents. Important drug related factors governing drug entry into brain and brain tumors include molecular weight, charge, protein binding, serum pharmacokinetics, and whether the agent is a substrate for efflux pumps. The integrity of the blood brain barrier varies in different regions of the tumor and may be influenced by external factors such as radiation, glucocorticoids, and anti-VEGF targeted therapies. Other tumor related variables may include necrosis, cyst, hemorrhage, and local edema, mass effect and pressure. **Results:** We have created (patent pending) a Drug Entry Simulator which displays a contrast enhanced MRI of the patient, a serum concentrations of drug over time, and an overlay of drug concentrations over time superimposed on the MRI. The area under the concentration time curve can be assessed for any region of interest. The accuracy of the model is continually refined using results from clinical trials employing microdialysis or surgical biopsies which measure concentrations of systemically administered agents within brain tumors. Examples will be presented to illustrate the information this simulator can provide using water soluble (methotrexate), more lipid soluble (temozolomide) chemotherapy, and targeted agents (imatinib). **Conclusions:** Pretreatment estimates of drug entry, maximal concentration, time over effective concentration, and area under the concentration time curve should result in more informed choices regarding the use of systemically administered agents in patients with primary and metastatic brain tumors and the best timing of radiation therapy.

O134

TARGETING OF THE TYROSINE KINASE RECEPTORS EGFRVIII AND C-MET IN GBM

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The most common mutation of the epidermal growth factor receptor (EGFR) in GBM is EGFRvIII. HGF/SF is the ligand for the receptor tyrosine kinase c-Met, both of which are often co-expressed in GBM. The presence of the HGF/c-Met axis or expression of EGFRvIII each independently enhances GBM growth and invasiveness. Using tyrosine kinase array technology, we show that expression of EGFRvIII in U87MG GBM cells (U87MG.de2-7 cells) leads to co-activation of several receptor tyrosine kinases, including PDGFRbeta; and c-Met. A fully human neutralizing antibody directed to HGF (AMG 102) did not inhibit this EGFRvIII mediated activation of c-Met, demonstrating that it is ligand independent. Treatment of parental U87MG xenografts with low doses of AMG 102 resulted in significant inhibition of tumor growth, while U87MG.de2-7 xenografts were profoundly resistant to AMG 102 treatment. Treatment with Panitumumab, a fully human antibody directed to the EGFR and EGFRvIII, was able to inhibit the EGFRvIII mediated activation of c-Met and significantly reversed the resistance to AMG 102. Indeed, combination therapy with Panitumumab and AMG 102 resulted in a marked increase in tumor cell apoptosis. An EGFRvIII molecule with an active kinase but incapable of autophosphorylation at the five major autophosphorylation sites involved in signal transduction was also able to mediate resistance to AMG 102, suggesting that the activation of c-Met by EGFRvIII occurs via a direct interaction rather than through these docking sites. Thus, expression of EGFRvIII leads to the promiscuous activation of several kinases, causing resistance to ligand inhibitory based strategies. Since Panitumumab can reverse this process, the combination of this antibody with AMG 102 may be an effective treatment for GBM patients.

O135

ASSESSMENT OF THE EFFICACY OF ETOPOSIDE AND TEMSIROLIMUS IN AN INTRACRANIAL RODENT MODEL WITH SPECIAL ATTENTION TO THE RELATIONSHIP OF TUMOR AND WHITE CELL P70S6 KINASE ACTIVITY DURING THERAPY

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PURPOSE: Clinical experience in malignant glomas suggests that agents targeting single molecular abnormalities do not control tumor growth . This investigation attempts to improve this situation by testing the efficacy the cytotoxic agent etoposide added to the mTOR inhibitor temsirolims in an intracranial tumor model. Additionally it evaluates peripheral blood monocyte (PBMC) p70S6 Kinase production and activity as a surrogate for brain tumor changes after mTOR inhibitor treatment. **METHODS AND RESULTS:** Nude mice with intracranial U87 tumors treated with etoposide or etoposide plus temisirolimus therapy survived significantly longer than a control vehicle group. The addition of temsirolimus to etoposide increased survival over etoposide alone. A substantial in vitro decline in U87 cell phosho-p70S6 Kinase protein expression occurred in a dose related manner to temsirolimus. U87 cell 70S6 Kinase activity as measured by a P32 based p70s6 Kinase assay declined to 22% of baseline after 30 minutes in 1 nM temsirolimus and 5% of baseline after 30 minutes in 10 nM temsirolimus. P70S6 Kinase activity in PBMCs, and concurrent intracranial and flank U87 tumors after temsirolimus (10 mg/kg) treatment was 29%, 39% and 34%, respectively, after 24 hours. These declined to 20%, 28% and 27%, respectively, after 72 hours. **CONCLUSIONS:** In the U87 model used, temsirolimus slightly improves the survival advantage provided by etopside. Temsirolimus decreases phospho-p70S6 Kinase protein and its activity in a dose dependent manner in vitro. P70S6 Kinase activity impairment in U87 intracranial and flank subcutaneous U87 tumors is paralleled by that in those same animals PBMCs. Thus this assay can be used to monitor biologic effect of this drug in this model. These results warrant (1) assessment of the combination of etoposide and temsirolimus clinically, and (2) correlative confirmation of the use of PBMCs as a surrogate monitor of the management of these patients.

O136

CDK4/6 SMALL MOLECULE INHIBITOR PD-0332991 DEMONSTRATES ANTI-TUMOR ACTIVITY AGAINST AN INTRACRANIAL GLIOBLASTOMA XENOGRAFT LACKING p16 FUCNTION

This Abstract nominated the Hoshino Award.
Please refer to P064 on page 152.

O137

RESULTS OF A PHASE IIB STUDY WITH TRABEDERSEN (AP 12009) IN RECURRENT OR REFRACTORY HIGH-GRADE GLIOMA PATIENTS

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Objective: High-grade gliomas (HGG) show an overexpression of TGF-beta 2. AP 12009 was developed for targeted suppression of TGF-beta 2. Aim of the Phase IIb study was to evaluate the efficacy and safety of AP 12009 compared to standard chemotherapy in recurrent or refractory HGG patients.

Methods: The Phase IIb study G004 was an international, open-label, randomized and active-controlled dose-finding study. Main objective was to compare 2 doses of AP 12009 (10 μM or 80 μM) and standard chemotherapy (TMZ or PCV) with regard to response rate, survival, and safety. Patients with recurrent or refractory HGG (AA WHO grade III and GBM WHO grade IV, N=145) were randomized into the 3 treatment groups. AP 12009 was administered intratumorally by convection-enhanced delivery with up to 11 treatment cycles (7-d-on, 7-d-off / cycle).

Results: In both AP 12009 treatment groups, long-lasting tumor responses were observed in AA as well as in GBM patients. For 10 μM AP 12009-treated AA patients, a significantly better overall response rate (CR+PR) at 14 months compared to control was observed (p=0.034). Also the tumor control rate (CR+PR+SD) differed significantly compared to control (p=0.003). The observed tumor responses correlate with superior overall survival. A median overall survival benefit of 17.4 months was noted for AP 12009-treated AA patients compared to the chemotherapy control. In the GBM subgroup, AP 12009 was as efficacious as standard chemotherapy regarding short-term survival and superior in terms of long-term survival.

Conclusion: The Phase IIb study revealed the superior long-term survival of recurrent or refractory HGG patients treated with AP 12009. A pivotal Phase III study in AA patients has started and a pivotal study in GBM patients is being planned. Clinical studies with AP 12009 are also ongoing in pancreatic and colorectal carcinoma and malignant melanoma.

O138

PHASE I STUDY OF TERAMEPROCOL IN PATIENTS WITH RECURRENT HIGH GRADE GLIOMAS: A NABTT CNS CONSORTIUM TRIAL

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Background: Terameprocol is a global transcription inhibitor that affects cell division (Cdc2, G2/M), apoptosis (survivin pathway), drug resistance (MDR1 and PGp), radiation resistance in hypoxia (HIF-1a), and hypoxia responsive genes (VEGF). **Methods:** A dose escalation study was conducted in heavily pretreated, recurrent, measurable, high grade gliomas. Terameprocol was administered intravenously for 5 consecutive days each month and discontinued for toxicity or progression. Patients taking and not taking enzyme inducing anticonvulsants (EIAC) were escalated independently. **Results:** 35 patients were accrued with a median age of 46 (29-71), KPS of 80 (60-100), number of prior chemotherapy regimens of 2 (1-6). 43% had glioblastoma and 57% had anaplastic gliomas. Pharmacology revealed no differences with coadministration of EIAC. Treatment related grade 3 or 4 toxicities began at 1700mg with one low phosphorus. In 8 patients treated at 2200mg, there was one metabolic acidosis (secondary to PEG formulation) and one ileus. The drug was reformulated to avoid acidosis and was well tolerated at 1700mg, but hypoxia and interstitial nephritis were seen at 2200mg. Thus, the recommended daily dose for future studies is 1700mg. No radiologic responses were seen but stable disease was noted in 9 of 32 (28%) evaluable pts. 5 patients (13%) remained on treatment for more than 6 months (6+, 8, 10, 10, and 21+ months). The median survival of this patient population was 5.9 months. **Conclusion:** This study established the toxicity profile of terameprocol, determined that EIASD do not affect its pharmacology, and identified the dose to be used in future studies. Encouraging long term stability was noted in this Phase I trial of heavily pretreated recurrent high grade gliomas. The toxicity profile and observed disease stability suggest that terameprocol could be safely combined with temozolomide and radiation and has the potential to impact survival in newly diagnosed glioblastoma.

O139

THE COMBINATION OF TEMOZOLOMIDE AND RTA203, A SELECTIVE H^+-ATPASE INHIBITOR, AUGMENT THE CYTOTOXIC EFFECT ON MALIGNANT GLIOMAS THROUGH BLOCKADE OF AUTOPHAGY

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Autophagy is organelles and proteins degradation system through the lysosomal machinery in eukaryotic cells. Accumulating evidences show that irradiation or some chemotherapy-agents cause autophagy in various types of tumor cells. However the autophagy may have opposite effects, that is, cell-kiling or cell-protective effect on tumor depending on type of tumor, of anticancer therapies. Therefore appropriate modification of autophagy, i.e., inhibition of cell-protective autophagy or promotion of cell-kiling autophagy could augment cytotoxicity caused by anticancer therapy in tumor cells. We previously reported Temozolomide (TMZ) caused not apoptosis but autophagy in malignant glioma cells. The purpose of this study is to clarify which type of autophagy is induced by TMZ in glioma cells and to determine the appropriate modification of TMZ-induced autophagy. Autophagy assays showed that TMZ induced cell-protective autophagy in glioma cells. When glioma cells were treated with combination of TMZ and RTA203 (RTA), a specific inhibitor of vacuolar type H^+-ATPase, TMZ-induced autophagy was blocked at the late stage by abrogating function of lysosome, resulting in augmentation of cytotoxicity. The combination of TMZ and RTA represented more than additive effect in cell viability assay. Apoptosis assays showed RTA alone induces a few apoptosis, while combination of TMZ and RTA significantly increased apoptosis. In animal study, the combination treatment shows significantly longer survival time compared with each single treatment. In conclusion, TMZ induce cell-protective autophagy in malignant glioma cells and inhibition of TMZ-induced autophagy by RTA enhanced cytotoxicity through increase of apoptosis.

O140

INTERIM ANALYSIS OF COMBINATION CHEMORADIOTHERAPY BY MEANS OF TEMOZOLOMIDE (TMZ) AND INTERFERON (IFN) BETA FOR MALIGNANT GLIOMA: MULTICENTER CLINICAL TRIAL (INTEGRA STUDY)

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Backgound: Previously, we demonstrated that Interferon (IFN) beta markedly enhanced chemosensitivity to Temozolomide (TMZ) in an in vitro study (Natsume, Cancer Res, 2005); this suggested that one of its major mechanisms is the downregulation of MGMT transcription via p53 induction. This effect was also observed in an experimental animal model (Natsume, Cancer Chemother Pharmacol, 2007). Following those preclinical studies, we conducted the Integrated Japanese Multicenter Clinical Trial of IFN beta and TMZ for Glioma in Combination with Radiotherapy (INTEGRA Study). Study design and **Results:** Twenty two patients with malignant gliomas (newly diagnosed and recurrent) were enrolled in this study. All the newly diagnosed patients received radiotherapy (fractionated focal irradiation in daily fractions of 2 Gy administered 5 days per week for 6 weeks, i.e., a total of 60 Gy) plus TMZ (75 mg/m^2/day, daily from the first to the last day of radiotherapy); and IFN beta (3 MIU/body, administered IV on alternate days during radiotherapy), followed by 6 cycles of adjuvant TMZ (200 mg/m^2/day, on days 1 to 5, administered every 28 days) and IFN beta (3 MIU/body, administered once every 28 days). Recurrent tumors were treated with TMZ (200 mg/m^2/day, on days 1 to 5, administered every 28 days) and IFN beta (3 MIU/body, administered once every 28 days). The preliminary results reveal that this combination therapy causes minimal toxicity. The most frequent toxic effect is the inhibition of hematopoiesis (leukopenia and neutropenia) that, in most cases, recovered within a month after the cessation of drug administration. In 15 patients with measurable malignant tumors, 3 had complete response, 3 had partial response, 5 had stable disease, and 4 had progressive disease. **Conclusions:** A combination of IFN beta and TMZ tolerated well, and has great potential of enhancing the efficacy of TMZ. Further clinical trials will be warranted.

O141

THE KOREAN EXPERIENCES OF CONCURRENT CHEMORADIOTHERAPY WITH TEMOZOLOMIDE FOR THE NEWLY DIAGNOSED GLIOBLASTOMA: METAANALYSIS OF PROSPECTIVE STUDY OF FOUR CENTERS

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Concurrent chemoradiotherapy using temozolomide (TMZ)demonstrated a clinical efficacy as primary therapy for the patients with newly diagnosed glioblastoma (GBL), and its effect was influenced by the molecular genetic factors such as MGMT status. The authors postulated a possible racial difference in the response to this therapy and performed a meta-analysis of 103 Korean patients with primary GBL treated from June 2004 to November 2007 in four major institutions (Yonsei University, Ulsan University, Seoul National University, The Catholic University of Korea). The patients received radiotherapy for a total 60 Gy plus continuous daily TMZ (75 mg/m2/day), followed by six cycles of adjuvant TMZ (150 to 200 mg/m2/day). The median age was 57 yr, and 77% of the patients underwent debulking surgery (more than subtotal resection). At a median follow up of 12 months, the progression free survival (PFS) was 9 months and 6-month PFS rate was 66.3%. The median OS was 19 months. The 12-month OS rate was 78.7% and 24-month OS rate was 48.9%. The response rate was 73% (21 CR and 32 PR of 73 evaluable patients). Median OS (19 months vs 14 months) as well as 6-month PFS (71% vs 47%) was significantly improved in the debulking surgery group. No grade 3 or 4 hematologic toxicity was noticed and grade I or 2 toxicity developed in only 8.3% of the patients. Concomitant chemoradiotherapy with TMZ appeared to prolong the survival of Korean patients with newly diagnosed GBL and was safe in terms of toxicities and tolerability. However, further investigations are mandatory in larger number of patients, longer term follow-up, and molecular genetic analysis, which have already been undertaken.

O142

COMPARISON OF BOLUS AND METRONOMIC CHEMOTHERAPY ON TUMOR CHARACTERISTICS

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Purpose: Few studies have compared the effects of low dose frequent administration of chemotherapeutic agents, metronomic therapy, versus bolus chemotherapies on tumor bed vasculature and other tumor characteristics. In this study, we evaluated tumor control, anti-angiogenic effects, and tumor cell characteristics in a rodent glioma model in response to these two methods of drug administration.

Methods: Rat 9L gliosarcoma cells were stably transduced with the luciferase gene, allowing cells to be monitored using Bioluminescent Imaging (BLI). BLI admits tumor response to treatment to be observed in animals both immediately and repeatedly imaged over time. Fifty thousands tumor cells were inoculated into the right striatums of 15 Fisher 344 rats. 10 days after inoculation, animals were randomly divided into 3 groups. Group 1 received no treatment. Group 2 received 10 mg of carboplatin via bolus intraperitoneal (i.p.) administration every 2 weeks. Group 3 received 2mg of carboplatin i.p 5 times per week. Tumor growth was measured every 2 to 3 days. To observe tumor vascularity and reaction to hypoxia, immunohistochemical assessments were carried out on brain specimens excised from rats 4 weeks after initial chemotherapeutic treatment.

Results: Metronomic and bolus administration of carboplatin both controlled tumor growth and vascularity. However, levels of hypoxic reaction and malignant mutation were significantly lower in tumors treated with metronomic therapy; represented by hypoxia inducible factor-1 alpha (HIF-1α) and cMet protein, respectively.

Conclusion: Recent evidence has shown that tumor cells in a hypoxic condition may survive with malignant changes which appear to be triggered by cMet protein activation. Our results suggest bolus administration of drug is more likely to result in cells becoming hypoxic, and acquiring malignant mutations. Metronomic therapy may provide a better alternative for treatment, as it appears to produce lower levels of hypoxia and malignant mutations.

O143

PHASE II TRIAL OF LOW-DOSE CONTINUOUS (METRONOMIC) TREATMENT OF TEMOZOLOMIDE FOR RECURRENT GLIOBLASTOMA

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PURPOSE: We performed a phase II trial of low-dose continuous (metronomic) treatment using temozolomide for recurrent GBMs. **PATIENTS AND METHODS:** Temozolomide-refractory patients with GBM who experienced disease recurrence or progression during or after cyclic treatment schedule of temozolomide after surgery and standard radiotherapy were eligible. This phase II trial included two cohorts of patients. The initial cohort, comprising 10 patients, received temozolomide at 40 mg/m^2 everyday. After this regimen seemed safe and effective, the metronomic schedule was changed to 50 mg/m^2 everyday. The second cohort, comprising 28 patients, received temozolomide at 50 mg/m^2 everyday. **RESULTS:** The 6-month progression-free survival in all 38 patients was 32.5% (95% CI: 29.3-35.8%) and the 6-month overall survival was 56.0% (95% CI: 36.2-75.8%). One patient developed a grade 3 neutropenia, grade 2 thrombocytopenia in three patients, and grade 2 increase of liver enzyme (GOT/ GPT) in three patients. Of all patients included in this study, four patients were withdrawn from this study because of side effects including sustained hematological disorders, cryptococcal infection, and cellulitis. In a response group, quality of life measured with SF-36 was well preserved, as compared with the pre-treatment status. **CONCLUSION:** Metronomic treatment of temozolomide is an effective treatment for recurrent GBM which is even refractory to conventional treatment of temozolomide and has acceptable toxicity.

O144

A CONTINUOUS SCHEDULE OF TMZ FOR GBM AT FIRST RELAPSE MAY OVERCOME THE THERAPEUTIC DISADVANTAGE OF NON-METHYLATED MGMT: RESULTS FROM THE PHASE II RESCUE TRIAL

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Background: Most pts with newly diagnosed GBM progress after standard RT with concomitant and adjuvant temozolomide (TMZ) and there is no standard second-line regimen. Continuous dosing and dose intensification of TMZ are thought to reduce levels of O-6-methylguanine-DNA methyltransferase (MGMT), which has been associated with TMZ resistance. Altering the schedule of TMZ may be one strategy to re-induce response. A prospective phase II study was conducted to investigate this approach; we report the survival analysis and MGMT data.

Methods: Patients with GBM (n=90) and anaplastic glioma (n=30) who failed standard TMZ (5/28d adjuvant regimen) received continuous dose-intense TMZ 50 mg/m^2 for 28/28 days for up to one year. The primary endpoint was 6-mo progression-free survival (PFS).

Results: 120 pts were enrolled at 11 centres. Pts were divided into four cohorts: recurrence during the first 6 mo of adjuvant therapy (early group); after 6 mos of therapy (extended group); after stopping adjuvant TMZ (completed group); and anaplastic glioma. No enrollment was permitted in the first 3 mos after RT in order to minimize the influence of pseudoprogression. Six-mo PFS was 28% (early), 13% (extended), 29% (completed), and 33% (anaplastic). Non hematological toxicity was mild and easily managed. Progressive lymphopenia was seen in 40% but no serious opportunistic infection was observed.

MGMT promoter methylation was tested in 71/120 pts of which 51 could be evaluated. 22 (43%) and 29 (57%) had a methylated and unmethylated promoter, respectively. These 2 groups were similar in age and surgical resection but more non-methylated patients were ECOG 0 (45% vs 32%). They received a median of 6 cycles of continuous TMZ with a median TTP of 4.6 (meth) vs 5.4 (non-meth) months and median time to death on study of 8.8 (meth) vs 9.4 (non-meth) months. 5/17 (29%) MGMT promoter methylated GBM pts reached >6mo PFS compared to 7/22 (32%) non-methylated.

Conclusions: Continuous dose-intense TMZ 50 mg/m^2 administered on a 28/28 day schedule is active and well tolerated after failure of the conventional 5/28 day regimen. Efficacy compares favorably to other commonly used second-line agents. This analysis revealed similar on-treatment time, 6 mo PFS, TTP, and survival for GBM patients regardless of MGMT promoter methylation. Continuous TMZ given at first recurrence of GBM may overcome the disadvantage of high MGMT expression. Further studies of this extended TMZ schedule are warranted.

O145

THE MIR-17/92 POLYCISTRON IS AMPLIFIED AND UP-REGULATED IN SONIC HEDGEHOG-DRIVEN MEDULLOBLASTOMAS AND INDUCED BY N-MYC IN SONIC HEDGEHOG-TREATED CEREBELLAR NEURAL PRECURSORS

This Abstract nominated the Hoshino Award.

Please refer to P043 on page 143.

O146

EPIGENETIC SILENCING OF KRUPPEL-LIKE FACTOR 4 (KLF4) IN MEDULLOBLASTOMA

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The genetic and epigenetic events involved in the initiation and progression ofmedulloblastoma (MB) are poorly described. To gain a better understanding ofMB pathogenesis, we have analyzed 201 primary MBs and 11 MB cell lines using a high-density single nucleotide polymorphism (SNP) array platform. We identified a discrete area of homozygous deletion on 9q31 in a primary MB sample containing 9 known genes. As loss of heterozygosity (LOH) at this locus is relatively frequent in MBs, we focused on one of the genes, Kruppel-like factor 4 (KLF4) as a possible tumour suppressor gene in MB. KLF4 is a zinc-finger transcription factor that regulates the expression of genes involved in differentiation and cell-cycle arrest and has recently been shown to undergo promoter methylation and LOH in gastrointestinal cancer. To determine if KLF4 is similarly inactivated by epigenetic mechanisms in MB, we profiled its epigenetic status using multiple complementary techniques. KLF4 expression was restored in MB cell lines after treatment with 5-aza-deoxycytididine, as shown by semi-quantitative RT-PCR. Bisulfite sequencing analysis showed methylation of the promoter region of KLF4 in MB cell lines and not in normal cerebellum. We examined the DNA methylation status of KLF4 in 45 primary MBs by methylation-specific PCR and determined that 18% of the tumours showed methylation of the KLF4 promoter region. We re-expressed KLF4 in KLF4-negative human MB cell lines and observed a decreased growth rate of MB cells in vitro, and extended survival of mice harboring intracranial xenotransplanted human MB cells in vivo. Our results suggest that KLF4, which appears to be inactivated frequently in MBs through genetic and/or epigenetic mechanisms, may be a novel factor in MB carcinogenesis.

O147

"EPENDYMOMA TREATMENT: AN UPDATE"

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PURPOSE: To review state of art and relevant advances in the management of ependymomas. **RECENT FINDINGS:** Ependymomas are uncommon neoplasms of the central nervous system, and may occur either in the brain or the spinal cord. Compared with intracranial ependymomas, spinal ependymomas are less common. Studies performed on genetic changes in ependymoma provide some insight into the pathogenesis and prognostic markers and yield new therapeutic targets. Almost all clinical studies have shown a major impact of extent of resection; thus, a complete resection must be attempted, whenever possible, at first surgery or at reoperation. Involved field radiotherapy is the recommended adjuvant therapy. The lowest limit age than radiotherapy can be used safely, and, the need for radiotherapy for completely resected supratentorial non-anaplastic ependymomas are controversial issues that are examined in clinical trials. Craniospinal irradiation is used occasionally for metastatic disease. Chemotherapy is reserved for young infants in order to avoid or delay the use of radiotherapy or in an attempt to facilitate second look surgery. Data from relevant studies will be presented. Patients with relapsed disease have very prognosis with variable occasionally chronic course with multiple relapses. Re-operation can provide improvement in progression free survival. Small series without long term toxicity data have shown that re-irradiation following relapse can occasionally result in long term survival in relapsed patients. Chemotherapy regimens have been used in relapsed ependymoma with only occasional responses without providing benefit in overall survival. New agents are desperately needed for this rare disease. Molecular biology studies suggest some potential new therapeutic targets.

O148

MEDULLOBLASTOMA IN INFANTS

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Infants with malignant embryonal brain tumors have poorer survival compared with older children. Surveillance, Epidemiology and End Results (SEER) Program data on the survival of all children with medulloblastoma/ primitive neuroectodermal tumor (PNET) confirms this pattern of poor survival rates in the youngest group of patients, less than three years of age, with an overall survival rate of 39% at five years and median survival of 1.8 years. Previous studies in large cooperative study groups consistently report this same trend, with collective survival rates ranging from 22% to 47%.

There have been divergent strategies across international cooperative groups, strategies such as prolonged post-operative chemotherapy; intraventricular administration of chemotherapy; intensive frontline chemotherapy with further intensification with stem cell rescue; and adjuvant focal conformal radiation. Metastatic disease at diagnosis remains problematic but there is evidence to suggest that intensive therapy with stem cell rescue is encouraging. The incidence of the histologic subtype, desmoplastic medulloblastoma, among the infant population is notable and their potential improved outcome as compared to the classic variety is of great interest. The ability to identify infants with medulloblastoma from those with atypical teratoid rhabdoid tumor must be considered a confounding factor. Recent molecular profiling data on medulloblastoma will guide therapy in the future, thus impacting the approach to treating young infants.

The predominant issue that surrounds therapy for infants is the well established risks of radiation therapy in terms of neurocognitive development, far outweighing the benefits of therapeutic response. Focused developmental assessments should be a required aspect in determining the "success" of any future therapy.

An international collaborative effort investigating the varying treatment strategies in concert with measuring long-term neurocognitive outcomes is currently underway.

O149

RECURRENT MEDULLOBLASTOMA- RECENT ADVANCES IN TREATMENT

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Medulloblastoma is the commonest malignant brain tumor in childhood with an annual incidence of around 400 cases per year in the United States. In recent years significant strides have been made in the treatment of this tumor with long-term survival of 85% in children with average risk disease and up to 70% in those with disseminated tumor. However, the outcomes in infants and those with recurrent disease remains poor. While high-dose chemotherapy with stem cell rescue has been the main stay of salvage therapy at recurrence with or without radiation therapy, it is far from clear if this approach can be successfully employed in all children at recurrence. A profusion of studies have focused on the pathology and molecular characteristics of this tumor. Identification of histologic subtypes, alterations in the Sonic Hedgehog (SHH) and WNT signaling pathways, over-expression of ERBB2, OTX-2 gene amplification, and the recent efforts to identify the putative tumor initiating cell in medulloblastoma have helped in the understanding the biologic basis of this disease, assessing prognosis, and to begin devising a more biology-based therapy of children with this tumor. A significant proportion of children suffer from leptomeningeal disease (LMD) at relapse. Therapeutic options are currently limited for this group and more effective strategies are needed. This discussion will focus on the recent advances in the treatment of children with recurrent medulloblastoma with specific emphasis on HDC with stem cell rescue, treatment of LMD, molecular targeted therapies, immunotherapy, and other innovative strategies that are already being tested in the clinic or actively being explored in the laboratory.

O150

MEASURING PERFORMANCE STATUS IN PEDIATRIC BRAIN TUMOR STUDIES - EXPERIENCE OF THE HIT-GBM-C PROTOCOL

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Introduction: Measuring children has proven to be a challenge. We have previously described the FMH, a questionnaire for pediatric brain tumor protocols. Here we report the experience in the treatment protocol HIT-GBM-C which included intensive simultaneous radiochemotherapy followed by further chemotherapy for high grade glioma and diffuse intrinsic pontine glioma **Method:** The FMH ("Fertigkeitenskala Muenster/Heidelberg (FMH)") is a quantitative measure of health status, which includes 56 simple questions between 2 and 15 words to be answered with yes or no. The number of positive answers is translated in age dependent percentiles (FMH%). Physicians were also asked to rank the handicap in a 5 point scale: normal, mild handicap, age normal activity severely reduced but not in bed, in bed but not in ICU, and ICU. No financial compensation was offered. **RESULTS:** Questionnaires were available from 50 of 97 eligible patients. 5 Patients scored initially over 40 on FMH%, four of which survived. 16 Patients scored less than initially 39%, and 15 of them died. The physicians judged the patients as normal in 20.4% initially, which decreases to 16.3% in the middle of the protocol. In reverse only 16.3% had activity severely reduced, which increased to 30.6% after receiving the half of the treatment. The FMH% correlated well with the physicians judgments (p less than 0.005). **Conclusion:** The FMH scale is valid as it correlated with the physician assessment. Patients with poor performance at treatment start had a poor prognosis. The number of questionnaires sent back was insufficient for answering a treatment related question. If a treatment comparison is to be answered, an incentive needs to be provided.

O151

DEVELOPMENT OF THE JAPANESE VERSION OF PEDIATRIC QUALITY OF LIFE INVENTORY™ BRAIN TUMOR MODULE

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The Pediatric Quality of Life Inventory™, PedsQL™, is a widely-used modular instrument for measuring health-related quality of life in children aged 2 to 18. The PedsQL™ Brain Tumor Module is comprised of six subscales: cognitive problems, CP, pain and hurt, PH, movement and balance, MB, procedural anxiety, PA, nausea, NA, and worry, WO. Here, we developed the Japanese version of the PedsQL™ Brain Tumor Module. **[METHODS]** Translation equivalence was confirmed using the standard back-translation method, and content validity was assured by cognitive debriefing tests of both children and parents separately. Self-reported or interviewer-administered questionnaires including both the PedsQL™ Generic Core Scales and the State-Trait Anxiety Inventory for Children, STAIC, were completed by 137 children with brain tumors, 90 self-reported, 47 interviewer-administered, and 166 parents (all self reported). Average child age was 9.8 years. Forty-seven children had embryonal tumors (29.0%), 36 had germ cell tumors (22.2%), 25 had high-grade glioma (15.4%) and 39 had low-grade glioma (24.1%). Sixty-five children (39.2%) were under treatment. **[RESULTS]** High internal consistency (Cronbach's α= 0.75-0.84 for children, 0.80-0.96 for parents) and sufficient test-retest reliability (Intraclass Correlation Coefficient [ICC] = 0.67-0.77 for children, 0.74-0.95 for parents) were demonstrated for all subscales except child-reported PH (α=0.50, ICC=0.45). Factorial validity was supported through exploratory factor analysis (factor-item correlation = 0.33-0.96 for children, 0.55-1.00 for parents). Known-groups validity confirmed that CP was sensitive for developmental disorders, MB for paresis, and NA for currently undergoing chemotherapy. Convergent and discriminant validity with the PedsQL™ Generic Core Scales and STAIC were acceptable. Child-parent correlation was 0.41-0.65 for five of the six subscales and 0.18 for WO. **[CONCLUSION]** The Japanese version of the PedsQL™ Brain Tumor Module is suitable for the measurement of health-related quality of life of children with brain tumors in clinical trials and research.

O152

A NEW ASSESSMENT TOOL FOR MEASURING NEUROLOGICAL AND BEHAVIORAL FUNCTION ACROSS LIFESPAN: THE NIH TOOLBOX

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AIMS: The NIH Toolbox, part of the NIH Blueprint for Neuroscience Research, seeks to develop brief, comprehensive assessment tools measuring motor, cognitive, sensory and emotional health. The ultimate goals of the Toolbox include the provision of: 1) uniformity among measures across disease groups, and 2) measures that demonstrate stable psychometric properties across the lifespan. Upon completion, the Toolbox will be available for use in longitudinal epidemiologic studies and clinical trials for people ages 3-85. In this paper, we describe the Toolbox developing process using motor and cognitive function as examples. **METHODS:** We first conducted both on-line surveys and in-depth interviews with clinical research experts in each domain. Specifically, experts were asked to nominate essential constructs to be assessed for motor and cognitive function and rank them based on conceptual and clinical relevance. A follow-up consensus meeting was held to finalize the constructs to be included in the Toolbox. **RESULTS:** 147 responded to an on-line survey; additional 12 and 8, respectively, completed in-depth interviews for motor and cognition. The results were reviewed by the NIH project team and external advisory group. The follow-up consensus meeting led to the inclusion of locomotion, balance, dexterity, strength, and endurance for the motor domain, and attention, episodic memory, executive function, language, processing speed and working memory for the cognition domain. **CONCLUSIONS:** We are currently revising existing instruments and developing additional instruments to assess the selected constructs, which will be completed by spring 2009. Normative data from the US general population will be completed in 2010, and with the final Toolbox to be available in 2011. The final Toolbox will be a valuable research resource, improving the ability to compares results from different studies. Use of Toolbox assessments holds the promise of improving our understanding of the overall health of neuro-oncology patients.

O153

DETAILED PROSPECTIVE NEUROPSYCHOLOGICAL, ACTIVITY OF DAILY LIVING AND ENDOCRINE FUNCTION ASSESSMENT IN CHILDREN WITH CRANIOPHARYNGIOMA TREATED WITH STEREOTACTIC CONFORMAL RADIOTHERAPY

This Abstract nominated the Hoshino Award.

Please refer to P054 on page 148.

O154

MANGING CHALLENGING BEHAVIOURS AFTER BRAIN CANCER: A RESOURCE FOR PATIENTS, CARERS AND HEALTH PROVIDERS

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Purpose: Between 17-50% of people with brain tumour (BT) display some degree of challenging behaviour (CB) including executive impairment, behavioural disturbance, or social/emotional dysfunction. However, little information on management strategies is available for patients and/or their carers to assist in coping with these problems. A web-based review identified 17 key English language information resources (web-based or hard copy) on BT from across Australasia, North America, and the United Kingdom. Although 47% contained lists of cognitive/behavioural impairments, 71% provided either no or minimal information (1-2 sentences) on management strategies. This project aimed to develop patient/carer oriented Fact sheets to address this information gap. **Methods:** A literature review regarding behavioural / cognitive impairments after BT was conducted. Resources developed for other neurologic groups were reviewed. Combining data from these sources with the clinical experience of the project team, draft fact sheets were devised and presented to a focus group of patients and carers for feedback. Drafts were also circulated to a state multidisciplinary health provider BT network convened by the New South Wales Cancer Institute. Readability statistics were also calculated to ensure that the resources met public health standards for comprehension. **Results:** Sixteen Fact sheets (web-based and hard copy) were developed addressing apathy, anger, stress/anxiety, fatigue, high level thinking problems (executive impairment), inappropriate social/sexual behaviour, lability, perseveration, disorientation/confusion, egocentricity, impulsiveness, depression, neglecting personal care, memory difficulties, attention/concentration and communication. Each sheet contains a definition of the problem, prevalence statistics, symptom list, de-identified case-study, management strategies for the person with BT and carer(s), key questions to ask health professionals and web-links to further information. After only three months of release, substantive requests for these resources from across Australia have been received. **Conclusion:** Results suggest that these Fact sheets fill an important information gap, providing an important resource for patients, carers and health providers.

O155

PATIENT FUNCTIONAL STATUS IS STRONGEST CORRELATE OF CHALLENGING BEHAVIOUR AFTER BRAIN TUMOUR

This Abstract nominated the Hoshino Award.

Please refer to P053 on page 148.

O156

MOUSE MODELS OF CNS EMBRYONAL TUMORS

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Background: CNS embryonal tumors are devastating cancers in children, consisting of medulloblastomas, supratentorial primitive neuroectodermal tumors (sPNETs), and atypical teratoid/rhabdoid tumors. Based on the genetic alterations found in human, multiple models of human CNS embryonal tumors have been developed in genetically engineered mice. The objectives of this study were to create novel mouse models for discovering efficient therapy and to understand brain tumor biology. **Methods:** The RCAS/tv-a system was used for modeling brain tumors in p53 null mice. The experimental retrovirus RCAS that has an inserted oncogene c-Myc or β-catenin was injected into neonatal mouse brains. Transgenic mice expressing RCAS receptor tv-a under the control of the GFAP promoter (Gtv-a mice) allow cell type-specific RCAS infection and gene transfer *in vivo*. Symptomatic mice were sacrificed and examined histologically. We also reviewed published reports of the molecular and cytogenetic abnormalities in human CNS embryonal tumors. **Results:** sPNETs were generated in Gtv-a $p53^{-/-}$ mice by forced c-Myc expression with 34% incidence. Combined β-catenin activation with c-Myc promoted tumor progression and induced divergent differentiation. This sPNET was histologically similar to human large cell/anaplastic variant of medulloblastomas and when injected into cerebellum, large cell/anaplastic medulloblastomas formed at higher incidence. By reviewing published reports, we found that human sPNETs seemed to share the several genetic abnormalities with other CNS embryonal tumors. The strongest similarity of the molecular/cytogenetic profile was observed between sPNETs and large cell/anaplastic medulloblastomas in terms of chromosome 1q gain, c-Myc and N-Myc amplification, β-catenin stabilization, and p53 inactivation.Conclusions: These findings indicate that common genetic abnormalities are seen in variants of human CNS embryonal tumors, and multiple histologic variants of these tumors can be generated from a single set of genetic abnormalities in mice. These data provide insight into the biology and classification of CNS embryonal tumors.

O157

MULTIPLE RECURRENT GENETIC EVENTS CONVERGE ON CONTROL OF HISTONE LYSINE METHYLATION IN MEDULLOBLASTOMA

This Abstract nominated the Hoshino Award.

Please refer to P044 on page 144.

O158

CLONAL SELECTION AND PARALLEL EVOLUTION DEFINE THE HIERARCHICAL STRUCTURE OF METASTATIC MEDULLOBLASTOMA

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We modified the Sleeping Beauty (SB) system so that SB is mobilized in the EGL under the influence of the Math1 enhancer. Nearly 100% of Math1-SB11, SB transposon donor, ptch +/- mice develop medulloblastoma with leptomeningeal metastases by 3 months, where-as 10-20% of Ptch +/- mice develop localized medulloblastoma by 8 months. Mice carring Math1-SB11, or transposon donor, or both do not develop cancer. We sequenced over one million insertions mapping to over 158,000 sites from over 140 SB-induced primary medulloblastomas and leptomeningeal mets using 454 technology. Statistical analysis identified 29 common insertion sites (CISs) in primaries and 44 CISs in metastases including well-known medulloblastoma genes such as CREBBP, and PTEN, as well as other cancer genes such as FHIT, CACNA2D1, and ERAS. CISs also frequently targeted genes involved in neural stem cell biology such as NFIA and NFIB.All primary/met pairs arose from a common ancestor as they share common clonal insertions. Some clonal events present in the mets are present in only a small subclone of the primary, supporting the clonal selection theory of metastasis and suggesting that some of these events affect metastasis virulence genes. Similarly, some highly clonal events seen in the primary tumor are not seen in the metastases. These likely represent events that have arisen post-metastatic dispersion, demonstrating that paralell evolution also occurs. Most importantly, comparison of insertions from primary tumors, and matched spinal and frontal lobe leptomeningeal metastases reveals that in some cases the mets share a clonal insertion that is only present in a very minor subclone of the primary tumor. This highly suggests that leptomeningeal dissemination only occurs once. Our results suggest reasons for the failure of medulloblastoma therapies developed against targets discovered in the primary tumor. Further understanding of the clonal hierarchy of medulloblastoma metastases will help design future effective therapies.

O159

INTEGRATED GENE EXPRESSION AND COPY-NUMBER ANALYSES IDENTIFY AGGRESSIVE PEDIATRIC CENTRAL NERVOUS SYSTEM PRIMITIVE NEURO-ECTODERMAL TUMORS

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Primitive neuroectodermal tumors (PNET) represent one of the largest groups of malignant pediatric brain tumors and include medulloblastoma as well as CNS-PNET arising in the cerebral hemispheres. Despite histologic similarities, CNS-PNET exhibit worse outcomes than medulloblastoma. In contrasts to medulloblastoma, CNS-PNET remain poorly characterized with limited studies of less than 10 cases reported to date. Prior studies indicate CNS-PNET differ genetically from medulloblastoma, however, specific biological features of CNS-PNET remain to be defined. In this study, we performed high resolution gene expression and copy number analyses on 40 primary CNS-PNET using the Illumina Human-6 V2 BeadChip expression and the Affymetrix 500K GeneChip Mapping arrays. Data analysis revealed absence of isochromosome 17q and MYCC amplification, and only 1 case of MYCN amplification in 40 CNS-PNET. 1/5 of CNS-PNET had chr22q loss that did not involve the rhabdoid tumor IN1/SMARCB1 locus. These data support the distinct identity of CNS-PNET. Copy number analysis respectively identified 28 and 13 regions of focal deletions and amplification in CNS-PNET involving known and novel tumor suppressor and oncogenic loci. Remarkably although CNS-PNET exhibited heterogeneity in copy number profiles, integrated gene expression and DNA copy number analysis demonstrated segregation of CNS-PNET into 2 major subgroups with specific molecular and clinicopathologic characteristics. Notably, a distinct subgroup, characterized by recurrent copy number alterations of chr 2, 3 and 19 was associated with younger age at diagnosis (less than 4yrs, p=0.026) and significantly poorer survival (mean of 4+/-1.3 vs 44+/-12.8 months; p=0.0001). Interestingly, gene expression profiles of these aggressive CNS-PNET indicated significant enrichment (p=0.00001) of genes associated with pluripotentcy and downregulation of pro-apoptotic loci. In summary, through integrated genomic analysis we have identified genetic features that define sub-groups of CNS-PNET. Our data leads us to propose that aggressive CNS-PNET are characterized by early developmental lineages and pro-survival phenotypes.

O160

GENOMIC PROFILING OF INTRACRANIAL GERM CELL TUMORS WITH SINGLE NUCLEOTIDE POLYMORPHISM ARRAY

This Abstract nominated the Hoshino Award.

Please refer to P058 on page 150.

O161

PATHWAYS AND GENES ASSOCIATED WITH EPENDYMOMA LOCATION, GRADE AND SUBGROUPS.

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Purpose of the study: Unravelling specific signatures of ependymoma sub-groups and understanding molecular alterations involved in their tumorigenesis. **Materials and Methods:** A series of 34 frozen ependymomas were analysed using Affymetrix HG-U133 Plus 2.0 arrays. Obtained data were compared with histological features. Results: Spinal cord ependymomas demonstrated a HOX gene signature, whereas intra-cranial tumors had Notch, Hedghog and BMP pathways upregulation. High expression levels of the oncogenes SOX2, HDAC1, NPM1, YAP1 and JAG1 were observed in PF ependymomas when compared to normal brain. Grade II ependymomas were associated with up-regulation of dyneins, and grade III with activation of the Wnt and E2F1 pathway, cell cycle, adherens junction dysfunction, apoptosis and angiogenesis. Hierarchical clustering and Correspondance analysis established the presence of three molecular sub-groups of posterior fossa (PF) ependymomas, while WHO only recognizes two. One of them regrouped tumors with specific histology characterized by biphasic and cerebriform appearance, which is either WHO grade II or III. They also demonstrated activation of genes implicated in glycogene metabolism and central nervous system development. We suggest to name this newly identified sub-group of children PF ependymomas: biphasic-cerebriform ependymoma. **Conclusion:** These data illustrated heterogeneity of ependymomas and assigned gene signatures to tumor locations and grades. They reinforced the notion of these tumors to derive from cancer stem cells bearing radial glial cell phenotype. In this context, Hox, Notch and Hedghog pathways play a role in maintenance and renewal of cancer stem cells, and BMP in their differentiation. This, together with the genes up-regulated in posterior fossa ependymomas, pinpoints Notch pathway as a major player of ependymoma tumorigenesis, and Wnt of high grade oncogenesis. Identification of three molecular subgroups in PF ependymomas, among which, the newly identified biphasic-cerebriform pattern, may partly account for discordance in histological ependymoma grading.

O162

GENETIC PROFILING OF PEDIATRIC NON-EPENDYMAL GLIOMAS ASSOCIATED WITH TEMOZOLOMIDE RESISTANCE

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Background: Temozolomide is now widely used as the first line chemotherapy for gliomas. However, efficacy of temozolomide is virtually based on reports on adult gliomas, and recent results for pediatric gliomas have not necessarily demonstrated similar efficacy of temozolomide shown in adult gliomas. Pediatric gliomas are characterized by distinct chromosomal abnormality from adult gliomas, and it is of interest whether any genetic or epigenetic characteristics of pediatric gliomas cause distict sensitivity to temozolomide. **Methods:** We examined several factors that affect sensitivity to temozolomide in pediatric gliomas with comparison to adult gliomas. We studied expression of MGMT (O6-methylguanine methyltransferase) and DNA mismatch repair gene MSH 6 (mutS homolog 6) by immunohistochemistry, presence or absence of promoter methylation of the *MGMT* gene by methylation-specific PCR, and genetic aberrations by comparative genomic hybridization. **Results:** Expression of MGMT in pediatric astrocytic tumors was significantly higher than those of adult tumors by immunohistochemistry. Moreover promoter region of *MGMT* gene was unmethylated in almost all pediatric astrocytic tumors by methylation-specific PCR. However, there was no significant difference in expression of MSH6 between pediatric and adult tumors. Codeletion of 1p and 19q was not observed in pediatric gliomas, and the most frequent aberration in those tumors was gain on 1q, with 10 of 23 cases showing the chromosomal abnormality. Gain on 1q showed tendency towards poor survival and aggressive behavior of the tumors regardless of grade. **Conclusions:** Pediatric gliomas appear to have distinct genetic profiling associated with temozolomide resistance. Higher expression of MGMT, absence of codeletion of 1p and 19q, and frequent gain on 1q in pediatric gliomas might be related to differential sensitivity to temozolomide from their adult counterparts.

O163

THE MAPK PATHWAY IS ALTERED IN THE MAJORITY OF PILOCYTIC ASTROCYTOMAS

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Pilocytic astrocytomas (PAs) are the most frequently occurring brain tumor in 5-19 year-olds. Little is known about the genetic alterations underlying their development. We carried out a genome-wide genetic analysis in 44 PAs to elucidate the molecular mechanisms underlying the development of these tumors. Array-CGH analyses using a combination of a 1Mb and a chromosome 7 tile path BAC-array as well as a custom-designed oligonucleotide array identified two types of tandem duplication, one being very common and the other rare. The former spanned approximately 2Mb at 7q34 and was found in 29 out of 44 (66%) PAs. This rearrangement was not observed in any of 244 higher-grade astrocytic tumors. PCR confirmed that the duplication resulted in a novel fusion gene between KIAA1549 and BRAF. The fusion gene encodes a novel, in-frame fusion protein that lacks the BRaf regulatory domain, has constitutive kinase activity, and confers anchorage independent growth on transfected NIH3T3 cells. The other tandem duplication was found at 3p25 in a single tumor without the BRAF fusion gene. This resulted in an in-frame oncogenic fusion between SRGAP3 and RAF1, which lacks the Raf1 autoregulatory amino-terminus but retains the kinase domain. It shows elevated kinase activity when compared with wild-type Raf1 and transforms NIH3T3 cells. Among the remaining 14 PAs without the BRAF or RAF1 fusion genes, three tumors had BRAF mutations, including one with a novel 3bp insertion. Three other patients without RAF gene alteration had clinical features of NF1. Mutations of the NF1 gene in this syndrome lead to hyperactive Ras signaling and Raf activation. Our results thus show that the great majority of PAs (36 out of 44, 82%) have alterations of the MAPK pathway. This highlights the central importance of this pathway in PA tumorigenesis and underlines its potential as a therapeutic target.

O164

PATIENT-SPECIFIC VIRTUAL CONTROLS CAN BE USED TO SIMULATE AND PREDICT RESPONSE TO RADIATION THERAPY IN INDIVIDUAL GLIOBLASTOMA PATIENTS

This Abstract nominated the Hoshino Award.

Please refer to P065 on page 152.

O165

CAN WHOLE BRAIN IRRADIATION (WBI) REALLY IMPROVE NEUROLOGICAL STATUS AND QUALITY OF LIFE OF PATIENTS WITH BRAIN METASTASES (BM)? A PROSPECTIVE MULTICENTER ANALYSIS OF THE BARCELONA GLIOMA GROUP. FINAL RESULTS

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Purpose: to prospectively analyze neurological status (NS) and quality of life (QL) of patients affected with BM treated with WBI. **Method:** Patients with prognostic RPA classes I, II, IIIa-b (Lutterbach, 02), any age, KPS 50-100, no chemotherapy during WBI, and informed consent were included. Exclusion criteria were class IIIc, poor medical or psico-social conditions, or leptomeningeal dissemination.NS was based on modified cerebral stroke scale with the following items: headache, seizures, speech, visual, motor, brainstem, sensitive, gaiting disorders, and MMSE. EORTC QLQ C-30 and BN-20 were used for each patient. WBI consisted on total dose of 30 Gy/ 10d.Changes of NS were defined as follows: Clinical improvement was the reduction of 25% of scoring and equal or less dose of DXM; clinical progression was the increase of 25% of scoring or more dose of DXM or both, and clinical stability was defined as other situations **Results:** 278 patients were registered; 103 (37%) accomplished inclusion criteria. Most patients had lung (57%) or breast cancers (23%). 98/103 patients finalized WBI. At the first month, 80.6% of intention-to-analyze cases (79 patients) fulfilled neurological evaluation. NS improvement or stabilization at the first month was 64%. Maintained good MMSE (29-30) influenced NS (p=.04); none of the following variables did not: age, gender, histology, number of BM, BM location, RPA class, radiological response, dose of DXM, or baseline NS. Median progression to NS deterioration was 2.3 m. QL improved in emotional (significant) and cognitive fields (trend), and worsened in physical and fatigue fields (both significant). **Conclusions:** 1- 63% of the entire cohort were not able for the study. 2- Only 80.6 % were able to be scored at the first month. 3- NS improvement or stabilization at the first month was 64%. 4- Neuro-cognitive function could determine NS. 5- QL did not decrease after WBI.

O166

INTENSITY MODULATED RADIATION THERAPY FOR CRANIOSPINAL IRRADIATION USING HELICAL TOMOTHERAPY: FROM PLANNING TO DELIVERY

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Purpose: To establish feasibility of doing Intensity Modulated Radiation Therapy (IMRT) for craniospinal irradiation (CSI) using Helical TomoTherapy (IMRT_Tomo) and report challenges in clinical implementation. A dosimetric comparison with conventional IMRT (IMRT_LA) and three-dimensional conformal radiotherapy (3DCRT) is also reported. **Methods:** Initially, computed tomography (CT) datasets of 4 previously treated patients of medulloblastoma were used to generate 3DCRT; IMRT_LA; and IMRT_Tomo plans for CSI. Standard CSI dose of 35 Gy/21 fractions was prescribed to planning target volume (PTV). Subsequently, 5 patients were treated on an ongoing prospective protocol of IMRT for CSI using Helical TomoTherapy. **Results:** The mean volume of PTV receiving at least 95% of prescribed dose (V95%) was more than 98% in all techniques. All techniques resulted in comparable dose homogeneity index (DHI) for PTV_brain. For PTV_spine, IMRT_Tomo achieved highest mean DHI of 0.96 as compared to 0.91 for IMRT_LA and 0.84 for 3DCRT. The best dose conformity index was achieved by IMRT_Tomo for PTV_brain (0.96) and IMRT_LA for PTV_spine (0.83). IMRT_Tomo was superior in terms of reduction of maximum and mean doses to almost all OARs. The average beam-on time was significantly longer in IMRT_Tomo. Issues in implementation included whole-body immobilization, areas to be imaged daily, co-registration efficiency, intrafraction motion, and impact of differential shifts, which were handled using appropriate methodology. **Conclusion:** Helical TomoTherapy seems to be ideally suited for CSI avoiding junctions, field-matching and abutment dosimetry. It is favorable in terms of target coverage, dose homogeneity, conformity, and OAR sparing. Challenges in successful implementation of IMRT_Tomo for CSI can be circumvented provided they are preempted during the planning phase. The longer beam-on time in IMRT_Tomo raises concerns about intrafraction motion and secondary carcinogenesis.

O167

AGE AND RADIOTHERAPY DOSES TO LEFT TEMPORAL LOBE PREDICT NEUROCOGNITIVE OUTCOMES IN YOUNG PATIENTS WITH BENIGN AND LOW GRADE BRAIN TUMOURS: DATA FROM A PROSPECTIVE TRIAL OF STEREOTACTIC CONFORMAL RADIOTHERAPY

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Background: To report the effect of age and RT doses to volumes of different normal structures on neurocognitive outcomes in young patients with benign and low grade brain tumours treated prospectively with stereotactic conformal radiation therapy (SCRT). **Materials and methods:** Twenty eight patients (median age 13 years; 23 male and 5 female) with low grade and benign residual/progressive brain tumours (10 craniopharyngioma, 8 cerebellar astrocytoma, 8 cerebral low grade glioma) were treated with SCRT to a dose of 54Gy/30Fr/6weeks with at least 2-years follow up were analyzed. Prospective neuropsychological assessment were done at baseline pre-radiotherapy (RT) and at subsequent follow up with an age-appropriate neuropsychological battery of tests. The change in intelligent quotient (IQ) scores was correlated with the age and dose-volumes to normal structures using logistic regression analysis. **Results:** While the overall mean full-scale IQ (FSIQ) at baseline pre-RT was 80 and remained unchanged at 2-year follow up, a third of patients had &gt10% drop in FSIQ over baseline. Patients &lt15 years of age had a significantly higher chance of developing &gt10% drop in FSIQ [53% vs. 10%, p=0.03]. Comparison of dosimetric data in patients showing a significant drop in IQ with patients with maintained IQ revealed that patients receiving &gt42.5 Gy (80% of the prescribed dose) to &gt13% of volume (p=0.048) and &gt27 Gy (50% of the prescribed dose) to &gt50% of the volume of left temporal lobe were the ones to show significant drop in FSIQ (p=0.06). Calculation of the RT doses to other normal structures including supratentorial brain and right temporal lobe did not reveal any significant correlation. **Conclusion:** Our detailed dosimetric data shows younger age and radiotherapy doses to left temporal lobe to be predictors of neurocognitive decline, and could be used as possible dose constraints for high precision RT planning.

O168

CARBON ION RADIATION THERAPY FOR PAEDIATIC PATIENTS AND YOUNG ADULTS TREATED FOR TUMORS OF THE SKULL BASE

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Purpose To evaluate the outcome of carbon ion radiotherapy (RT) in children and young adults with skull base chordomas and chondrosarcomas. **Patients and Methods** We treated 394 patients with carbon ion RT at GSI in Darmstadt, of which 17 were younger than 21 years and were treated for chordoma or low-grade chondrosarcomas of the skull base. Irradiation was performed with a median total dose of 60 Gy E in a fractionation of 7x3 Gy E per week. **Results** The median follow-up time was 49 months. Acute side effects were only minor. Alopecia occurred in the periauricular region in a patient with a large chondrosarcoma with the tumor extending to the lateral skull base. Skin erythema was observed at the left cheek in a 6 years old boy with a chondrosarcoma of the left nasal cavity and maxillary sinus together with CTC grade 1 conjunctivitis in the left eye. Two patients developed mucositis of the soft palate(CTC grade 1 and 2). One patient developed temporary middle ear effusion. All symptoms resolved completely. No patient developed new neurological deficits or temporal lobe damage.In a 6 year old boy with a chondrosarcoma of the left nasal cavity and maxillary sinus a slight facial asymmetry could be observed. No other growth abnormalities or secondary malignancies developed.Prior to RT, 10 out of 17 patients presented with neurological deficits. None of these symptoms worsened after carbon ion RT. In one patient, paresis of the facial nerve resolved after carbon ion RT. Only one patient with chordoma developed tumor progression 60 months after RT. **Conclusion** Further evaluation of carbon ion RT is required in this patient population. Randomized studies comparing the outcome after carbon ion RT with proton RT are needed to evaluate the role of particle beams in the treatment of skull base tumors.

O169

PSEUDOPROGRESSION IN BORON NEUTRON CAPTURE THERAPY

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Pseudoprogression has been recognized and widely accepted in the treatments for malignant gliomas, as transient increase in volume of enhanced area just after the chemo-radiotherapy, especially using temozolomide. We experienced the similar phenomenon in the treatments of malignant gliomas and meningiomas using boron neutron capture therapy (BNCT). BNCT is high linear energy transfer, cell-selective particle radiation. Here we introduce the representative cases and analyze this pathogenesis. Fifty two cases of malignant glioma and 13 cases of malignant meningioma who were treated by BNCT were reviewed retrospectively mainly in MRI. Twelve out of 52 malignant gliomas and 3 out of 13 malignant meningiomas showed transient increase of enhanced volume in MRI within 3 months after BNCT, without any chemotherapy. For these cases peak tumor doses were given by BNCT between 234.7 to 1774.2 Gy-Equivalent as given in daily 2-Gy by fractionated XRT. In these cases, 6 glioma cases underwent operation for the suspicion of relapse. In histology, most part of the specimen showed necrosis with small amount of tumor cell residual. Ki-67 labeling index showed decreased positivity in comparison with previous samples of the individuals. Fluororide-labeled boronophenylalanine positron emission tomography (PET) was applied in 5 and 2 cases of malignant gliomas and meningiomas, respectively at the time of transient increase of lesions. These PETs showed decreased lesion/normal brain ratio in all cases in comparison with those obtained prior to BNCT. With surgery or without surgery, all lesions were decreased or stable in size during observation. Transient increase in enhanced volume in malignant gliomas and meningiomas immediately after BNCT seemed to be pesudoprogression. This pathogenesis was considered as treatment-related intra-tumoral necrosis in subacute phase after BNCT. Pseudoprogression has been recognized as good prognosis landmark with intensive treatments. Therefore pseudoprogression in BNCT suggested potential therapeutic effects of this particle radiation methodology.

O170

DIAGNOSIS AND TREATMENT OF RADIATION NECROSIS IN THE BRAIN

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Recently, new radiation therapies, such as IMRT, BNCT and other particle radiation modalities using high absorbed doses to tumor tissue have been developed for patients with malignant gliomas, mainly glioblastomas (GB), with good clinical results. While, clinically, the differential diagnosis of the tumor progression (TP) of GB and radiation necrosis (RN) has become important, as has the assessment of tumor activity after adjuvant therapy. We determined how to evaluate tumor activity before and/or after radiation therapy using positron emission tomography (PET) with boronophenylalanine (BPA) as a tracer. F-BPA-PET images were obtained from histologically verified 38 GB, 8 RN, and 5 RN cases with partial residual tumors. The lesion/normal (L/N) ratios for these groups were 4.2±1.4, 1.5±0.3, and 2.0 ±0.3, respectively. Also Repeat-PET imaging was found to be useful for evaluating changes in GB-associated tumor activity with respect to the treatment received. For the treatment, we experienced 20 RN cases in these 3 years. Anticoagulants and vitamine E alone improved or stabilized RN in one third of the RN cases, especially for relatively small lesions in Gd-enhancement. Also this treatment was effective for prevention of RN. In 11 RN cases, lesionectomy was applied with 5-ALA-guided microsurgery. Four out of 11 cases showed improved KPS one month after surgery, while 2 cases showed aggravation and the remaining 5 were unchanged and stable in KPS. Steroids could be decreased in amount in all 11 cases. 5-ALA guided resection showed a clear boundary between the necrotic tissue and surrounding normal brain in surgery. Recently we experienced the treatment of 3 medically-refractory RN in eloquent area using humanized anti-VEGF antibody, bevacisumab. This treatment showed rapid shrinkage of perilesional edema and enhanced lesion in all cases only by single intravenous administration of bevacisumab at 5mg/kg. Let me show the representative cases in my talk.

O171

BRIEF REPORT: ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (ACGH) CONFIRMS FLUORESCENT IN SITU HYBRIDIZATION (FISH) 6Q DELETION IN POOR-PROGNOSIS DIFFUSE LARGE B-CELL, CD20 POSITIVE PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL).

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Our data suggested that deletion of chromosome locus 6q22-23 was a characteristic of poor prognosis PCNSL patients. In this report we describe a pilot study of aCGH in combination with FISH to confirm deletion status and assess copy member changes. Frozen tumor samples were retrieved from Mayo Tumor Registry (IRB 08-001933). All cases were reconfirmed as PCNSL; as having diagnostic tissue without hemorrhage or necrosis; and as EBV-encoded RNA (EBER) negative. Each case was interrogated using well validated and published protocols for FISH, aCGH; and immunohistochemistry (IHC) for protein tyrosine phosphatase kappa (PTPRK), the gene product of 6q22-23. The cohort comprised two male and three female immunocompetent patients. Two of the four patients with sufficient frozen tumor samples had 6q deletion by aCGH, one complete and one partial. The patient with complete deletion had homozygous deletion by FISH. The patient with partial 6q deletion by array CGH had no FISH abnormalities. Other recurrent abnormalities included partial 1p and partial 1q loss, partial 6p loss, and partial or complete gains on chromosomes 7, 12, 13, 18 and 19q; as well as deletions affecting well characterized tumor suppressor genes p16 and Rb1. This combination of complimentary techniques was chosen on the premise that it would better ascertain whole genome chromosomal imbalances, ploidy, and chromosome integrity. This combination strategy is the most logical next step in the determination of genomic signatures consistent with chromosomal instability mechanisms in PCNSL. This approach may provide important insights concerning the mechanisms responsible for generating complex genomes. The resulting phenotypic diversity can generate tumors with a propensity for an aggressive disease course. A better understanding of the underlying mechanisms leading to PCNSL development could result in the identification of prognostic markers and therapeutic targets.

O172

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS DERIVE FROM B-CELL IN TRANSITION STAGE FROM GERMINAL CENTER B-CELL TO POST GERMINAL CENTER B-CELL.

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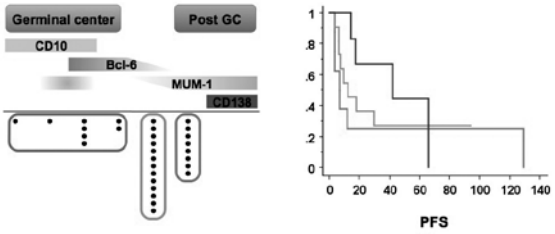
Although most primary central nervous system lymphomas (PCNSL) are diffuse large B-cell lymphomas (DLBCL), biological characteristics of PCNSL are not fully understood. We have attempted to investigate the origin of PCNSL with respect to the stage of B-cell differentiation and challenged the hypothesis that the tumor's stage of differentiation could impact the patient's prognosis. Clinical data of 33 patients treated under the diagnosis of DLBCL of the CNS were retrospectively collected. In 32 patients, immunohistochemical study for the following antigen was performed for classifying B-cell differentiation: CD10, BCL-6, MUM1 and CD138. The median PFS of all 33 patients was 17.2 months and median OS was 63.3 months. 40% of the cases showed CD10-/BCL-6+/MUM1+ indicating that the tumor was originating from the transition stage from germinal center B-cell (GCB) to post-GCB. 25% cases were CD10-/BCL-6-/MUM1+, suggesting post-GCB origin. 25% cases were CD10+ or CD10-/BCL-6+/MUM1-, suggesting GCB origin. When B-cell stages of the tumor were compared to clinical outcomes, late B-cell origins (post-GCB) showed a statistically significant better prognosis than early B-cell origins (pure GCB type and transition stage from GCB to post-GCB type) with a median PFS of 42.4 and 9.6 months respectively. We suggest that PCNSL often derive from B-cell in a differentiation stage from GCB to post-GCB and that classifying PCNSL as above could be beneficial for predicting the patient's outcome and provide more insights into the biology of PCNSL.



O173

NEUROLYMPHOMATOSIS (NL)IN HEMATOLOGIC MALIGNANCIES:AN INTERNATIONAL PRIMARY CNS LYMPHOMA COLLABORATIVE GROUP REPORT.

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Background: Neurolymphomatosis (NL) is defined as a clinical neuropathy induced by infiltration of peripheral nerves, nerve roots, plexus, or cranial nerves by any hematologic malignancies. Given the rarity of this condition, a retrospective review with collaboration across many centers is underway. Objective: to describe the demographics, diagnostic details, management, and outcome of NL. **Methods:** 40 patients (diagnosed 1993 to 2008) were retrospectively assembled from 10 centers in 5 countries. **Results:** Median age was 55.5 years and 60% were males. NL was related to systemic lymphoma in 80%, to acute lymphoblastic lymphoma in 12.5% and to primary CNS lymphoma in 7.5%. It occurred as the initial manifestation of the malignancy in 30% of cases. The most common manifestations were related to peripheral nerves involvement observed in 55%, followed by spinal nerve roots (42.5%), cranial nerves (37.5%) and plexus (35%) with multiple site symptomatology in 50% and painful neuropathy in 78%. Diagnostic evaluations included imaging, CSF studies (36 pts) and biopsies (19 pts). Imaging studies suggested the diagnosis in 78% of cases with 70% (26/37) of MRI and, 82% (14/17) of CT-PET being positive. CSF cytology was positive in 36% and nerve biopsy confirmed the diagnosis in 16/19 (84%). Therapeutic approaches in 39 patients included high-dose methotrexate and/or Ara-c in 61.5%, intra-CSF chemotherapy in 50% and radiotherapy in 36%. Response to treatment by MRI in 21/39 cases revealed 13(62%) CR, 3(14%) PR, 1(5%) SD and, 4(19%) PD. Response by neurological score in 36/39 (92%) patients showed improvement/stabilization in 27(75%). Median survival was 15 months with 31% alive at 56 months. **Conclusions:** NL is a challenging diagnosis associated with an overall dismal prognosis. 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 30% of the affected patients.

O174

SALVAGE CHEMOTHERAPY WITH TEMOZOLOMIDE IN REFRACTORY OR RELAPSED PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Purposes: High-dose methotrexate (HD-MTX) has been considered as the most effective agent in the initial treatment of primary central nervous system lymphoma (PCNSL). However, some patients have refractory lymphoma to initial treatment and another patients relapse easily. Since treatment options for patients with progressive or recurrent PCNSL are limited, their prognoses are very poor. Temozolomide is well-tolerated oral alkylating agent and easy to permeate the blood brain barrier. The effectiveness of temozolomide is reported on not only malignant glioma, but also recurrent PCNSL. In this study, we evaluated the results of treatment with temozolomide for the patients with refractory or relapsed PCNSL. **Methods:** Immunocompetent patients with refractory or relapsed PCNSL were eligible after the initial treatment with HD-MTX (3.5 g/m2) with or without irradiation. All of the patients were treated with temozolomide 150 to 200 mg/m2, for 5 days in 28 days. This treatment was continued until disease progression. **Results:** Seventeen patients with a median age of 68 years (ranged from 49 to 88 years) were included in this study. Four patients were used as second line treatment, ten as third and three as fourth. Five complete remissions (median treatment 12 times; ranged from 4 to 35 times), five partial response and stable disease and seven progressions were observed. No major toxicities were observed, apart from grade 3 thrombocytopenia in one patient. **Conclusions:** Although some patients were elderly and had been heavily pre-treated, temozolomide yielded 29% objective responses and was well tolerated without any major toxicity.

O175

THE ARGENTINE PEDIATRIC ONCOLOGY REGISTRY AS A TOOL TO EVALUATE THE SITUATION OF CHILDREN WITH CNS TUMORS IN ARGENTINA.

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Objectives
The Argentine Pediatric Oncology Registry - KALEIDOS Foundation (ROHA) has recorded since 2000, cancer cases in children less than 15 years of age in Argentina. Our aim is to highlight the usefulness of a population-based cancer registry (with significant participation of hospital-based cancer registries) and its ability to evaluate the situation of children with CNS tumors at a national level.

Methods
ROHA has developed numerous children's hospital cancer registries. ROHA includes not only cases from hospital cancer registries (its major source of data) but also from: all-ages population-based regional cancer registries, some cooperative groups for the treatment of specific tumors, the National Mortality Database, the National Bank of Antineoplastic Drugs and from private physicians' practices. We evaluated the early mortality in meduloblastoma and low-grade glioma as indirect markers of the care of children with CNS tumors.

Results
Between 2000 and 2006, the average number of cancer cases annually was 8,741 with a crude incidence rate of 128.2 per million, with estimated coverage of 93%, total CNS tumor cases of 1,634, with a crude incidence rate of 23.7 per million. On average, 86% of the children were cared for in public institutions. The percentage of children who sought specialized centers at some stage during treatment, varied according to their province of residence (6% to 100%) and to the tumor type (49% cases of CNS tumors and 29% of leukemia). For a total of 294 evaluable medulloblastoma cases, 140 are dead and 63 (21.4%) died during the first 6 months following diagnosis. For a total of 136 evaluable low-grade glioma cases, 46 are dead and 21 (15.5%) died during the first 6 months following diagnosis.
After evaluating the situation of cancer patients in the northwestern area of the country, government entities (national and provincial), with support from non-governmental entities, have developed a collaborative program between the national referral hospital (Hospital Prof. Garrahan, HPG, Buenos Aires) and the pediatric hospital in the Tucumán province, to optimize oncology services and develop then as a model reference in the region. ROHA has developed, as a tool for this program, a booklet "When to suspect cancer in children", accompanied by an audio-visual (both published by the Fundación Garrahan and of free distribution). In addition, ROHA will participate in a multi-center study on the mortality of pediatric oncology patients in public hospitals. The origin of this study, (HPG coordinated), was the observation of lower cancer survival rates in Argentine children, especially those with CNS tumors, compared with that expected from international publications.

Conclusions
The preponderance of hospitals as source of data and the population-based coverage, make ROHA particularly valuable. The diagnostic data from each institution provide useful information to improve hospital activities. The early mortality in medulloblastoma and low -grade glioma is very high, and is representative of the rest of childhood CNS tumors. We have observed: delayed diagnoses; delayed time to surgery and deficits with neuro-surgical experience; difficulties in defining pathological anatomy in patients and in interdisciplinary communication The analysis of national data has provided tools for the design of health policies for children with cancer in Argentina. The booklet "When to suspect cancer in children", will facilitate improvements in earlier diagnosis and allow the implementation of less intense and expensive treatments. The study on hospital mortality will support enactment of measures to increase survival. National cancer registries, as exemplified in Argentina by ROHA, can be of great value if their data can be used to optimize the quality of childhood cancer patient management.

O176

TREATMENT OF OPERABLE HIGH-GRADE GLIOMA WITH SITIMAGENE CERADENOVEC GENE THEREAPY AND GANCICLOVIR: RESULTS OF A RANDOMIZED PHASE III TRIAL

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Background: High-grade glioma (HGG) continues to have a dismal prognosis with limited survival despite multimodal therapy. This study assesses the impact of the delivery of the HSV-tk gene into the healthy tumour bed of resected HGG using a serotype 5 adenoviral vector (sitimagene ceradenovec). This gene induces the expression of thymidine kinase, which phosphorylates the systemically administered ganciclovir to be effective locally against malignant cells. **Methods:** 250 patients with operable HGG were recruited from 38 centres in Europe and Israel between November 2005 and April 2007. Patients were randomized to receive standard therapy, with or without sitimagene ceradenovec injections. On Days 5-19 post-operatively, the active group received ganciclovir (5 mg/kg i.v. twice-daily). Adjuvant treatment with temozolomide was permitted, but not obligatory, depending on local treatment practice. Patients are currently being followed for reintervention-free survival, overall survival (OS) and MRI progression, as well as standard safety monitoring. The primary outcome measure is time from surgery until reintervention or death. The principal secondary outcome was time to all-cause mortality - OS. **Results:** The study completed in May 2008. Updated final results from the OS outcome will be available in early Q1 2009 and will be presented at the meeting, along with demographics of the study population, efficacy of the therapy (as Kaplan-Meier survival plots and statistical analyses) and the overall time-based safety profile. **Conclusions:** Conclusions will be derived from the results to provide an overall risk and benefit assessment of this form of gene therapy in operable HGG.

P001

CD133 : STILL A MARKER OF BRAIN TUMOR STEM CELLS?

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China

The brain tumor stem cells (BTSCs) hypothesis presumes that BTSCs are responsible for the initiation, progression and recurrence of several typies of malignant brain tumors, such as glioblastoma multigorme(GBM), medullblastoma(MB). They can proliferate, self-renew infinitely and differentiate into diverse populations such as neuronal, astrocytic and oligodendroglial cells. Treating the differentiated brain tumor cells will not cure the tumor so the therapeutic strategy should target on the stem cells.

Till now, the most popular reported and applied cell marker of BTSCs is CD133. Several brain tumor researchers demonstrate that CD133 positive cells can produce tumors in nude mice that recapitulate the original tumor and possess the capacities for resistence to chemotherapy and radiotherapy. In our previous studies, we also found CD133 positive cells have a higher proliferation index when compared to CD133 negative cells. But the CD133 expression can not always be detected in malignant brain tumors which, on the contrary, include a proportion of cells with significant capacities for neurosphere forming, clonegenecity and tumorgenecity. Here we discuss the relationship between the cell surface antigen CD133 and BTSCs in GBM, MB and pituitary adenomas. We hope to explore a better approach to study BTSCs in both CD133 positive and negative brain tumors and finally to make an individual therapeutic strategy that target on BTSCs.

Introduction

P002

CD133 NEGATIVE GLIOMA SUBPOPULATIONS GROUPED BY DIFFERENT CELL TYPE MARKERS DISPLAY TUMORGENICITY IN VIVO

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Purpose: The cancer stem cell hypothesis implies that intratumor heterogeneity is created by rare cancer stem cells. These cells have the capacity to differentiate into various tumor cell types as well as to self renew. In brain tumors, CD133 has been proposed as a marker for cancer stem cells, although this has been challenged by a series of studies demonstrating tumor formation from CD133 negative glioma cells as well. **Methods:** To further elucidate the heterogeneity seen in Glioblastoma Multiforme (GBM), we created lineage specific lentiviral reporter constructs that expressed GFP under the Nestin, glial fibrillary acidic protein (GFAP) or neuron specific enolase (NSE) promoter. The U373 and U87GBM cell lines were infected with these constructs, and separated with simultaneous removal of CD133 positive cells using fluorescence-activated cell sorting (FACS). **Results:** This way, we obtained glioma subpopulations expressing different cell type markers, and subsequently implanted these cells in NOD-SCID mice. Finally, we repeated the experiments using two primary GBM biopsies instead of cell lines. In the cell lines, all glioma cell populations gave rise to tumors at similar rates. For the biopsies, both nestin + and nestin- cells grew tumors, while none of the lineage specific cells (GFAP and NSE) produced tumors. **Conclusions:** We conclude that different glioma subpopulations exhibit tumor initiating ability, although glioma cells expressing mature markers may be relatively less tumorogenic than other glioma cell populations.

P003

NOVEL TECHNIQUE FOR ESTABLISHMENT OF GLIOBLASTOMA STEM CELL LINE AND PATHOLOGICAL VERIFICATION OF DISTRIBUTION AND DIFFERENTIATION STAGE OF TUMOR CELLS ON ITS XENOGRAFT.

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We successfully apply the monolayer culture method to establish glioma stem cell line from surgical sample (paper in submission). We present our novel technique for establishment of GBM stem cell line and some particular feature of its xenografts. **Methods** We used 3 glioblastoma surgical samples. We cultured cells from each sample on monolayer methods with EGF/FGF without serum. After conformation every cell lines fulfill the criteria of glioma stem cell, we inject cells into NOD/SCID mouse brain and observed the pattern of invasion and differentiation of tumor cells pathologically on its xenograft. **Results** We established 3 GBM stem cell lines from 3 samples successfully. Every lines fulfill the criteria of glioma stem cell. They express stem cell markers such as Sox2, Nestin and have ability of multilineage differentiation. All lines have tumor initiating ability in vivo. Although ratio of CD44 expression is almost 100% in all lines, interestingly ratio of CD133 expression varied as follows; 96.5%, 1.4%, 43.6%. Xenograft have gliobalstoma specific feature such as necrosis/pseudopallisading. The undifferentiated GFAP(-)Nestin(+) tumor cell showed strong invasive capacity, invaded contralaterally even in the early stage. Undifferentiated GFAP(-)Nestin(+) tumor cell favored invasion to subventricular zone (stem cell niche). In all xenogarft, invasive front is formed by undifferentiated GFAP(-)Nestin(+) tumor cell. The higher CD133 expressed line has the stronger invasive capacity. Lower CD133 line formed somewhat well margined mass. Differentiated GFAP(+) tumor cell remained injection site and expanded and formed tumor bulk. **Conclusions** The novel monolayer culture method is useful to establish glioma stem cell line and provides enough chances to study glioma stem cell from surgical sample. The xenografts of GBM stem cell lines recaptured the pathological feature of original sample patient. Undifferentiated stem-like tumor cell have strong invasion ability and formed invasive front of tumor.

P004

THERAPEUTIC EFFECT OF GENETICALLY ENGINEERED MESENCHYMAL STEM CELLS IN RAT EXPERIMENTAL LEPTOMENINGEAL GLIOMA MODEL

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Purpose: Disseminating disease of high grade gliomas is difficult to treat. We examined, in this study, the therapeutic effect of intrathecal administration of mesenchymal stem cells transduced with herpes simplex virus-thymidine kinase gene (MSCtk) in experimental leptomeningeal glioma model.

Methods: Leptomeningeal glioma model was produced by injecting C6 glioma cells into the cisterna magna in Sprague-Dawley rats. Mesenchymal stem cells were isolated from 6-week-old rats and MSCtk cells were established using retrovirus transduction system. First, to examine in vivo bystander effect, rats were intrathecally co-injected with a mixture of MSCtk and C6 cells and then, intraperitoneally administered with gancicloir (GCV) or saline for 10 days (co-injection model). Next, to examine the therapeutic effect of MSCtk/GCV therapy, MSCtk cells were intrathecally administered one day after C6 injection and then, GCV or saline was administered (treatment model). Tumor volume was measured on day 14 in one group and survival time was measured in the other group for each model.

Results: GCV administration significantly reduced tumor sizes both in the co-in jection model (0.41 ±0.22 vs 3.10 ±0.97 mm2, p p<0.01) and in the treatment model (0.73 ± 0.29 vs 2.84 ± 0.82 mm2 , p p<0.01). Survival was also significantly prolonged in GCV group both in the co-injection model (29.17 ± 3.27 vs 18.83 ± 0.79 days, p p<0.01) and in the treatment model (21.50 ± 1.48 vs 17.17 ± 0.48 days, p<0.01).

Conclusions: This study provided a novel treatment strategy for leptomeningeal glioma dissemination using intrathecal MSCtk injection followed by systemic GCV administration.

P005

CHLOROQUINE SUPPRESSES GLIOMA CELL VIABILITY AND SENSITIZES RADIORESISTANT STEM-LIKE GLIOMA CELLS TO IONIZING RADIATION AND CHEMOTHERAPY

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Study purpose
The purpose of this study was to evaluate the effects of chloroquine on glioma cell viability in vitro and in vivo, and to explore the therapeutic potential of chloroquine as an adjunct to glioma therapy.

Methods
Human glioma cell lines with known p53 status or primary cultures derived from surgical specimens of human glioblastomas were propagated in the presence of serum or under serum-free conditions as neurospheres. Radioresistant populations were selected from neurosphere cultures of glioma stem-like cells by repetitive exposures to clinically relevant doses of radiation. The tumorigenic potential was evaluated inan orthotopic glioma mouse model. Assessment of cell death was performed by trypan blue exclusion, by immunofluorescence detection of cells positive for activated caspase-3 or TUNEL (TdT-mediated dUTP-nick end labeling). The transcriptional activity of p53 was assessed by western blot.

Results
ClQ potently induces death response in glioma cells in vitro and inhibits growth of experimental gliomas in an orthotopic glioma mouse model. We demonstrate that accumulation of the p53 protein, activation of p53 transcriptional response and induction of p53-dependent apoptosis are the mechanisms underlying induction of cell death by ClQ in cells with wild type p53. Unlike DNA damage, chloroquine does not induce p53 posttranslational modifications contributing to p53 stabilization through phosphorylation. In addition to its p53-activating effects, chloroquine is also able to suppress glioma cell growth via p53-independent mechanisms. We found that chloroquine inhibits growth of glioma cells that express mutant p53, possess stem-like properties and are capable of establishing highly invasive tumours. Furthermore, chloroquine treatment renders radioresistant cell populations selected from glioma stem-like cultures susceptible to radiation, and chemotherapy with BCNU or temozolomide.

Conclusions
Chloroquine possesses therapeutic potential and can be envisaged as an adjunct for glioma therapy.

P006

ESTABLISHMENT AND CHARACTERIZATION OF AN EXPERIMENTAL MODEL OF INVASIVE GLIOMA WITH HIGHLY RADIORESISTANT PHENOTYPE.

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Study purpose
A distinct sub-population of tumorigenic cells exhibiting properties of neural stem cells, generally termed as brain tumour initiating cells (BTICs), has recently emerged as the major cellular component determining the invasive and radioresistant phenotype of malignant brain tumours. There is an urgent need for experimental models that would recapitulate the hallmark features of human glioblastoma multiforme (GBM) such as histological heterogeneity and a highly invasive, radioresistant phenotype. The purpose of this study was to develop an invasive orthotopic GBM mouse model using human glioma cells with defined genetic background and the hallmark features of stem-like BTICs.
Methods
Stem-like BTICs were selected from the human glioma cell line G112 and propagated in the presence of serum or under serum-free conditions as floating neurospheres. Cell viability was assessed by using a neurosphere assay and trypan blue exclusion. The BTICs stem-like phenotype was assessed in vitro by immunofluorescence staining of cultured cells and in vivo by immunohistochemical examinations.

Results
The human glioma cell line G112 shows a stem-like BTIC phenotype under serum-free culture conditions. Genetic analyses indicate that G112-BTIC derive from a single genotype. The G112-BTIC neurosphere cultures are capable of long-term self-renewal, show immunoreactivity for neural stem cell markers nestin and CD133 and display a radioresistant phenotype in vitro and in vivo. Tumours formed by G112-BTIC cells show an extremely aggressive growth pattern, exhibit the hallmark features of human GBM and show an impaired radio-response. In contrast, non-BTIC tumours show significant reduction upon treatment with identical radiation doses.

Conclusion
The G112-BTIC glioma model provides an adequate experimental platform for the testing of potential therapeutic efficacy of existing treatments and developing new strategies to target radio- and chemoresistant glioma cell populations.

P007

FUNCTIONAL RESULTS AFTER RESECTION OF GLIOMA INVOLVING THE SUPPLEMENTARY MOTOR AREA

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[OBJECTIVE] Surgically induced supplementary motor area (SMA) deficiency syndrome has been reported, and reversibility of the SMA deficiency syndrome has been emphasized. In this report, we aimed to confirm functional results after the resection of gliomas involving the SMA. **[METHODS]** Twelve patients with gliomas of the SMA treated by surgery between 2002 and 2007 were studied. The nondominant side was affected in 7 cases and the dominant side in 5. Degree and duration of postoperative deficits were evaluated and correlated with the extent of tumor resection on the postoperative magnetic resonance imaging and intraoperative neuromonitoring findings. **[RESULTS]** Postoperatively, motor deficits were evident in 11 of 12 patients and speech deficits in 4 of 5. Recovery of motor function began between a few hours and 7 days after the surgery, and significantly improved within 2 months. Five patients in whom the posterior resection margin was at a distance of less than 0.4mm from the precentral sulcus experienced a persistent drop in skilled motor function. Two patients who complained of cognitive dysfunction subjectively after the surgery were assessed with WAIS-III, and a diminishing in processing speed was recognized in both patients. **[CONCLUSIONS]** Preserved muscle tone and recovery from the distal part of the limbs were characteristic of SMA deficiency syndrome compared with damage to the pyramidal tract. A good correlation existed between the severity and duration of postoperative motor deficits and the distance of the resection margin from the precentral sulcus. The follow-up of cognitive dysfunction, characterized a diminishing in processing speed, is necessary.

P008

THE DIAGNOSTIC IMPACT OF RADICAL RESECTION IN THE MANAGEMENT OF LOW-GRADE GLIOMA

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OBJECTIVE: The extent of resection in low-grade glioma (LGG) still remains controversial to be concerned with patient outcome. A part of LGGs behave progressive growth and malignant transformation, however, there are no diagnostic tool to distinguish their clinical natures. In this study, we assess the diagnostic role of radical resection in the management of LGG. **METHODS:** A series of 40 patients has been diagnosed as LGG, World Health Organization (WHO) grade II, by modern diagnostic imaging since we introduced awake surgery into LGG resection in 2004. The high intensity area in FLAIR images was defined as LGG. The tumor was radically resected within the limits avoiding neurological deficits. The widespread histopathological analyses were performed at the various loci of resected LGGs. **RESULTS:** 2 patients were diagnosed with stereotactic biopsy because of difficulties of tumor resection. Although the diagnostic images show characteristic features of LGG, the widespread histopathological studies demonstrated local malignancy in a part of LGGs. In the series of 40 LGGs, there are 9 diffuse astrocytomas, 10 diffuse astrocytomas with mitosis, 5 anaplastic astrocytomas, 4 dysembryoplastic neuroepithelial tumors, 5 oligodendrogliomas, 2 oligoastrocytomas, and 1 cortical dysplasia. **CONCULSION:** The widespread histopathological study with radical resection of LGGs helps us to detect local malignancy. This finding suggests that extensive surgical resection and widespread histopathological study in large specimen are recommended to decide the management of LGG.

P009

EXPERIENCE WITH SPECTROSCOPY-SUPPORTED STEREOTACTIC BRAIN BIOPSY

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OBJECTIVE Comparative evaluation of the diagnostic efficacy of stereotactic brain biopsy performed with and without additional use of spectroscopic imaging (SCI) for target selection was done. **METHODS** From 2002 to 2006, 39 patients with parenchymal brain lesions underwent frame-based image-guided stereotactic biopsy based on structural neuroimaging, whereas in 30 others SCI-supported procedures were performed. Comparison of the diagnostic yield of the procedure in these subgroups was done. Additionally, the diagnostic accuracy was evaluated in 37 lesions, which were surgically resected within 1 month after initial tissue sampling. **RESULTS** Definitive histopathological diagnosis was established in 57 cases, diagnosis of low-grade glioma without specific tumor typing in 8 cases, and in 4 cases the biopsy findings were non-specific. In 5 out of 8 cases with incomplete histopathological diagnosis and in all non-diagnostic cases target selection was done without use of SCI (P=0.2073). The diagnostic yield of stereotactic biopsy was 90±5% in cases based on structural neuroimaging compared to 100% in SCI-supported procedures (P=0.1268). Among lesions surgically resected thereafter 10 cases of major diagnostic disagreement in histopathological diagnosis were met. The diagnostic accuracy was 79±9% in cases based on structural neuroimaging compared to 67±11% in SCI-supported procedures (P=0.4756). **Conclusion** It seems, that use of SCI-detected metabolic information during stereotactic brain biopsy may increase the diagnostic yield of the procedure, but optimal selection of the metabolic target for improvement of its diagnostic accuracy remains unclear.

P010

USEFULNESS OF FDG-PET-CT-GUIDED STEREOTACTIC BIOPSY FOR BRAIN TUMORS

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Objectives: PET image is useful for detecting biologically active lesions. Sometimes it is difficult to distinguish neoplasms from inflammatory changes by MR-guided biopsy. We report the usefulness of FDG-PET-CT-guided stereotactic biopsy. **Materials and Methods:** We performed FDG-PET-CT-guided stereotactic biopsy for seven patients (age 47-78 years, M:F=5:2). Komai-type head ring was applied to patients. CT and FDG-PET images were obtained sequentially after injection of FDG. A biopsy target was set on a lesion of the highest FDG accumulation using spatial coordinates calculated from CT scan data. Single biopsy tract was designed not to pass the ventricles and eloquent areas. **Results:** In two cases, the targets that we planned on MR images had little FDG accumulation, so we changed the target. All seven biopsies got histological diagnoses. Small bleeding was observed at the target in two cases on postoperative CT: one had noSymptoms, the other showed transient hemiparesis. Measurement of radioactivity revealed our specimens equal to the background of the operating room. **Conclusions:** With FDG-PET-CT images, a biological active lesion can be accurately obtained leading to a correct diagnosis. Careful management of radioactive materials is mandatory with multidisciplinary cooperation for this method.

P011

NEURONAVIGATION AND BRAIN BIOPSY. SURGICAL TECHNIQUE AND COMPARISON WITH TRADITIONAL ESTEREOTAXIC METHOD.

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Since the introduction and generalization of Neuronavigation, brain biopys had to be performed with stereotactic procedures such Leksell frame. Classic technique had inconvenients like having to perform a CT scan the day of procedure, placing the frame and carry the patient to make the CT. In the last eigthteen months we have performed brain biopsys with the Navigus system by Medtronic (optical mode) in thirteen pacients, all of them with intracerebral tumors (low grade astrocitoma and glioblastoma).We have analyzed the surgical procedure, problems and results of these surgerys and compared with the results of the last twenty brain biopsys with Leksell frame.We have concluded that, in our experience, Neuronavigation provides visual assited guide and accuracy and the possibility of variation of target if desired.

P012

THE ROLE OF DIFFUSION TENSOR IMAGE OF THE TUMOR RESECTION

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Introduction Diffusion tensor image plays a role based on the anisotopy of the hydrogen ions in the interesting area; In the current state, the DTI tractography can predict intracranial structure especially corticospinal tract, optic radiation, ...et.al.the preoperative recognition of the tarctcan avoid iatrogenic damage to the eloguent area. **Methods** From year 2002-2008, We applied 15 cases of brain tumors who obtained well-studied data and benefit from the DTI analysis;there were 6 meningiomas with compression on the motor strip; 5 gliomas with presentral gyrus involved; the others were matastatic tumor feasible for surgical resection.The preoperative tractography were analyzed and incoporated into navigation system(Brain Lab). **Result** There is no major motor deficit after the DTI-assisted resection for 24-month follow up except trasient edema by manipulation on 2 glioma cases; there is one mortality carried out by the underlying lung malignancy after 5 months. **Conclusion** The DTI is poved to be the good example of neuroradiology incoporation with clinical application;it provides a exact guidance of surgical margins and avoids hazadous sequalae. Familiarity with the new technology and well practice make a promising chapter on neuro-oncology.

P013

USEFULNESS OF FIBER TRACT NAVIGATION IN TUMOR SURGERY ADJACENT TO ELOQUENT AREA

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PURPOSE: There is a significant risk of neurological deficits after tumor surgery near the eloquent areas. The purpose of this study was to minimize the postoperative injuries by means of the magnetic resonance based intraoperative neuronavigation with fusion of the preoperative diffusion tensor imaging, which was used for tracking of the white matter fibers, such as the motor cortex, the pyramidal tract and the optic radiation. **METHODS:** From September 2007 to December 2008, there were 15 patients who underwent surgery for brain tumor adjacent to the eloquent area. In each case, the DTI combined neuronavigation was performed preoperatively. **RESULTS:** There were 10 female patients and 5 male patient. Their mean age was 43.6 years old. The location of the tumor was adjacent to the motor cortex in 11 patients, the pyramidal tract in three patients, and the optic radiation area in one patient. The pathologic diagnosis was glioblastoma in 8 cases, anaplastic astrocytoma in 3 cases, diffuse astrocytoma in 3 cases, and atypical choroid plexus papilloma in one case. Gross total removal was performed in 10 patients and subtotal resection was done in 5 patients. There were three cases of patients whose hemiparesis aggravated after surgery in which one of them was permanent and the others recovered within 4 weeks. **CONCLUSION:** It is considered very safe and useful for the surgery of tumors located near the eloquent area when functional neuronavigation is utilized. However, limitations of the neuronavigation such as brain shifting during the operation. Moreover, development of imaging techniques for the visualization of surgical field is demanded.

P014

PREOPERATIVE FUNCTIONAL IMAGING AND INTRAOPERATIVE MONITORING FOR IMPROVING RESECTION RATE OF GLIOMAS AND FUNCTIONAL OUTCOME OF THEIR PATIENTS

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Purpose To examine how the preoperative functional imaging of the MRI and intraoperative physiological monitoring contribute to the improvement of the resection rate of the tumor and postoperative functional outcome in the glioma surgery. **Cases and Methods** Resection rate of the glioma and functional outcome of the patients were compared with the early cases (12 cases) (group A) between 2001-2005.11 with later cases (26 cases) (group B) after December, 2005. We used MRI, CT, DSA and SPECT as preoperative neuro-radiological images, functional MRI, tractography and SPGR as functional images, VEP, ABR and ECHO as intraoperative physiological monitoring and 5-ALA, ICG as fluorescent markers. We compared the functional outcome of group A with group B by neurological symptoms and KPS at discharge. **Results** The glioma in eloquent area was 3 cases in Group A (30%), 11 cases in Group B (42.3%). SEP, MEP and Echo were done as intra-operative monitors in 3 cases of group A, and in15 cases of group B. Grossly total and subtotal removal of the tumor were done in each 3 cases (50%) of group A, while those removal of the tumor were performed in 19 cases (73%) of groupB. Histologically, 6 GBMs, 4 AAs and 2 benign gliomas in group A, while 19 GBMs, 5 AAs and 2 benign gliomas in group B. Functional outcome at discharge was 3 improved(25%) and 4 deteriorated(33.3%) in group A, while 14 improved (54%) and 6 deteriorated (23%) in group B. **Conclusion** Our results demonstrated that preoperative evaluation on the tumor characteristics by functional images and intraoperative physiological monitoring as well as fluorescent tumor labeling made it possible to remove gliomas as much as possible without serious neurological deficit, followed by better functional outcome.

P015

AWAKE CRANIOTOMY AND ELECTROPHYSIOLOGICAL MAPPING IN A HIGH FIELD INTRA-OPERATIVE MRI

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Objective and importance: Awake craniotomy and electrophysiological mapping (EPM) are established techniques to facilitate resection in or adjacent to eloquent cortex. Intraoperative MRI (iMRI) is increasingly used to aid resection of intracranial lesions. Awake craniotomy and EPM with low-field iMRI has been reported but awake craniotomy and EPM with high-field iMRI has not. Here, we describe a simple technique for awake craniotomy, EPM, and high-field iMRI. **Clinical presentation:** A 57 year-old right-handed man presented with new onset generalized seizures and one episode of speech arrest. MRI demonstrated a large left temporal intra-axial mass with a small area of contrast enhancement. A biopsy at an outside hospital showed anaplastic astrocytoma. **Technique:** The patient underwent an awake, left frontotemporal craniotomy in the supine position. EPM demonstrated a single critical area for speech in his inferior frontal gyrus. After an initial anterior temporal lobectomy and tumor debulking, the scalp flap was loosely approximated, the operative field was covered with a sticky Steri-Drape, and the excess drapes were trimmed. The patient was kept in position and transferred to an adjacent 1.5 Tesla GE scanner. An intraoperative MRI was obtained. The image-guidance system was re-registered and the patient was re-draped. Further resection of residual tumor was performed, allowing an extensive removal. The patient tolerated this well without any new neurological deficits. **Conclusion:** Prior protocols for positioning and draping in high-field iMRI units made awake craniotomies problematic. This technique for combined awake EMD and iMRI is simple and can facilitate safe removal of large lesions in eloquent cortex.

P016

NEW INTRAOPERATIVE MRI SYSTEM IN BRAIN TUMOR SURGERY

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Background of Study: Extensive surgical removal will contributes to longer life expectancy for brain tumors. As dynamic changes occurred during surgical procedures, intraoperative MRI system is important tool for safe and maximum resection of tumors. In July 2008, new operation room with intraoperative high-field MRI (1.5 T) system, neruonavigation, and fluorescence diagnosis system (Surgical Suite) opened at the Yamagata University Hospital. This new suite is also compatible for intraoperative angiography. **Methods:** Preoperatively, the 3T MR studies including morphological study, multivoxel MR spectroscopy, tractography and functional MRI was performed. If the tumor located in or near eloquent area, we performed MEP (motor evoked potential) / SEP (sensory evoked potential) monitoring and/or awake surgery using cortical and subcortical stimulation. Intraoperative MRI is performed after total resection or to up-dated data (to get information of brain shift) during removal of the deep sheeted tumors. **Results:** Using this new suite, we treated safely brain tumors including gliomas, metastatic brain tumors, menigniomas and pituitary adenomas. Intraoperative MR images contribute to improvement of the tumor resection rate. **Conclusion:** In this paper, we present our first 10 months of experience using this suite, and discuss its advantages and limitations. We also present the relation of the results of preoperative chemical shift, intraoperative photodynamic diagnosis and pathological findings.

P017

NOVEL SURGICAL TECHNIQUE OF RADICAL REMOVAL FOR GLIOMAS

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Despite the improvement in surgical technique, more powerful sources of radiation and development of new chemotherapeutic agents, the prognosis for patients with malignant gliomas remains very poor. One of the most important factors which limits our ability to successfully treat these patients is the diffuse and infiltrative nature of glioma growth. Although extensive radical resection fails to cure the malignant gliomas, it does both improve the quality of life for these patients and increase the duration of survival for selected patients. In my series of 125 patients with malignant gliomas, the extent of surgical excision as judged by postoperative enhancement on MRI was one of the strongest factor for survival. The median survival with patients with glioblastoma was 36.3months in extensive removal, 15.4 months in subtotal removal and 12.5months in biopsy group. Many retrospective studies have reported that there is an improvement in both median survival and long-term survival with extensive surgical resection for malignant gliomas. Because the reduction of tumor mass provides space for postoperative brain swelling, the extensive resection can be less risky than open biopsy or limited resection. To enable safe and radical resection of malignant gliomas, especially those adjacent to eloquent brain areas, I used the tiny special markers which were inserted into the tumor margin using navigation systems. And awaken surgery with intraoperative brain mapping can remove tumor safely and adequately without postoperative cerebral edema and neurological deficits. In summary, Extensive radical surgery is one of the most prognostic factors for the patients with malignant gliomas. I will discuss the detail surgical techniques.

P018

DISSECTION AND SEPARATION OF HIGH GRADE GLIAL TUMORS FROM BRAIN TISSUE BY USING METHALIC DISSECTOR WITH THE CAPABILITY OF PRODUCING VIBRATION

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Purpose: Previously, some different techniques had been presented in terms of precise dissection and separation of tumors from surrounding brain. Purpose of this study was to describe the effect of vibration in the surgical dissection and separation of malignant brain tumors. **Method:** We recently developed an instrument for micro-dissection and separation of brain lesions from brain tissue. This instrument was produced in a medical instrument producing factory in Samsun. This instrument has the capability of producing mechanical vibration. The holding and using of this instrument was described. **Results:** In this study, we used this instrument in five high grade glial tumors for dissection and separation of these lesions from surrounding brain tissue. Dissection and separation were graded as poor, moderate and good. Vibrato-dissection technique was superior to those of conventional technique in the aspect of dissection and separation of brain tissue. **Conclusion:** The capability of dissection and separation with this instrument during surgical treatment of brain tumors may enhance dissection and separation of lesions. **Key Words:** Micro-vibration, glial tumors, dissection, separation.

P019

THE OPERATION OF PIAL SYNANGIOSIS IN THE SURGICAL TREATMENT OF MALIGNANT GLIAL TUMORS

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Purpose: The primary treatment of malignant glial tumors is surgical intervention for maximally reducing and/or total removing of tumors. The purpose was to describe pial sinangiosis for diversion of blood brain barrier for enhancing of systemic blood-brain tissue penetration and the effect of chemotherapy. **Method:** We developed a new surgical technique in the surgical treatment of malignant glial tumors for enhancing of systemic blood-brain tissue penetration. Preoperative and postoperative magnetic resonance images were obtained to evaluate peri-lesional edema and complications. **Results:** This technique was used in three cases of malignant glial tumors. There were no operative mortality and morbidity related to this technique. **Conclusion:** This technique may be used in the surgical treatment of malignant glial tumors to enhance penetration of chemotherapeutic agents into the brain tissue. **Key Words:** Pial synangiosis, malignant glial tumors, surgical treatment.

P020

THE CLINICAL RESULTS OF FIVE CASES OF INSULAR TUMOR RESECTION

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PURPOSE: Because of its complex and critical neurological function, the insular lobe has been considered to be difficult to approach. However, insular glioma could be controlled and complication could be prevented with exact anatomical knowledge. The purpose of this study is to introduce our clinical results of five cases of insular tumor and to present anatomical consideration. **MATERIALS AND METHODS:** Five patients who had intraaxial tumors in insular lobe were selected. Three were men and two were female. Chief complaint was seizure and there was no neurologic deficit. Tumor located entirely in insular lobe in 2, with frontal extension in 1, with temporal extension without medial temporal invasion in 1 and with medial temporal invasion in 1. Follow up period was 15 months. **RESULTS:** Tumor resection was performed under G/A in four patients and there was one awake anesthesia case. Subtotal resection was possible in four and tumor was partially removed in one. Four cases were WHO Grade III or IV glioma and one was WHO grade II glioma. None had postoperative neurological deficit. There was no progression of tumor during follow up period. Seizure was disappeared in 3 and worthwhile improved in 2. **CONCLUSIONS:** Both tumor control and seizure control are possible with detailed understandings of anatomy and dissection plane. Insular glioma is no more difficult tumor, if surgeon follows anatomical caution.

P021

KEYHOLE SURGERY IN THE TREATMENT OF GLIOMAS

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Backgrounds: Recent innovation of diagnostic and therapeutic modalities such as tractography and neuronavigation system (NNS) have contributed the progression in glioma surgery, and sufficient resection of tumor has been achieved. Therefore, we initiated keyhole surgery as less invasive treatment of gliomas in 2005. The aim of this analysis is to evaluate the clinical significance of this treatment. **Methods:** Under the guide of NNS, the outlines of the tumor and risk organs were visualized in the microscope through scalp before surgery, and the minimum craniotomy area, 3 to 5 cm in majority of cases, was designed on the scalp. These small craniotomies required only linear scalp incision. 103 gliomas were resected with this method (Group K). The surgical results were compared with 36 gliomas removed by standard navigation surgery (Group S). Tractography was evaluated before surgery and SEP/MEP was performed if necessary in both of the groups. **Results:** The median extent of resection was 95.2% in Group S and 98.0% in Group K (p=0.212). Among the independent pts, 3% (2/67) deteriorated function and became dependent after surgery in Group K, while 14% (3/22) in Group S (p=0.095). In the dependent pts, 32%(12/38) improved function and became independent after surgery in Group K, while 21% (3/14) in Group S (p=0.731). Indeed the statistical significance was not proved, the overall survival time of glioblastoma in Group K (median: 37months) was superior to that in Group S (median: 13 months, p=0.153). On the other hand, both the operation time (median: 215 min. Group K and 265 min. in Group S) and hospitalized period (12 days in Group K and 24 days in Group S) were significantly decreased by our keyhole manner (p=0.022 and 0.005). **Conclusions:** Keyhole surgery was time-effective and less invasive for brain and scalp, while keeping the sufficient extent of resection.

P022

SIGNIFICANCE OF PORPHYRINS IN BLOOD AND URINE OF BRAIN TUMOR PATIENTS THAT UNDERWENT 5-ALA ADMINISTRATION

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Protoporphyrin IX is known to accumulate in tumors when 5-ALA is administered to brain tumor patients followed by the tumor resection using protoporphyrin IX fluorescence. However, there is no report on measured blood and urinary porphyrins during 5-ALA administration in humans. This study reviewed the significance of such measurement.Fifteen brain tumor patients and 8 adult volunteers were given 5-ALA orally. Blood and urinary porphyrins were measured before and at 4 hr after 5-ALA was administered. Urinary levels of coproporphyrin and blood levels of uroporphyrin were significantly high in the brain tumor patients compared with the volunteers.A high urinary coproporphyrin level or blood uroporphyrin level after 5-ALA administration suggests the possibility that urine coproporphyrin level or blood uroporphyrin level could become a tumor marker. These high levels indicated 5-ALA hypermetabolism of the lesions in the patients.

P023

ELECTROLOADED DENDRITIC CELLS WITH WHOLE TUMOR LYSATE ELICIT ENHANCED INDUCTION OF TUMOR-SPECIFIC IFNγ PRODUCING CELLS IN PATIENTS WITH GLIOBLASTOMA

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We demonstrate in vitro using peripheral blood (PB) and tumor lysates derived from patients with glioblastoma that tumor lysate-loaded dendritic cells (DCs) by electroporation (Lysate-EP DCs) can elicit better induction of IFNγ producing cells, which may be associated with anti-tumor immunity than tumor lysate co-cultured DCs (Lysate-Coculture DCs). Further this higher induction of IFNγ producing cells by Lysate-EP DCs could be enhanced by co-incubations of Lysate-EP DCs with Zoledronate. The number of IFNγ-producing cells in lymphocytes following stimulation with autologous Lysate-EP DCs determined by ELISPOT assay significantly increased in the all patients so far tested in comparison with using Lysate-Coculute DCs. This enhancing effect of Zoledronate was observed also in all the patients, in which expansion of Nγδ T cells may be partly contributed to the enhancement of IFNγ producing cells. Tumors expressing higher HLA class I molecules seemed to be associated with higher induction of IFNγ producing cells, in which HLA class I-restricted CD8+ CTLs might be major components. These data suggest that Lysate-EP DCs may be extensively useful to induce more effective immune responses in immunotherapy, including DC therapy.

P024

CYTOTOXIC T LYMPHOCYTES INDUCTION WITH HLA-A2402-RESTRICTED GLIOMA SPECIFIC PEPTIDES

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[Purpose] Glioma is a malignant brain tumor with poor prognosis. Recently, basic and clinical study showed that immunotherapy using cytotoxic T lymphocytes (CTLs) against tumors is expected to be effective for the treatment. Successful immunotherapy against tumors needs identification of HLA-restricted tumor-specific antigenic peptide. Unfortunately, a few glioma-specific antigenic peptides bound to HLA-A2402, major haplotype in Japanese population, have been identified. Therefore, we searched glioma specific antigen-derived and HLA-A2402-restricted peptides which are effective for induction of tumor-specific CTLs. **[Methods]** We analyzed HLA-A2402-restricted glioma specific peptides using the combination of two computer algorithms. CD8+ T cells and DCs were prepared from HLA-A2402 positive PBMCs from healthy donors. Glioma antigenic peptide-specific CTLs were induced by culturing CD8 T cells with DCs pulsed with these four peptides. Effective tumor antigenic peptides were screened for their ability of CTLs by 51Cr release assay and intracellular IFN--&gamma staining. **[Results and Discussion]** We selected glioma antigenic peptides, which showed high affinity to HLA-A2402 molecules. CTLs induced by the stimulation with DCs pulsed with both peptides showed strong cytotoxicity against tumor cell lines and also produced IFN-&gamma. Additionally, the peptides specific CTLs killed glioma cell line, YKG1 (an HLA-A2402 positive glioma cell line) . Therefore, these peptides could be ideal candidates for DCs- or CTLs- based immunotherapy against glioma.

P025

CHARACTERIZATION OF A NOVEL TUMOR-ASSOCIATED ANTIGEN FOR TARGETED TOXIN THERAPY IN GLIOMAS.

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Therapies targeting specific tumor-associated antigen have shown promising initial results in the treatment of glioma patients. A desirable target antigen should be unique for tumor cells, abundantly expressed and readily available for antibody binding. One of the main obstacles is the identification and characterization of such specific targets, especially since most antigens in gliomas exhibit quantitative rather than qualitative alterations. The genetic heterogeneity and large capacity for immune escape in gliomas also makes identification of several distinct targets an important task for the successful development of targeted therapies. We describe the identification and characterization of the Ku70/80 protein complex as a novel target in gliomas. The Ku70/80 protein has previously been reported to be abundantly expressed in the cell nuclei of normal cells, but localize to the plasma membrane specifically in some transformed cell types. We show that the antigen localizes to the cell surface of glioma tumor cells, and that it is prominently expressed in human glioma tissue, while no expression could be found in normal human brain tissue. We further demonstrate that upon antibody binding the antigen-antibody complex undergoes endosomal internalization, and that this internalization can be efficiently used for delivery of toxins in cultured glioma cells. These results pinpoints Ku70/80 as one important novel glioma-associated antigen for antibody based therapy.

P026

TUMORICIDAL BYSTANDER EFFECT-MEDIATED SUICIDE GENE THERAPY OF GLIOMA USING GENETICALLY ENGINEERED BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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In our previous study, we successful treated an established C6 brain tumor using neural stem cells transduced with the herpes simplex virus-thymidine kinase gene (HSVtk) and ganciclovir in the rat. In the present study, we investigated the use of mesenchymal stem cells transducerd with HSVtk (MSCtk) instead of neural stem cells because mesenchymal stem cells are much easier to obtain from the adult subjects. In vitro co-culture experiment revealed a sufficient bystander tumoricidal effect between MSC and tumor cells and only 1/32 MSCtk cells were needed for complete tumor eradication. In vitro bystander effect was also observed in a real-time fashion using a culture microscope and it was shown that only tumor cells that had contact with MSCtk cells died. In vivo treatment of an established C6 brain tumor with an intratumoral injection of MSCtk cells followed by systemic ganciclovir administration resulted in a significant reduction of the tumor size and a significant survival prolongation. The treatment strategy using MSCtk and ganciclovir ("MSCtk therapy") is more feasible for clinical application that the method using neural stem cells.

P027

AN EFFICIENT TARGETED GENE THERAPY USING BRAIN TUMOR-SPECIFIC PROMOTER

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Despite many efforts to develop effective therapy, the outcome of malignant glioma remains poor. Gene therapy for this disease using retroviral vector is attractive, because the virus can infect only mitotic cells. Previously, we reported the eradication of mouse glioma by retroviral-mediated gene therapy. In this study, a tumor-specific targeting system was studied to develop the effective and safe gene therapy. We searched for genes expressing at high frequency in brain tumors but not in normal human astrocyte (NHA) among cancer testis antigen (CTA) genes. MAGEA3 and brain tumor-specific gene 2 (BTSG2) were identified as tumor-specific genes. The promoter of both genes was cloned into luciferase reporter vector and the activity was measured in glioma, telomerase-immortalized fibroblast and normal human astrocyte cells. The BTSG2 promoter but not MAGEA3 showed the tumor-specific activity. The minimal promoter of BTSG2 were defined as a 256 bp fragment upstream of transcriptional start site. In order to define a useful tumor-specific promoter for targeting in context of retroviral-mediated gene therapy, the BTSG2 promoter were used to restrict the expression of suicide gene HSVtk in retroviral vector. The glioma cell lines transduced with the retroviral vector were killed efficiently by addition of ganciclovir (GCV), but telomerase-immortalized fibroblast BJ-5ta cells were not. Mouse glioma RSV-M cells transduced with the retroviral vector were transplanted into S.C. of syngeneic mouse. The administration of GCV suppressed the tumor growth completely. These results suggest that the promoter of BTSG2 is useful for tumor-specific gene therapy, and promising the safe and effective retroviral-mediated gene therapy.

P028

LONG-TERM OUTCOME OF 23 CONSECUTIVE CHILDREN WITH PRIMARY INTRADURAL SPINAL TUMORS TREATED AT THE MEDICAL UNIVERSITY OF VIENNA (1997-2008)

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Primary intradural spinal tumors are rare in childhood and there is little information on therapy and outcome in this location. We report on the histology, management and outcome of 23 consecutive patient with intradural spinal tumors treated at our institution over the past 11 years.

Patients: From 1997 to 2008, 23 patients (10 m, 13 f) aged between 2 and 17 years (median 10) were treated for intradural spinal tumors. 18/23 tumors were intramedullary (gangliocytoma, n=2, pilocytic astrocytoma, n=5, diffuse astrocytoma WHO grade II, n=1, anaplastic astrocytoma WHO grade III, n=2, glioblastoma WHO grade IV, n=1, ependymoma WHO grade II, n=5, atypical teratoid rhabdoid tumor, n=2) and 5 were extramedullary (myxopapillary ependymoma, n=2, neurofibroma, n=3). Total or subtotal resection was performed in 16 patients, partial resection in three and biopsy only in four. Surgery was the only treatment in 14 patients. The three patients with high-grade gliomas received chemo-and radiotherapy as did the patient with progressive diffuse glioma. Two patients with ependymoma WHO grade II, who were irradiated postoperatively received additional radio- and chemotherapy for secondary metastases. One patient with myxopapillary ependymoma was irradiated for secondary leptomeningeal dissemination. The two patients with atypical teratoid rhabdoid tumor received chemo- and radiotherapy.

Results: In January 2009, 20/23 patients are alive with a median survival time of 5.5 years (range 8 months to 11 years). The two patients with atypical teratoid rhabdoid tumor and the patient with glioblastoma died of their disease. In all patients preexisting neurological deficits improved after treatment and no permanent postoperative worsening occurred in any of the patients.

Conclusion: Surgery only may suffice for ependymoma and low-grade glioma when gross total removal is confirmed by immediate postoperative MRI and long-term survival may be achieved with combined chemo-radiotherapy in patients with disseminated ependymoma and nonpilocytic astrocytoma.

P029

EFFECTIVENESS OF MULTI-STAGED OPERATIONS WITH COMBINED CHEMOTHERAPIES FOR HUGE MALIGNANT INFANTILE BRAIN TUMORS IN THE FIRST YEAR OF LIFE

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Purposes: Infantile brain tumors are extremely rare and mostly malignant, and difficult to be treated because they are almost huge and easy to bleed. We performed multi-staged operations with combined chemotherapies for malignant infant cases which were massive and hypervascular. We estimated effectiveness and limitation of this procedure. **Materials and Methods:** Out of pediatric cases treated in the past seven years at our related institutions, six cases with infantile brain tumors in the first year of life were picked up. Out of six, three cases were neonate cases. All cases showed hydrocephalus and symptom of increased intracranial pressure. In five cases, intraventricular hemorrhages or intratumoral hemorrhages were observed at the first examination. All tumors were huge, irregular and heterogeneous on MRI imaging. **Results:** We at first performed biopsy intentionally for histopathological diagnosis. Histopathological diagnosis included three central PNETs, one immature teratoma, and one choroid plexus carcinoma, one desmoplastic infantile astrocytoma. After biopsies, following adjuvant chemotherapies were done in four cases. In all, Volume of tumors were reduced, and decrease in MIB-1 index and intratumoral vessels were seen as histopathological changes after chemotherapies. During chemotherapies, multi-staged removals were performed. Two cases needed removal at two times. In huge immature teratomas, operation from two directional approach were needed. Intraoperative blood loss was less and hemostasis was safer than the first operation. In all cases, gross total removal or complete remission (CR) was obtained. After CR, postoperative adjuvant chemotherapies were added without radiation therapy. Treatment for hydrocephalus was done in five. As complication, One meningitis and a subdural abscess was seen. Two malignant cases suffered from relapse at brain stem, additional chemotherapy and radiation was needed. **Conclusion:** Multi-staged operations with combined chemotherapies for malignant infantile brain tumors is safer and more effective especially in huge and hypervascular cases.

P030

WHICH THERAPY WORKS BETTER IN METASTATIC CHOROID PLEXUS CARCINOMAS?

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Background: Metastatic choroid plexus carcinomas (CPCs) have a dismal prognosis. Besides, the role of surgery is not addressed yet. **Material and Methods:** The database registering all cases of CPTs recorded in the National Library of Medicine (PubMed) until 2008 March was created using the method previously described. **Results:** Of 906 documented CPTs patients, 361 were choroid plexus carcinoma (CPCs). There were 46/361patients that were found to be metastatic at diagnosis. In order to find the best therapeutic option in metastatic CPCs, patients that had surgery for certain, and those that did not have it for certain are compared using Kaplan Meier curves and log rank test. Patients with gross total resection after surgery were found to have a significantly better Kaplan Meier curves and log rank tests (p=0.021). However, an effect of radiotherapy or chemotherapy on survival could not be demonstrated using Kaplan Meier curves and log rank test (p&lt0.05). With a further Cox regression analysis, we have demonstrated that gross total resection has a positive effect of on metastatic CPCs, regardless of other prognostic factors namely, the patients age, sex, location of the tumor or if the patients' were given or not given radiation or chemotherapy. **Discussion:** This literature database analysis confirms that maximal efforts to resect even metastatic CPCs are well supported.

P031

99 TC SESTAMIBI SCAN DIFFERENTIATES TUMOR FROM OTHER CONTRAST ENHANCING TISSUE IN CHOROID PLEXUS TUMORS - CASE REPORT AND REVI+EW OF LITERATURE

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Background: Choroid plexus tumors (CPT) are rare brain tumors which account for 0.4-0.6 % among brain tumors. Tumor resection is known to be of large prognostic impact, and re-resection of residual tumors is a part of standard care. However, after multiple resections it can become difficult to differentiate what is tumor, what is reactive tissue. 99 m TC Sestamibi scans show normal choroid plexus tissue, and may assist in differentiating neoplastic (sestamibi +) from non-neoplastic tissue (sestamibi -). **Case Report:** 6 month old girl presented with signs of poor feeding, weight loss and excessive crying. MRI showed a left parietal mass. Gross total resection revealed atypical choroid plexus papilloma (WHO II). The tumor recurred and she was enrolled into CPT SIOP 2000 protocol, re-resected, treated with chemotherapy, proton beam radiation, further chemotherapy, re-resected again, and finally completed the treatment protocol. On MRI, the location of the primary tumor still contained contrast enhancing tissue. Various imaging techniques were attempted to solve the problem but never completely resolved it. On Sestamibi scans normal choroid plexus enhanced on the right, and no enhancement on the left choroid plexus suggesting no active CPP but post-operative and post-RT reactive tissue. We decided against further resection. Further follow up showed the patient asymptomatic, on MRI the contrast enhancing tissue shrinking and the sestamibi scan remained negative. **Conclusions:** Previous literature showed sestamibi to be helpful detecting residual choroid plexus tumors resulting in further resection (Med Pediatr Oncol 36:323-5, J Nucl Med 43:1438-43) this is the first reported case to show that sestamibi scans can also help with the opposite decision. Sestamibi-scans should be included in the decision process for choroid plexus tumor treatments.

P032

ADJUVANT CHEMORADIATION FOR MIXED GERM CELL TUMORS

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Purpose: The aim is to demonstrate the efficacy of adjuvant chemoradiation for mixed germ cell tumors and the histology after this particular treatment.**Methods & Results:** There were six cases. Main regimens of chemotherapy (chemo.) was CBDCA and VP-16(CARE) or CARE and IFM. Histologically, they were examined by H&E and immunohistochemistry for AFP, β-hCG, p53, p21, and MIB-1. **Case 1:** 9 years-old girl presenting a HCG and AFP producing pineal tumor. After GKs and chemo., complete remission was achieved. However, she died 2 years later due to repeated recurrence. The autopsy revealed the histology of choriocarcinoma(Ch) and embryonal carcinoma(Ec). **Case 2:** 22 years-old man presenting an AFP producing pineal and thalamic tumor. The histology of the surgery was germinoma(G) with york sac tumor(Ys). During the chemoradiation, he was complicated by sepsis, and then died. The autopsy revealed no tumor cells. **Case 3:** 17 years-old man presenting an AFP producing tumor on the left basal ganglia. Although chemoradiation(8Gy) was tried, he deteriorated resulting in an emergent removal. The histology was Ys showing pleomorphism and huge necrosis. **Case 4:** 44 years-old man presenting a pineal immature teratoma(T) with G. After chemoradiation, the histology on the salvage surgery was mature T. Thereafter, he died 2 years later due to spinal metastasis. **Case 5:** 7 years-old girl presenting an AFP and HCG producing suprasellar tumor. After chemoradiation, the histology on the salvage surgery was partly immature T. Case 6: 8 years-old girl presenting an AFP and HCG producing suprasellar tumor. Although 2 courses of chemotherapy were tried, her hydrocephalus was deteriorated resulting in a removal. The histology was partly immature T. **Conclusion:** The results suggest that the efficacy of adjuvant chemoradiation for mixed germ cell tumors was better in G and Ys than in Ec, Ch, and immature T.

P033

OVER A 10-YEAR SURVIVAL AND COMPLETE RESPONSE OF A PATIENT WITH DIFFUSE INTRINSIC BRAINSTEM GLIOMA (DBSG) TREATED WITH ANTINEOPLASTONS (ANP)

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Our purpose is to report a case of a long-term complete response (CR) in a DBSG treated at our center and to discuss the factors contributing to the success. The patient received intravenous injections of ANP every 4 hours through a subclavian central venous catheter via a double channel infusion pump followed by PO ANP only. Response was assessed by gadolinium-enhanced MRIs of the brain. The patient is currently a $10^{1/2}$ year-old Caucasian female who, as a 6-week-old infant, was diagnosed with a DBSG on August 12, 1998. The tumor was inoperable and the pediatric oncology service felt that chemotherapy as well as radiation therapy would not be an option considering the potential toxicity and the age of the patient. On October 14, 1998, she began IV ANP under the FDA's special exception to phase II protocol BC-BT-11, was converted to PO ANP on June 8, 2000 and permanently discontinued ANP on July 8, 2004. She achieved CR in late February 1999 after discontinuation of dexamethasone and MRI of the brain showed resolution of the enhancing tumor. She developed only one episode of grade 3 vomiting which resolved within 3 days. Her most recent MRI of the brain on April 2, 2008, did not show any sign of recurrence. In November 2008, her father stated that clinically she was doing very well. ANP is a multi-gene-targeted therapy that is well tolerated with minimal and reversible adverse events and has multiple different mechanisms of action by affecting the AKT, RAS, TP53, p21 and PTEN pathways. This patient with DSBG achieved CR and over a 10-year survival after treatment with ANP. These promising results have already been confirmed in a larger group of children in phase II studies.

P034

IMPROVED RESPONSE OF PONTINE GLIOMA WITH SIMULTANEOUS RADIOCHEMOTHERAPY: RESULTS OF A RETROSPECTIVE IMAGE ANALYSIS

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Background: Magnetic resonance imaging (MRI) is the standard method of diagnosis for diffuse intrinsic pontine glioma (DIPG). Conventional fractionated radiation improves survival time of these patients; the benefit of adding of chemotherapy is frequently debated.

Methods: We conducted a retrospective analysis of DIPG from 2001-2008. The mediolateral diameter of the pons was measured horizontal in T2/Flair axial images. We calculated the differences of the pontine diameters before and after conventional fractionated radiation, and then compared the changes between patients with and without simultaneous chemotherapy. The hypothesis that the addition of chemotherapy improved tumor shrinkage was tested and $p<0.05$ (t tests) was considered statistically significant.

Results: From 2001 to 2008, 20 patients (10 males, 10 females), age 7.7 years (± 3.7 SD) with pontine gliomas were available. The mean time interval between the images was 70.2 ± 16.4 days. The average change of pontine diameter was 5.9 ± 4.9 mm (n = 20) for all patients. The shrinkage was larger when radiation was given with chemotherapy (8.3 ± 4.4 mm, n = 12) as compared to without chemotherapy (2.4 ± 3.4 mm, n = 8). This difference was statistically significant ($p=0.0028$).

Discussion: Tumor shrinkage was more prominent with chemotherapy. This might translate into improved quality of life, while overall survival improvement might depend on the velocity of the still inevitable tumor recurrence.

Conclusion: This provides evidence that irradiation with simultaneous chemotherapy resulted in tumor shrinkage advantages.

P035

TACTICS FOR DIAGNOSIS AND SURGICAL TREATMENT OF BRAIN STEM TUMORS

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Objective To explore the diagnosis and microsurgery indication,approaches and curative effective of brain stem tumor. **Methods** The cases of 32 patients with brain stem tumor were retrospectively reviewed, during 2002.1 to 2007.12. **Results** All patients were underwent microsurgrical treatment, attenuation of craninal nerves was obviously improved in 22 cases, no change in 7 cases, deterioration in 2 cases, respiration and circulation dysfunction in 1 case. **Conclusion** Preoperation MRI examination is the frist choice. The correct choice of microsurgery indications and approaches, precise operation, active precaution, management of postoperative complications are the key points to increase the curative effect.

P036

PROFILE OF PATIENTS WITH MEDULLOBLASTOMA AT THE PHILIPPINE GENERAL HOSPITAL: A SIX-YEAR REVIEW

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Background: Medulloblastoma is a primitive neuroectodermal tumor of the posterior fossa, and the most common malignant brain tumor in childhood. Local data on medulloblastoma are limited to epidemiologic studies of primary pediatric intracranial tumors. **Objectives:** To determine the local incidence of histologically confirmed medulloblastoma at the Philippine General Hospital (PGH), and to review the demographic, clinical features and patient outcome on discharge. **Material and Methods:** The incidence of medulloblastoma between 2000 and 2005 was determined from the database of the neurosurgical and surgical pathology sections of the PGH. Clinical charts of patients were reviewed, and demographic, and clinical features and outcome on discharge of the patients were summarized in a unified database. **Results:** In this 6-year review, the prevalence of medulloblastoma is 4.85% (n=62) of the total 1,279 primary CNS malignancy seen. Of these, 90.32% were children, and 9.68% were adults. The mean age at diagnosis was 9.82 (SD 8.06) years (median of 8 years). The male-female ratio was 1.21:1. The most frequent presenting clinical manifestations were headache and papilledema. The mean symptom duration prior to diagnosis was 3.39 months (median of 2 months). Majority of the tumors were midline (77.42%). Hydrocephalus was seen in 87.10% of patients, majority (92.59%) of which underwent CSF diversion. Total and subtotal resection was done in 51.61% and 48.39% of the patients, respectively. Seizure, CNS infection, and hemorrhage were the most common postoperative complications. Twenty-four (77.42%) of the patients were alive on discharge. **Conclusion:** This study determined the local prevalence of medulloblastoma at the PGH. A great majority of which were children, with slight male predominance. The most common clinical manifestations were headache and papilledema. Most patients had midline tumors and developed hydrocephalus. Total and subtotal resections were done with a 77.42% survival rate on discharge.

P037

RESULTS OF TREATMENT FOR MEDULLOBLASTOMA IN OUR INSTITUTE

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Objective : The result of treatment for medulloblastoma is improving by the novel therapy. We evaluated the curative effects for the medullobalastoma patients with three different protocols in Gifu University. **Materials :** Between 1976 and 2008, 16 medulloblastoma patients (8 boys and 8 girls) with median age of 8.5 years (range 4-16 years) received initial treatment with surgery followed by chemotherapy and craniospinal radiotherapy in our institute. The early 7 cases (group A) were underwent chemotherapy with nimustine (ACNU) and vincristine (VCR) and the total 39 Gy of fractionated external beam radiation therapy (EBRT) to the posterior fossa with craniospinal radiation. The middle 7 cases (group B) were with cisplatin (CDDP) and etoposide (VP-16) and total 52-56Gy of EBRT to the posterior fossa with craniospinal radiation. The recent 2 cases (group C) were not high risk group, treated with cyclophosphamide (CPM), VP-16 and carboplatin (CBDCA) and total 50Gy of EBRT to the posterior fossa with craniospinal radiation. **Results :** Median progression-free survival (PFS) was 36 months and median overall survival (OS) was 81 months. 5- and 10-year OS rate was 71 and 50%. The significant difference was not recognized between group A and group B in PFS (p=0.903) and OS (p=0.893). All patients, who passed from a first operation in 81 months, were alive. There were 3 patients with the long-term survival for more than 25 years. **Conclusion :** The treatment results for the medulloblastoma are improved, and some patients were considered to be cured during a long-term follow-up. In addition, it was considered that the appropriate therapies might contribute to a good functional prognosis.

P038

CAN ABSOLUTE LYMPHOCYTE COUNT REALLY BE A PROGNOSTIC INDICATOR FOR OVERALL SURVIVAL IN PATIENTS WITH MEDULOLBLASTOMA?

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Background: Recently, in a number of studies a link between absolute lymphocyte count and survival rate was shown. The purpose of this study is to determine if absolute lymphocyte recovery is an independent prognostic indicator in an overall survival in patients with medulloblastoma. **Methods:** After institutional review board approval, a retrospective chart review is being conducted for patients diagnosed with medulloblastoma treated at MD Anderson Cancer Center between years 1988-2008. The clinical data collection includes the absolute lymphocyte count (ALC), hemoglobin, platelet count, white blood cell count and absolute neutrophil count (ANC). **Result:** Data was available on 33 patients for analysis. Median age 9 years (14month-32 yrs), Males n=20, Females n=13, median survival 2.05 years. After analyzing the data using log rank test on the various blood parameters in different combinations no significant relationship could be established between the blood count and the survival rate. The only significant finding was in the sub-group of males who had ANC at day 15 of the treatment more than 1.5K/UL. Total n=19, ANC less than 1.5 = 4, ANC15 more than 1.5 = 15(median overall survival 1.81 years versus 3.52 years, p=0.001). No finding was significant for females with ANC15 over 1.5. **Conclusion:** No correlation could be found between ALC and survival rates in patients with medulloblastoma. This is different from almost all other malignancies examined so far and could be due to the use of steroids in patients with medulloblastoma decreasing the ALC count which is not the case in ALL, AML and Ewing sarcoma.

P039

INTEGRATING COMPLEXITY, THERAPEUTIC RECREATION, AND RADIOTHERAPY IN MULTIDISCIPLINARY MEDICAL TEAM ON REDUCING ANESTHESIA/SEDATION REQUIREMENT FOR TODDLERS AND YOUNG CHILDREN WITH BRAIN TUMOR

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BACKGROUND: Complexity theory has been pervasively applied in medical education to adapt multiple environmental changes and multidisciplinary collaboration in recently research. More than forty percent of children with brain tumor require radiotherapy to have better disease control and prognosis. Painless radiotherapy procedures always create tremendous psychological fear for young children, especially aged 3 to 5. General anesthetic (GA) or conscious sedation (CS) procedures were generally necessary for young children with malignant brain tumor undergoing radiotherapy. **PURPOSE:** Recent studies have called for humanizing GA/CS procedure and other better preparation to reduce the need of repeat GA or CS in 30- to 40-day radiation therapy to reduce related medical costs and to avoid potential physical or psychological side effects on children with brain tumor. The purpose of the study is to employ complexity theory as a theoretical framework, to develop therapeutic recreation interventions to assist communication among medical team, patients and family members, to create flow experience for children with brain tumor in radiotherapy process, and to humanize GA/CS protocol to reduce the need of repeat GA or CS. **METHOD:** Fifteen Children with Brain Tumor were selected and had individualized therapeutic recreation interventions, which included video viewing, art and crafts, story telling, and drama playing. **RESULTS:** After interventions, all of children were able to complete multiple radiotherapy sessions without GA or CS. **CONCLUSIONS:** Evolving from chaos theory, complexity theory not only keeps the unpredictability and nonlinearity but also develops mutual-adaptation, co-evolution, dynamic interaction and self-organization. **SUGGESTIONS:** According to the interviews from primary caregivers and related medical service professionals, positive recognition and feedback recommend that complexity theory are viable and valuable to offer further development of standard operating procedure, executive professional team training, and quantitative evaluation to ensure the quality of life for toddlers and young children with brain tumor.

P040

IMPLICATIONS OF COMPLEXITY: LEISURE PARTICIPATION ON PRIMARY CAREGIVERS AND HOSPITALIZED CHILDREN WITH BRAIN TUMOR

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This purpose of the study is to explore benefits of leisure participation on improvement of life quality for primary caregivers who have hospitalized children with brain tumor under the theoretical framework of complexity theory. Complexity theory has been pervasively applied in medical services to adapt multiple environmental changes and multidisciplinary collaboration to enhance the quality of life for clients. Primary caregivers of 3 children aged 3-7 with brain tumor were recruited and provided 4-week leisure activity interventions. After interventions, participants and children completed in-depth interviews. According to the results, the primary caregivers stated that they emphasized and cared their children too much to keep participating past leisure activities and expressed that leisure interventions during the hospitalization alleviated their life stress and empowered them to face their routine daily life enthusiastically. Complexity theory implicated that providing leisure activities for primary caregivers and their children with brain tumor not only conform to the challenges of unpredictability and nonlinearity in medical service system but also develops mutual-adaptation, co-evolution, dynamic interaction and self-organization to provide a better quality of medical services for primary caregivers and their children with brain tumor.

P041

ENDOTHELIAL CELL TRANSDIFFERENTIATION OF HUMAN GLIOMA STEM/ PROGENITOR CELLS IN VITRO

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Objective: The transdifferentiation of normal stem cells in many kinds of tissues or organs has been studied. However, whether tumor stem cells can transdifferentiate is seldom reported. Meanwhile, the mechanism of angiogenesis in tumors is in disputations, and it is still unknown that whether glioma stem/ progenitor cells (GSPCs) participate into angiogenesis in glioma. In this study, we tried to prove that GSPCs could transdifferentiate into endothelial cell-like cells, and participate into the angiogenesis of glioma. **Methods:** GSPCs were cultivated in endothelial differentiation medium, as well as on Matrigel, for 10 days, their morphological changes were observed. Meanwhile, a transmission electron microscope was used to detect the ultrastructural characteristics of GSPCs cultivated on Matrigel for 10 days. Furthermore, GSPCs were also cultured in hypoxia or oxygen-glucose deprivation (OGD) for 4 hours, then the transcriptions and expressions of endothelial cells markers, including CD31 and CD34, were detected by RT-PCR and immunocytochemical stain. **Results:** After culture for 10 days in endothelial differentiation medium, GSPCs presented to be the typical "flagstone" appearance of vascular endothelial cell (VEC); when cultivated on Matrigel, GSPCs gradually formed tubular-like structures in vitro; and cells composing of the tubular-like structures showed typical ultrastructural characteristics of VEC, and expressed CD31. Furthermore, when cultured in hypoxia or OGD, GSPCs transcribed and expressed CD31 and CD34. **Conclusion:** GSPCs could participate into angiogenesis of glioma by transdifferentiating into VEC-like cells.

P042

THE NEW DIAGNOSIS AND TREAMENT OF PITUITARY APOPLEXY

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Objects: To explore the pathogenesis of typical and atypical pituitary apoplexy for the sake of helping to formulate the treatment program reasonably and to select operation occasio correctly. **Methods:** 28 cases of pituitary apoplexy were diagnosed by the clinical manifestation and findings from operation or pathology. 24 presented typical manifestation, and the other 4 without clinical manifestation fit pathologic diagnosis. We analyzed the relationships between the course of disease and clinical manifestation, image study, operation and pathology, and the characters of them during all periods of course. **Results:** Based on the analysis between the course of disease and clinical manifestation, image study, operation and pathology, we suggested that the typical pituitary apoplexy should be divided into two stage: the early stage of hemorrhagic necrosis and the late stage of complete necrosis. The atypical pituitary apoplexy was proved the obsolete hemorrhage inside the tumor by image study, operation and pathology. The total removal rate was 75%;. **Conclusions:** the typical pituitary apoplexy was mainly caused by infarcted necrosis and subsequent hemorrhage of the tumor. The purpose of emergency operation is the remission of severe acute headache onset, visual acuity disorder, and consciousness disorder. The operation effect of the late stage of complete necrosis was obviously better than the early stage of hemorrhagic necrosis. The patients with no significant symptoms can be treated by hormone substitution conservative therapy. Most atypical pituitary apoplexy cases are characterized by the chronic bleeding and have no index of emergency operation. The typing and staging of the pituitary apoplexy are considerably important to its treatment strategy.

P043

THE MIR-17/92 POLYCISTRON IS AMPLIFIED AND UP-REGULATED IN SONIC HEDGEHOG-DRIVEN MEDULLOBLASTOMAS AND INDUCED BY N-MYC IN SONIC HEDGEHOG-TREATED CEREBELLAR NEURAL PRECURSORS

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Medulloblastoma is the most common malignant pediatric brain tumour and mechanisms underlying its development are poorly understood. We identified recurrent amplification of the miR-17/92 polycistron proto-oncogene in 6% of pediatric medulloblastomas by high-resolution SNP genotyping arrays and subsequent interphase FISH on a human medulloblastoma tissue microarray. Profiling the expression of 548 mature microRNAs in a series of 90 primary human medulloblastomas revealed that components of the miR-17/92 polycistron are the most highly up-regulated microRNAs in medulloblastoma. Expression of miR-17/92 was highest in the subgroup of medulloblastomas associated with activation of the Sonic Hedgehog (Shh) signaling pathway as compared to other subgroups of medulloblastoma. Medulloblastomas in which miR-17/92 was up-regulated also had elevated levels of MYC/MYCN expression. Consistent with its regulation by Shh, we observed that Shh treatment of primary cerebellar granule neuron precursors (CGNPs), proposed cells-of-origin for the Shh-associated medulloblastomas, resulted in increased miR-17/92 expression. In CGNPs, the Shh effector N-myc, but not Gli1, induced miR-17/92 expression. Ectopic miR-17/92 expression in CGNPs synergized with exogenous Shh and enabled them to proliferate in the absence Shh. We conclude that Hedgehog signaling promotes the transformation of cerebellar neural precursor cells, driven at least in part by over-expression of the miR-17/92 polycistron. Moreover, in a subset of medulloblastomas, excessive Sonic Hedgehog signaling drives oncogene addiction to miR-17/92, and selects for subsequent genomic amplification of the miR-17/92 polycistron.

P044

MULTIPLE RECURRENT GENETIC EVENTS CONVERGE ON CONTROL OF HISTONE LYSINE METHYLATION IN MEDULLOBLASTOMA

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Genotyping of 212 medulloblastomas (MBs) using high-resolution SNP arrays identified novel amplifications and homozygous deletions, including recurrent, mutually exclusive, highly focal genetic events in genes targeting histone lysine methylation, particularly histone 3, lysine 9 (H3K9). Proper control of histone lysine methylation is critical for transcriptional regulation and plays an essential role in the differentiation of stem and progenitor cell populations. We identified two tumors with focal homozygous deletions of EHMT1 on 9q34, whose protein product is an H3K9 methyltransferase. Similarly, we found focal deletion of SMYD4 on 17p13, another histone lysine methyltransferase (3 tumors). Conversely, we found amplification of JMJD2C (7 tumors) and JMJD2B (5 tumors) through the combined analysis of our SNP array data and FISH probing of a MB tissue microarray. The JMJD2 family genes encode H3K9 demethylases, whose effect is opposite to that of EHMT1 on H3K9. The ability of EHMT1 to methylate H3K9 is blocked by acetylation. We found amplification of MYST3 (4 tumors), a histone 3 lysine acetyltransferase. We also found deletions in polycomb genes that interpret the state of H3K9 methylation including L3MBTL3 (1 tumor), L3MBTL2 (4 tumors), and SCML2 (3 tumors). Re-expression of L3MBTL3 in a MB cell line where it is homozygously deleted resulted in decreased growth, and cell cycle redistribution. Re-expression of L3MBTL3 also increased H3K9 dimethylation of E2F6 target gene promoters as demonstrated by chromatin immunoprecipitation. Additionally, retroviral-mediated over-expression of JMJD2C in cerebellar granule cell progenitors caused a reduction in the levels of H3K9 dimethylation. Copy number aberrations of genes with critical roles in writing, reading, removing, and blocking the state of histone lysine methylation, particularly H3K9, suggest that defective control of the histone code contributes to the pathogenesis of medulloblastoma.

P045

MEDIP-CHIP AND MEDIP-SEQ IDENTIFY NOVEL EPENDYMOMA TUMOUR SUPPRESSOR GENES THROUGH DELINEATION OF THE EPENDYMOMA EPIMETHYLGENOME

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Ependymoma is the third most common pediatric brain tumor and little is known about its genetic underpinning. At the genetic level, many pediatric posterior fossa ependymomas have a balanced karyotype, suggesting that they are initiated and maintained by very small copy number aberrations, small sequence changes, or epigenetic events such as DNA promotor methylation or histone modifications. We fragmented genomic DNA from 27 primary pediatric posterior fossa ependymomas, 10 normal brain samples, and 4 ependymoma xenograft cell lines and subjected them to MeDIP (methylation dependant immunoprecipitation). The IP fraction (enriched for methylated DNA) was then compared to the wash fraction (impoverished for methylated DNA) by differential labeling and hybridization to Nimblegen Promotor tiling arrays (MeDIP-ChIP). Regions of the genome in which the ratio of IP/Wash DNA was >2 in one or more tumor samples, but not in normal brain samples were identified as methylated. We identified methylation of numerous genes involved in transcriptional regulation (HOXD5, FOXD4, RASSF2), apoptosis (BMF, TNF, TRADD, BCL2L2) in addition to several other gene families. We subsequently analyzed 4/27 of the primary ependymoma samples by MeDIP-Seq using a next generation sequencing strategy. Tumor DNA was subjected to MeDIP, and then subsequently analyzed by generating >30 million short (35 bp) reads for IP and wash fractions, followed by mapping and alignment to the genome on the UCSC genome browser. Genomic regions showing significantly more reads in the IP fraction versus the wash fraction were identified. There was a significant overlap between genes identified as methylated by MeDIP-ChIP and MeDIP-Seq. MeDIP-Seq also identified a number of regions of intragenic and intergenic tumor specific methylation that are currently of uncertain significance. Our data demonstrate that MeDIP-ChIP and MeDIP-Seq are useful tools to delineate the ependymoma epimethylgenome.

P046

CLINICAL DIAGNOSIS OF OPTIC NERVE HEMANGIOBLASTOMA

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Objective To study the clinical diagnosis and treatment of optic nerve hemangioblastoma. **Methods** A 38-year-old man with solitary hemangioblastoma in intracranial segment of optic nerve who was the initial case in our country was treated in Huashan Hospital, and analyzed based on the reviews on 17 reported cases of optic nerve hemangioblastoma in the world. **Results** Optic nerve hemangioblastoma might occur in any segment of the optic nerve. The initial symptoms were progressive visual loss and visual field defect. The preoperative differential diagnosis from optic nerve gliomas or meningiomas was quite difficult. Magnetic resonance imaging could provide more information than other radiological examinations. The hemangioblastoma at the peripheral optic nerve could be removed totally with the preservation of optic nerve and vision. **Conclusion** Solitary optic nerve hemangioblastoma is extremely rare, and preoperative diagnosis is difficult. The majority of the patients with optic nerve hemangioblastoma are complicated by Von Hippel-Lindou`s disease. The only way to cure optic nerve hemangioblastoma is surgical treatment.

P047

GENE EXPRESSION META-ANALYSIS IDENTIFIES GRADING AND SURVIVAL MARKERS IN ANAPLASTIC GLIOMA

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Purpose: Although molecular analyses have been shown to be useful for classifying tumors and formulating a prognosis for patients, current methods applied to glioma are mostly based on histopathology. To search for robust diagnostic and prognostic markers of high-grade gliomas (HGGs), we applied a meta-analysis that extracts the most reliable information from the available gene expression data. **Methods:** We obtained data sets from three studies in HGGs. We selected a consensus set of 267 patients. Differential analyses were performed separately for each study and we applied a meta-analysis approach base on non-parametric rank products to evaluate the combined data. The genes selected in both analyses were used to construct a gene classifier by logistic regression modeling. We performed survival analyses on 144 patients by fitting Cox proportional hazard model. Genes identified as both differentially expressed and correlated to survival were used to build an optimal survival model. Performances were evaluated on an independent data set comprising 54 patients. Several genes were validated using RT, Q-PCR and immunohistochemistry on a local cohort of 130 patients. **Results:** This inter-study cross-validation approach generated a set of 65 genes consistently and specifically differentially expressed in GBM. Functional annotation revealed a clear association with the nervous system development and the cell communication. The genes significantly associated with grading were mostly related to the extracellular matrix. The optimal survival model was built on a four genes signature. Kaplan-Meier curves and the log rank test (training: p = 2e-11, testing: p = 1e-4) indicated the high survival prognostic potential for this classifier. Finally, applied only to GBM it clearly outperformed previous reports, by grouping GBM in two subtypes with significant different prognosis (p = 3e-4). **Conclusions:** Meta-analysis allows the identification and validation of HGGs biomarkers that might represent good candidates for novel diagnostic and prognostic approaches in HGGs.

P048

MULTIPARAMETERIC MR APPROACH FOR TISSUE CHARACTERIZATION OF PATIENTS WITH GBM

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Aim: To use a combination of conventional and advanced MR methods, in order to improve diagnosis and definition of lesion boundaries. **Methods:** Study-groups: five patients with GBM and five healthy volunteers were scanned using 3T-GE scanner. MRI protocol: included conventional imaging: T1/T2 weighted images(WI), FLAIR, GE and T1WI post contrast. In addition advanced MR methods included: diffusion tensor imaging(DTI), dynamic susceptibility contrast(DSC) and calculated T1-maps. **Data analysis:** Data from all sequences and calculated maps were realigned and co-registered into the same space. In the healthy controls, several white and gray matter volumes of interest (VOIs) were defined in the occipital lobe and the mean value of each parameter was calculated separately. In the patients, VOIs were defined from enhanced lesion and pure and infiltrative edema. Normalized ratios relative to mean values of the contra-lateral hemisphere were calculated for all images having arbitrary units (such as T1/T2WI). Each VOI was defined by a multi-parameters pattern. **Results:** Healthy-controls: different multiparametric patterns differentiated between the white and gray matter. Patients-group: different multiparameters patterns distinguished between enhanced lesion, pure and infiltrative edema. In addition, within the peritumoral edema, different compartments were obtained characterized by varying multiparametric patterns. The main differences were in their 3DT1W post contrast intensity (although both areas presented as non-enhancing tissues); in the perfusion parameters and in its T1 values. Conventional imaging failed to detect any differences between these compartments. **Conclusion:** Those finding present specific finger-prints for normal and pathologic tissues using conventional and advanced MR methods. Such an approach may provide more accurate definition of tumor boundaries regarding the extent of infiltration. If so this tool will impact differential diagnosis, planning of not only biopsy but also radiotherapy, and the follow-up of these patients.

P049

NG2 EXPRESSION ESTABLISHES A LINK BETWEEN NEURAL PRECURSORS AND CANCER STEM CELLS IN GLIOBLASTOMA

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Introduction
NG2 expressing cells (NG2+ cells) are the largest group of neural cycling precursors in the adult brain and exhibit wide anatomical distribution and proliferative activity. Based on these characteristics, we hypothesised that NG2+ve cells are potential candidates as cancer stem cells (CSCs) that maintain the glioblastoma (GBM) growth.

Methods
GBM tumour-initiating cell (TIC) lines were derived from fresh clinical samples under serum-free (SF) conditions according to our Cambridge Protocol (Fael Al-Mayhani et al.; 2009). Cells were analysed and sorted using FACS. Comparative studies on NG2+ and NG2- cells were conducted in vitro and in vivo. Molecular studies were performed using microarray and comparative genomic hybridization (CGH).

Results
Microarray data showed that genes associated with neural precursors (NG2, Olig2, PDGFRa and NKX2.2) were expressed by all GBM TIC lines and the majority (90%) of GBM tumours tested (n=147). In vitro data show that NG2+ cells isolated from TIC lines exhibit high level of growth, proliferation and clonogenic potentiality compared to NG2- cells. Only NG2+ cells were able to form tumours in vivo. Array CGH demonstrate that both NG2 populations have typical GBM cytogenetic profile (e.g. Ch7 gain and Ch10 loss), however, NG2+ cells exhibit significant additional abnormalities.

Conclusion
Genes associated with neural precursors are widely expressed by GBM tumours and GBM TIC lines. NG2+ cells isolated from TIC lines appear to conserve the precursor status of their normal counterparts and have tumourigenic characteristics in vivo. In addition, NG2+ cells exhibit significant structural chromosomal abnormalities that differ from NG2- cells and among different samples reflecting GBM heterogeneity. These data indicate that NG2 can be used as CSCs marker in GBM and draw attention to possible clinical implications (for more details see abstract by Watts C. et al.)

P050

UNUSUAL CASE OF RADIATION-INDUCED GROWING TERATOMA SYNDROME

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Introduction
Intracranial Germ cell tumors (GCT) are rare. The mixed varieties require a multimodality therapy including chemotherapy. While on chemotherapy, radiologically progressing masses with decreasing tumor markers have been found to be teratomas . Such an occurrence while on radiotherapy is unusual and never reported before.Case

A 5-year-old boy diagnosed with a posterior third-ventricular mass underwent endoscopic biopsy and ventriculostomy. With a diagnosis of a pineal parenchymal tumor he was commenced on radiotherapy. After the first ten fractions (18Gy) he had progressive neurological deterioration. Repeat CT and MR were highly suggestive of a teratoma and he underwent emergency surgical excision .histology revealed features of a mature teratoma. He had a stormy postoperative course succumbing to deep venous infarction.Discussion

Intracranial mixed GCTs usually require multimodality therapy inclusive of chemotherapy which is usually effective for the malignant elements. The mature teratomatous components however being resistant, may persist and occasionally grow rather rapidly , a phenomenon described as Growing Teratoma Syndrome (GTS). 7 such cases have been reported. None of these however had this whilst on radiotherapy. Our patient had an erroneous diagnosis (a known limitation with sterotactic/endoscopic biopsy methods) for which radiotherapy was given leading to this occurrence. Whether this was a result of the radiotherapy or part of the natural history of this tumor remains conjectural. absence of any immature/ malignant transformation on histology coupled with the known indolent history of mature teratomas, most probably suggests a case of radiotherapy-induced GTS, much like the chemotherapy-induced GTS. This report also reiterates the fact that mature teratomas are best treated surgically, with favourable outcomes provided lesions are small and detected early before neurological compromise.

P051

CAUSES AND CORRELATES OF DISTRESS AND MAJOR DEPRESSION IN HIGH-GRADE GLIOMA

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Purpose To study causes of distress and how it relates to depression in malignant glioma, to help optimise future supportive and psychological care. **Methods** We studied 50 adults with histologically proven high-grade glioma attending two tertiary referral centres at the start of their radiotherapy. All patients completed the NCCN distress thermometer and received a clinical interview to diagnose DSM-IV Major Depressive Disorder (MDD). The frequency and causes of distress and its associated clinical characteristics were surveyed. Data were analysed with respect to clinical variables, including MDD, using Fishers exact test. **Results** Distress was high in 23 subjects (46%) and severe in 8 (16%). Mean distress score was 3.5 (SD 2.7; 95% CI 2.7-4.3). The commonest problems were physical: getting around (30%), fatigue (26%) and sleep (24%); and emotional: worry (22%), anger (20%) and sadness (12%). Distress scores were higher in patients with MDD (p=0.001). However, depressed glioma patients tended not to complain specifically of depression. Emotional sources of distress were more common in patients with MDD (82% vs 38%; p=0.02), and in women (74% vs 32%; p=0.008). There were no other statistically significant differences in distress score or source in respect of MDD, age, sex, physical function, cognitive status or presence of epilepsy. **Discussion** Distress scores vary widely in patients with high-grade glioma at the start of radiotherapy. Perhaps surprisingly, most patients (54%) report low distress. Women and depressed patients report more emotional problems, but the broad causes of distress otherwise seem similar across different groups. Anger is surprisingly prominent and may easily be missed unless enquired about.Overall distress score, and the presence of emotional sources of distress, may aid in deciding which patients to screen for MDD. Assessing only those patients who complain of depression risks missing cases. A larger sample size may clarify these findings.

P052

BEVACIZUMAB PLUS ERLOTINIB IN RECURRENT HIGH-GRADE GLIOMA: A PHASE II TRIAL

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Background: Bevacizumab (B), a neutralizing VEGF monoclonal antibody, has anti-glioma activity as single agent and in combination with cytotoxic therapy. Erlotinib (E), an EGFR tyrosine kinase inhibitor, may exhibit anti-tumor activity in some high-grade glioma (HGG) patients. B plus E was associated with clinical benefit in several cancers. We performed a single-arm, phase II trial to assess the efficacy and safety of B and E in patients with recurrent HGG. **Methods:** The primary endpoint was 6-month progression-free survival (PFS-6). Radiographic response, pharmacokinetics and correlative biomarkers were secondary endpoints. E was orally administered daily at 200 mg/day for patients not on enzyme-inducing anticonvulsants (EIAC) and 500 mg/day for patients on EIAC. All patients received 10 mg/kg of B intravenously every two weeks. **Results:** Fifty-six patients with recurrent HGG (n=24 for glioblastoma multiforme [GBM] and n=32 for anaplastic gliomas [AGs]) were assessable for outcome. The PFS-6 rates were 25% for GBM and 50% for AGs. There was no survival difference between EIAC and non-EIAC groups. Rash (54% grade 1-2 and 38% grade 3) was the most common side effect. Fatigue, nausea and diarrhea were also common but mostly grade 1-2. Serious side effects were rare and included two patients with pulmonary embolism, single patients with either intestinal perforation, ischemic stroke, gastric bleeding or nasal septal perforation. Pharmacokinetic and tissue biomarker profiles are in preparation. **Conclusions:** Combination of bevacizumab and erlotinib is tolerated and associated with anti-tumor benefit among heavily pretreated recurrent high-grade glioma patients.

P053

PATIENT FUNCTIONAL STATUS IS STRONGEST CORRELATE OF CHALLENGING BEHAVIOUR AFTER BRAIN TUMOUR

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Purpose: Few studies have documented behavioural disturbance, impairments in executive functioning as well as emotional/social dysfunction (as distinct from mood disturbance) associated with brain tumour (BT). This study investigated (i) the prevalence of such challenging behaviours (CBs) and (ii) potential correlates among patient, tumour, treatment variables and functional status. **Methods:** A consecutive series of 54 patients with BT recruited from two Sydney-based hospitals completed a series of self-report surveys documenting patient impairment. Thirty seven carers derived from this series also participated. Measures of CBs included the Frontal Systems Behaviour Rating Scale (FRSBE), Overt Behaviour Scale (OBS), and Emotional/Social Dysfunction Questionnaire (ESDQ). Functional measures included the Sydney Psychosocial Reintegration Scale (SPRS; self-report scale documenting function in occupational, relationship, and independent living domains) and clinician-rated Karnofsky performance scores (KPS; 31% at 90-100; 34% at 80; 35% at 50-70). **Results:** Median patient age was 51 years (range 18-91) and time since diagnosis was 4 months (range 1-82). Patients had high grade (39%), low grade (22%) or benign tumours (39%) and 60% underwent radiotherapy. Patient self-report found prevalence rates of CBs ranging from 17% - 50% across various domains including verbal aggression, physical aggression, lack of initiation, disinhibition, apathy and executive impairments. No consistent pattern of correlates was found between CBs (patient or carer report) and patient demographics, tumour features (benign versus malignant diagnosis, grade, location), treatment timing or modality (surgery, radiotherapy or chemotherapy). However, functional status (Karnosfsky and SPRS scores) demonstrated significant correlations (.30-.50 range) with various carer-rated CB variables including FRSBE apathy and executive impairment sub-scales; ESDQ emotional dysfunction, fatigue, inappropriate behaviour, and poor insight sub-scales, and OBS total score. **Conclusions:** Patients functional status may be the most useful indicator of challenging behaviours after brain tumour. Karnofsky scores could be linked to further screening questions to help identify and subsequently manage challenging behaviours in brain tumour patients.

P054

DETAILED PROSPECTIVE NEUROPSYCHOLOGICAL, ACTIVITY OF DAILY LIVING AND ENDOCRINE FUNCTION ASSESSMENT IN CHILDREN WITH CRANIOPHARYNGIOMA TREATED WITH STEREOTACTIC CONFORMAL RADIOTHERAPY

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Purpose: We report prospective neuropsychological and neuroendocrine function in young patients with residual/recurrent craniopharyngioma treated with stereotactic conformal radiotherapy (SCRT). **Material and method:** Eighteen patients (median age 14 years) treated with SCRT underwent prospective detailed serial evaluation of neurocognitive, Barthel index (BI), LOTCA and neuroendocrine function. **Result:** Among eighteen patients (14 males, 4 females; mean follow up 28.4 months), 16 (89%) were controlled and two (11%) patients died with disease progression. At pre-RT, 16 (89%) patients had full scale IQ (FSIQ) below normal. Pre-RT mean verbal IQ (VQ), performance IQ (PQ) and FSIQ were 74.12, 82.42 and 76.53 respectively. Mean FSIQ at 6 month and 2 year were 80.5 and 77.7; PQ score 87.5 and 86.6; VQ score 86.33 and 82.88 respectively. Mean trait anxiety (C2) score was poor at baseline (29.67) and improved to 21.83 at 2 year follow-up. Mean BI score at baseline, 6 month and 2 year were 95.64, 99.81 and 100 respectively. LOTCA scores were maintained at 2 year in orientation, spatial-perception, thinking and attention concentration domain. At baseline, 83.3% (15) patients had hormone deficiency in at least one axis. Growth hormone, corticosteroid, thyroid and sex hormone axis impairment were in 67% (12), 61% (11) 33.3% (6) and 5% (1) patients respectively. Pre-RT BI score was significantly lower in visually handicapped ($p=0.007$), low KPS score (0.004), poor neurological function status (NPS) ($p=0.014$) and in patients with severe hydrocephalus ($p=0.031$). FSIQ score was significantly lower in patients with thyroid replacement ($p=0.05$). LOTCA scores were lower in visually impaired ($p=0.071$) and in patients with poor neurological function ($p=0.021$). **Conclusion:** Craniopharyngioma patients have poor neuropsychological and endocrinal function even before starting RT. However, there is no further decline upto 2 years follow-up. Factors other than RT such as tumor, patient-related factors and surgery may influence baseline neurocognitive function.

P055

HUMAN NEURAL STEM CELLS CAN TARGET AND DELIVER THERAPEUTIC GENES TO BREAST CANCER BRAIN METASTASES

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The tumor-tropic properties of neural stem cells (NSCs) led to the development of a novelstrategy for delivering therapeutic genes to tumors in the brain. To apply this strategy to thetreatment of brain metastases, we made a human NSC line expressing cytosine deaminase(F3.CD), which converts 5-fluorocytosine (5-FC) into 5-fluorouracil, an anti-cancer agent. In vitro,the F3.CD cells significantly inhibited the growth of tumor cell lines in the presence of the prodrug5-FC. In vivo, MDA-MB-435 human breast cancer cells were implanted into the brain ofimmune deficient mouse stereotactically, and F3.CD cells were injected into the contralateralhemisphere followed by systemic 5-FC administration. The F3.CD cells migrated selectively intothe brain metastases located in the opposite hemisphere and resulted in significantly reducedvolumes. The F3.CD and 5-FC treatment also decreased both tumor volume and number oftumor mass significantly, when immune deficient mouse had MDA-MB-435 cells injected into theinternal carotid artery and F3.CD cells were transplanted into the contralateral brain hemispheresterotactically. Taken together, brain transplantation of human NSCs, encoding the suicideenzyme CD, combined with systemic administration of the pro-drug 5-FC, is an effectivetreatment regimen for brain metastases of tumors.

P056

STEROID REQUIREMENTS DURING RADIOTHERAPY FOR MALIGNANT GLIOMAS

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Objective: To assess the steroid requirements during Radiation Therapy (RT) in patients with malignant gliomas.

Introduction: Radiotherapy is the mainstay of treatment for high-grade gliomas. However, even with optimal RT regimen, toxicity may develop, such as brain oedema and worsening of neurological symptoms. Steroids are currently used for the management of these complications. Steroid requirement has been mainly described in patients with brain metastasis, but not in adult patients with malignant gliomas.

Design/Methods: We evaluated prospectively all patients with malignant gliomas addressed for RT to Avicenne Hospital from January 2007 to September 2008. Age, sex, initial Karnofsky performance status, tumor localization and histology, type of surgical resection, clinical target volume, total dose and duration of RT, concomitant treatment with temozolomide and steroid dosage during RT and at 1 and 3 months after RT, were recorded in all patients.

Results: Out of 49 patients, 27 (55%) required instauration or increase of steroids during RT because of headache, vomiting, seizures, neurological decline or intracranial hypertension. The median time to steroid increase was 5 days. 31% of patients were free of steroids at the beginning of RT, 14% by the end of RT and 33% at 3 months after RT. The median dosage of prednisone was 40 mg during RT and 20mg 3 months after RT. Poor performance status, biopsy, concomitant TMZ and young age, but not clinical target volume, total dose and duration of RT, were associated with higher dosage of steroids.

Conclusions/Relevance: In our series, half of the patients required instauration or increase of steroids during RT. Poor performance status, young age, biopsy and combination of temozolomide to RT seem to be factors that require more steroids.

P057

MUTATIONAL PROFILING OF HIGH GRADE GLIOMAS REVEALS INACTIVATING MUTATIONS IN IDH1

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High grade gliomas (HGG) are malignant brain tumors for which no cure is available. To identify new therapeutic targets we performed an extensive mutation analysis. We sequenced 39 genes in 113 HGG (including 109 glioblastoma) tumor samples and 16 HGG cell lines. We analysed 200 exons belonging to 35 kinases, isocitrate dehydrogenase 1 (IDH1), NRAS, PTEN and TP53 genes. At least one somatic mutation was found in 90 out of 129 HGG samples (70%). Most mutations were observed in genes belonging to the PI3K-AKT pathway; in more than 45% of HGGs we detected a mutational activation of the pathway, indicating that the PI3K-AKT axis represents a relevant therapeutic target for HGG. In addition, five different types of somatic mutations affecting the IDH1R132 residue were detected in 20% (23 of 113) HGG tumor samples. IDH1 mutations were predominantly observed in secondary glioblastoma (11 of 94 vs. 11 of 15, p-value =0.0000016). It has been established that IDH1 mutations occur early in gliomagenesis and that the overall survival in both primary and secondary glioblastoma is better in patients with IDH1 mutations. However, it remains to be determined whether the mutations functionally activate or inactivate the IDH1 enzymatic activity. To address this issue we performed metabolic mapping (enzyme histochemistry) on wild-type and mutated HGG samples. Our initial results suggest that IDH1 mutations are inactivating. These data have relevant implications to exploit IDH1 mutations for diagnostic, prognostic and therapeutic purposes.

P058

GENOMIC PROFILING OF INTRACRANIAL GERM CELL TUMORS WITH SINGLE NUCLEOTIDE POLYMORPHISM ARRAY

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Intracranial germ cell tumors (GCTs) are relatively rare brain tumors affecting primarily adolescents. Although the survival rate of pure germinoma is excellent (>90%), optimal volume and dose of radiation or the role of chemotherapy is not clear, and the risk factors of relapse is unknown. In contrast, the outcome of nongerminomatous germ cell tumors (NGGCTs) is poor (30-40% survival) and the standard treatment for NGGCTs remains controversial. The lack of progress in improving the outcome of these patients is due to our poor understanding of the biology of intracranial GCTs. To better characterize underlying genetic alteration of intracranial GCTs, copy number aberration (CNA) analysis using genome-wide genotyping microarray was performed.

We have analyzed the DNA samples of 55 intracranial GCTs by GeneChip Human Mapping 100K Array. After applying stringent quality control criteria, 30 cases with both tumor and matched blood samples (18 pure germinomas and 12 NGGCTs) were selected for further analysis using. the Copy Number Analyzer for GeneChip (CNAG).

Overall, copy number gains are observed more frequently than losses. The most frequently observed CNAs include gain of chromosomes 1q, 2, 7, 8, 12p, 21, and X as well as loss of chromosomes 11q and 13. Validation of these results are ongoing using real time quantitative PCR method. The CNAs which are specific to histologic subtypes were also identified. Loss of choromosomes11q and 13 were specific to germinomas. Gain of chromosome 2 was observed more frequently in germinomas, whereas gain of chromosomes 1q and 12p were more frequently observed in NGGCTs.

We identified multiple frequently altered loci of intracranial GCTs genome. Similar CNAs have been observed in intracranial GCTs as well as extracranial counterparts. These findings will serve as the focus of further search for the genes involved in tumorigenesis of intracranial GCTs.

P059

PRESENCE OF AN ALTERNATIVE LENGTHENING OF TELOMERE (ALT) MECHANISM AS A FAVORABLE PROGNOSTIC MARKER IN PATIENTS WITH GLIOBLASTOMA

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Objectives: During proliferation of normal human somatic cells, telomeres progressively shorten, leading eventually to senescence. However, the majority of cancers evade this by activating either telomerase or the alternative lengthening of telomeres mechanism (ALT) to prevent telomere shortening. Why ALT, rather than telomerase, is switched on in certain tumors is unknown and its presence has been associated with good and bad prognosis, depending on tumor type. Our goal in this study was to examine whether the presence of ALT in GBM tumors has prognostic significance. **Methods:** We performed a retrospective review and analysis of 432 cases of GBM diagnosed from 1998-2007 at Royal North Shore and North Shore Private Hospitals, Australia and Auckland Hospital, New Zealand. To assess ALT, ALT-associated promyelocytic leukemia (PML) bodies (which are a known hallmark of ALT) were detected by combined PML immunofluorescence and telomere fluorescence in situ hybridization. **Results:** Of the 432 cases, 61 patients received surgery only, 110 patients were postoperatively treated with radiotherapy (RT), while 261 patients were postoperatively treated with RT plus chemotherapy administered either concurrently with RT or post RT. ALT was detected in tumors from 61 patients (14.1%). In all treatment arms, ALT positive patients displayed longer median survival when compared to patients without ALT: No treatment: 3.8 months compared to 2.2 months, p=0.696; RT only: 10.1 months compared to 7.3 months, p=0.026; RT + Chemotherapy: 17.8 months compared to 13.9 months, p=0.051. Approximately 33% of ALT positive patients survived more than 2 years post diagnosis as opposed to 12% of all ALT negative patients treated with RT and chemotherapy. **Conclusion:** The presence of ALT in GBM tumors correlates with patient survival. ALT positivity in GBMs not only predicts better outcome for these patients but also identifies a subgroup that will not benefit from telomerase inhibitors.

P060

LONG TERM FOLLOW UP OF PATIENT WITH ACROMEGALY AFTER LINAC RADIOSURGERY

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Purpose The authors retrospectively reviewed the long term outcome of linear accelerator radiosurgery for patients with acromegaly in our hospital in the view of biochemical remission and further analyzed the associated factors. **Materials and Methods** We reviewed patients who received LINAC radiosurgery between 1994 and 2004 due to residual or recurrent growth hormone (GH)-secreting functional pituitary tumor in our hospital. All patients were followed &gt 3 years. The residual or recurrent of the tumor were defined persisted high level of growth hormone or insulin-like growth factor-1 (IGF-1). The biochemical remission was defined as basal growth hormone &lt 5ng/ml with a normal sex-and-age adjusted IGF-1. **Results** Total 22 patients were included in our reviewed. The mean follow up after radiosurgery was 94.68 months.The overall mean biochemical remission time was 49.2 months (median 42 months). Nineteen patients (86.4%) achieved the biochemical remission throughout the follow up period. The 3, 5, 8 years biochemical remission rate were 36.4%, 54.5% and 72.7% respectively. The inital GH at diagnosis and the pre-SRS GH correlated with biochemical remission (p=0.005, p&lt 0.0001). Overall post SRS hormone deficit was in 5 patients (22.7%). **ConclusionIn** comparison to other radiosurgery facilities, LINAC radiosurgery also provided a competent outcome. SRS has maximun effect in the first two years. Moreover, SRS showed long term biochemical effects and needs longer follow up for better biochemical remission.

P061

DO MEDULLOBLASTOMAS IN ADOLESCENTS HAVE A DISTINCT MOLECULAR SIGNATURE & DIFFERENT TUMOR BIOLOGY?

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Purpose
Incidence of invasive cancer rises from adolescence (SEER Data). Brain tumor types change with age; embryonal brain tumors become less common in adolescence (CBTRUS data). Our hypothesis is that medulloblastomas (MB), an embryonal tumor, is biologically different in adolescence due to differences in host biology. Adolescence is defined as >/=9, </=15years in this study.

Methods
mRNA expression profiles of 71 MB and 6 normal brain specimens were characterised using Affymetrix HG-U133plus2.0 Gene-Chips. Unsupervised hierarchical clustering identified 4 clusters. Cluster C comprised of 6 normal brain tissues and 1 tumor (non-adolescent). Class comparison was performed between adolescents and non-adolescents, between clusters to establish significant differences in gene expression (using FDR=0.001, CI=95%). Differentially expressed genes were categorized in GeneOntology groups using the DAVID Tool.

Results
Adolescents accounted for 44.1% (15/34) of Cluster D, Cluster A (6/27), Cluster B (0/9), p=0.019 (Fisher,s Exact Test). There were 21 adolescents; 71.4% of adolescents have Cluster D signature. Cluster D has 3 subgroups; majority of adolescents occurred in Subgroup D1 (11/20), D2 (1/11), D3 (3/3).
No significant difference in gene expression of adolescent MB compared to non-adolescents across clusters.
Tumor Clusters A, B, D compared among each other; Cluster A significantly overexpressed genes involved in brain development, organ, cellular, neurite morphogenesis, neurogenesis and axon guidance, compared to Cluster D (p=0.0000007-0.04).
Class comparison between Subgroup D1 and D2 revealed 153 significantly differentially expressed genes. D1 significantly underexpressed genes of light perception (p=0.0008), pathways (p=0.0004-0.001) involved in T-cell receptor (TCR) locus.

Conclusions
Adolescent MB have different molecular signatures, majority showing the D1 signature, underexpressing genes of light perception and TCR as compared to D2. The presence of T-cells and its potential significance in the prognosis of MB is being explored.

P062

ABERRANT EXPRESSION OF GDF-15 CONTRIBUTES TO PROLIFERATION AND IMMUNE ESCAPE OF MALIGNANT GLIOMAS

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Growth and differentiation factor (GDF)-15 is a member of the transforming growth factor (TGF)-beta family. Its biological function has been linked to neuronal survival and tumor cell apoptosis. Here we demonstrate that glioma patients display elevated serum levels of GDF-15 compared to healthy controls. GDF-15 mRNA and protein are also expressed in human glioma cells in vitro. Suppression of GDF-15 expression by RNA interference reduces the proliferation of malignant glioma cells. Intriguingly, GDF-15-depleted glioma cells are more susceptible towards NK and T cell-mediated cytotoxicity. Moreover, GDF-15-deficient glioma cells are less tumorigenic than control cells in syngeneic mice in vivo. GDF-15 is thus a novel aberrantly expressed molecule which contributes to proliferation and immune privilege of human malignant gliomas.

P063

PERSONALIZED CHEMOTHERAPY BASED ON O6-METHYLGUANINE-DNAMETHYLTRANSFERASE (MGMT) EXPRESSION PATTERN FOR GLIOMA PATIENTS: EXPERIENCE OF 57 CASES

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There were 57 malignant glioma patients (anaplastic oligodendroglioma, AO 3 cases, anaplastic astrocytoma, AA 36 cases, glioblastoma multiforme, GBM 18 cases) received chemotherapy in Cancer Center of Sun Yat-sen University, based on expression of O6-methylguanine-DNAmethyltransferase (MGMT) and response as well as survival time were evaluated. In which 36 were recurent cases, and 21 were initial chemotherapy. Twenty patients had received previous chemotherapy and 37 cases without previous chemotherapy. All the pateients had received radiotherapy before. Thirty-five patients with MGMT positive tumors received no-alklating agents chemotherapy regimen, which consisted of teniposide (VM-26), cisplatin (DDP), carboplatin(CBP), isophosphamide(IFO), etoposide(VP16), or cisplatin plus TMZ regimen. While 22 patients with MGMT negative tumors, there was no restriction on chemotherapy regimen. That was eigther nitrourea or TMZ had been used in the regimen, and the most commonly used regimen were PCV;TMZ+VM26; MeCCNU+VM26. There was no significant difference on objective response (OR) or response rate (RR)between MGMT positive and negative patients (p>0.05). By following up 0.7-53.4 months(mean 11.7 months), progressive-free survival (PFS) in MGMT negative and positive patients were 8.5 months(95% CI 4.8-19.3) and 6.7 months(95% CI 3.7-9.3), and overall survival(OS) were 20.3 months (95% CI 14.3-) and 16.1 months (95% CI 11.1-26.2, respectively. (p>0.05). Our results indicated that personized chemotherapy for glioma patients based on MGMT expression can obtain satisfactory results espercially for patients with MGMT positive gliomas.Key words: chemotherapy, glioma, O6-methylguanine-DNAmethyltransferase

P064

CDK4/6 SMALL MOLECULE INHIBITOR PD-0332991 DEMONSTRATES ANTI-TUMOR ACTIVITY AGAINST AN INTRACRANIAL GLIOBLASTOMA XENOGRAFT LACKING p16 FUCNTION

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PURPOSE: The purpose of this study was to achieve a preliminary assessment of whether glioblastomas (GBMs) with p16 homozygous gene deletions (approximately two-thirds of all GBM) are responsive to cyclin dependent kinase (CDK) 4 and CDK-6 small molecule inhibition, in the context of an orthotopic GBM xenograft therapy response model. **METHODS:** Luciferase-modified U87 GBM cells, which lack p16 function, were injected into the brains of athymic nu/nu mice that were randomized to CDK4/6 inhibitor PD-0332991 vs. vehicle only treatment groups, with oral administration of inhibitor 1x daily (150 mg/kg) for four consecutive weeks, beginning at day 13 subsequent to tumor cell injection. All animals were monitored 1-2x weekly using bioluminescence imaging (BLI) to assess tumor response to therapy, and each animal was followed until presentation of neurological symptoms indicative of excessive tumor burden that requires euthanasia. **RESULTS:** BLI monitoring revealed sustained anti-tumor activity for the entire period of therapy administration, which was followed by rapid tumor growth after completing the 4 week CDK4/6 inhibitor administration regimen. CDK4 inhibitor extended median survival by 18 days (60%) relative to vehicle treatment, with mean survival extended by greater than 19 days. One CDK4/6 inhibitor treatment group mouse showed no evidence of intracranial tumor following completion of treatment, and was euthanized at day 100 following tumor cell injection. Comparison of MIB-1 staining in tumor from the brain of one mouse that was sacrificed while on therapy vs. the MIB-1 staining in tumor from a control (untreated) group mouse revealed a greater than 8-fold reduction in tumor MIB-1 positivity resulting from CDK4/6 inhibitor treatment. **CONCLUSIONS:** Our results indicate that oral administration of CDK4/6 inhibitor PD-0332991 results in substantial anti-proliferative activity against an orthotopic GBM xenograft lacking p16 function, and support further investigation of this therapeutic for treating patients with GBM.

P065

PATIENT-SPECIFIC VIRTUAL CONTROLS CAN BE USED TO SIMULATE AND PREDICT RESPONSE TO RADIATION THERAPY IN INDIVIDUAL GLIOBLASTOMA PATIENTS

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Patient-specific virtual controls (VCs) are created for 26 patients with histologically diagnosed glioblastoma multiforme allowing testing and validation of a biomathematical model for glioma response to radiation therapy (RT). Individualized glioma growth kinetics, described by the mathematical model parameters quantifying net rates of invasion and proliferation of glioma cells, are determined from serial magnetic resonance imaging prior to treatment for each patient[1]. Knowledge of these patient-specific growth kinetics allows for simulation of untreated VCs for each patient against which therapeutic response can be assessed. Serial post-treatment imaging is used to assess response to RT. The net proliferation rate is strongly correlated (r = 0.84, p &lt 0.01) with the radiation efficacy (&alpha from the classical linear-quadratic radiobiology model). This correlation was tested as a means of predicting radiation efficacy from the pre-treatment proliferation rate using a leave-one-out-cross-validation analysis yielding a mean error between simulated and actual post-RT tumor radius of 3 mm. In a survival analysis, pre-treatment growth kinetics and radiation response metrics was a significant predictor of survival even when controlling for the prognostic value of recursive partitioning (RPA) class established by the radiation therapy oncology group (RTOG). The fundamental result from the investigation is the ability to predict response to therapy prior to treatment using a variety of clinically established and novel biomathematical model-based metrics. Ultimately, directing treatment with knowledge of VCs allows for improvement in the design of individualized patient therapies, such as RT, as well as a novel technique to assess response for any therapies since an untreated VC provides comparison for the actual tumor behavior. [1] HLP Harpold, EC Alvord, Jr, KR Swanson: The Evolution of Mathematical Modeling of Glioma Growth and Invasion. Journal of Neuropathology and Experimental Neurology, 66(1):1-9, 2007

P066

AUTOPHAGY INDUCED BY VALPROIC ACID IS ASSOCIATED WITH OXIDATIVE STRESS IN GLIOMA CELL LINES

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Autophagy represents an alternative tumor-suppressing mechanism to overcome the dramatic resistance of malignant gliomas to radiotherapy and proapoptotic related chemotherapy. In this study, we reported that valproic acid (VPA), a widely used anti-epilepsy drug, induces autophagy in glioma cells. Autophagy, crucial for VPA-induced cell death, is independent of apoptosis, even though apoptotic machinery is proficient. Oxidative stress induced by VPA occurs upstream of autophagy. Oxidative stress also activates the ERK1/2 pathway, whereas blocking ERK1/2 pathway inhibites autophagy and induces apoptosis. VPA-induced autophagy can not be alleviated by inositol, suggesting a different mechanism from lithium. Moreover, VPA potentiates autophagic cell death, but not apoptosis, when combined with other autophagy inducers such as rapamycin, Ly294002 and temozolomide in glioma cells, which may warrant further investigation toward possible clinical application in patients with malignant glioma.

P067

MANAGEMENT OF PITUITARY ADENOMA WITH STEREOTACTIC RADIOTHERAPY AT PRINCESS MARGARET HOSPITAL.

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Purpose: To evaluate local control and toxicity for pituitary adenomas treated with stereotactic radiotherapy (SRT).

Methods: Patients with pituitary adenomas referred and treated 1997-2007 were retrospectively reviewed (n=83). Median age was 47 years (range: 14-73), with 46 males and 37 females. Twenty patients had functional and 63 had non-functional tumors. Median follow up was 42 months (range: 1-137). Two patients received SRT as their primary treatment, 38 received it postoperatively and 9 for raised hormones. Thirty-four patients received SRT for radiological progression despite prior surgery with median time to progression following surgery being 12 months (range 1-275). Before SRT, hormone replacement therapy was observed in 37% (thyroid), 35% (cortisol), and 30% (testosterone, males only). SRT dose was 50Gy in 25 daily fractions using the GTC frame, and CT-MR fusion for planning (Radionic™). The GTV and sella contents were treated, with no expansion from CTV for PTV margin. The prescription guideline was > 95% coverage of the CTV by a minimum dose of 47.5 Gy, and maximum dose < 52.5 Gy.

Results: The 3-year progression free survival for functional and non-functional adenomas was 94% and 92% respectively (p=0.90). Four patients had progression (3 nonfunctional and 1 functional); among these, 2 had metastatic spread. One patient had salvage excision, 1 had radiosurgery, 1 patient required temozolamide for lepto-meningial disease and 1 required palliative radiation to treat lumbar bony metastases. Post SRT 43 patients (52%) had hypothyroidism, 35 (42%) required cortisol and 20 (24%) required testosterone. 1 patient had severe optic neuropathy. To date there were no second cancers.

Conclusion: Though with a relatively short follow up, this study suggests fractionated stereotactic radiotherapy with a narrow margin is safe and effective for the treatment of pituitary adenomas.

Reference and Updating Password: doctor

P068

GENOMEWIDE ANALYSIS OF LOSS OF HETEROZYGOSITY IN GLIOBLASTOMA USING THE SNP MAPPING ARRAY AND THE PCR WITH MULTIPLE MICROSATELLITE MARKERS

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[PURPOUSE] Loss of heterozygosity (LOH) analysis is an effective approach to classify the malignant gliomas. Recent development of genomewide analysis revealed that the LOH extent could have different consequences for clinical courses. Thus, LOH analyses with limited numbers of markers cannot evaluate LOH status accurately. **[MATERIALS and METHODS]** We analyzed LOH status of 92 malignat gliomas (61 glioblastoma (GBM), 16 anaplastic astrocytoma (AA), 15 anaplastic oligodendroglioma (AO)) using the PCR with multiple microsatellite markers. Furthermore, we applied 50K SNP mapping array for comprehensive analysis of LOH status in 14 glioblastomas. For the conventional PCR analysis, we set six markers for 1p region, ten for chromosome 10 (ch10) and four for 19q region, respectively. We distinguished between partial and total LOH for 1p and ch10 region. **[RESULTS]** Total loss of ch10 was observed in 38 GBMs (66%) and 4 AAs (25%). All AO cases except two cases showed 1p total loss combined with 19q loss. In contrast, only seven GBM cases showed 1p total loss. Moreover, ten GBM cases showed 1p partial loss. Only GBM with 1p total/ 19q loss showed favorable overall survival compared with the others. SNP array analysis can detect both copy number and allelic imbalance simultaneously. In total, we identified 254 LOH loci in 14 GBM cases. Interestingly, 143 out of 254 loci (56.3%) showed copy number neutral LOH (cnLOH). Thus copy number analyses such as FISH and CGH may underestimate LOH loci compared with SNP array analysis. The PCR analysis with multiple mirosatellite markers is accurately detected LOH loci identified by SNP array analysis. **[CONCLUSIONS]** The PCR with multiple microsatellite markers is practically useful approach to evaluate LOH status. LOH pattern and cnLOH should be considered for accurate LOH analysis.

P069

MOLECULAR ALLELOKARYOTYPING OF MALIGNANT GLIOMA BY HIGH-RESOLUTION SINGLE NUCLEOTIDE POLYMORPHISM (SNP) OLIGONUCLEOTIDE GENOMIC MICROARRAY

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Purpose: Glioma is a CNS tumor resulting from accumulation of genetic alterations. Whole genome analysis clarifying the correlations between genetic abnormalities and clinical features in gliomas may provide a new strategy for determining prognosis and treatment of glioma patients. **Patients and Methods:** A robust technology, single nucleotide microarray (SNP-chip) was used for the whole genome analysis in all grade gliomas. We examined 73 samples with GBM, 58 with grade III glioma, 18 with low grade glioma and their matched blood samples at 50000/250000 SNP sites using an SNP-chip platform. Correlations between genetic alterations and prognosis in malignant gliomas were also analyzed. In this analysis we focused on several genetic alterations such as loss of heterozygosity (LOH) of chromosome 10, 1p, 19q, amplifications sites of PI3KC2B, PDGFRA, EGFR and LOH of CDKN2B locus on chromosome 9p21, p53 locus on 17p13, RB locus on 13q14. **Results:** Frequent changes detected in GBM were LOH of Chromosome 10 (49/73, 67%) and LOH of CDK2B locus (41/73, 56%). On the other hand LOH of 1p or 19q was frequently found in anaplastic oligodendroglioma (20/21, 95%) or oligo-astrocytoma (11/22, 50%). Amplification of EGFR or PDGFRA was found in approximately 30~40% of GBM cases but none in pure oligodendroglioma (grade II & grade III). LOH in 17p13 (p53) locus was equally found in grade III glioma and GBM (15/58, 26% in grade III, 18/73 25% in GBM). LOH in 13q14 (RB) locus was found in 22% (16/73) of GBM. Multiple chromosome alterations (at least 5) were dismal prognosis factor in GBM and 1p and 19q LOH was good prognosis factor in grade III glioma. No Genetic alteration was found in low grade gliomas. **Conclusion:** Although there are various genetic alterations, common genetic alterations were observed in all grade gliomas.

P070

AN ANOCEF GENOMIC AND TRANSCRIPTOMIC MICROARRAY STUDY OF THE RESPONSE TO RADIOTHERAPY OR TO ALKYLATING FIRST-LINE CHEMOTHERAPY IN GLIOBLASTOMA PATIENTS.

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Context: The molecular characteristics associated with the response to treatment in glioblastomas remain largely unknown. Methods: Here, we compare the gene expression (n=56) and genomic profiles (n=67) of responders and non-responders to either first-line chemotherapy or radiation therapy alone. Results: We show that the characteristics associated with treatment response are different according to the type of treatment received. In patients treated with radiation therapy alone, the response was associated with the differential expression of microenvironment-associated genes, where the expression of immune genes was associated with prolonged progression-free survival, whereas the expression of hypoxia-related genes was associated with early relapse. In patients treated with first-line chemotherapy, we found a significant association between the treatment response and p16 locus deletions, in particular in patients without MGMT promoter methylation. This association was further validated in an independent data set. Our results demonstrate that, according to the tumor genomic characteristics, some patients benefit more from chemotherapy and others more from radiotherapy. Conclusion: These results encourage the combination of both treatment strategies to increase the likelihood of glioblastoma response and suggest that further work could identify specific markers to predict chemo- or radiotherapy efficacy.

P071

SELECTIVE EXPRESSION OF A SUBSET OF NEURONAL GENES IN OLIGODENDROGLIOMAWITH CHROMOSOME 1P LOSS

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Gliomas are classified based mainly on microscopic resemblance to their presumed glial origin such as astrocyte and oligodendrocyte. However, more objective diagnostic criteria are indispensable for the precise treatment of patients. For instance, loss of the short arm of chromosome 1 (1p) in oligodendrogliomas is recognized as an important marker for better response to chemotherapy and longer survival of the patients. To gain insight into their molecular biological background and to identify genes characterizing each subgroup, we investigated gene expression profile of the 4 glioma subsets, oligodendroglioma with and without 1p loss, diffuse astrocytoma and glioblastoma using DNA microarray. Remarkably, most of the genes showing distinctive expression in oligodendroglioma with 1p loss were also highly expressed in normal brain tissues and had neuron-related function, which included MYT1L, INA, RIMS2, SNAP91 and SNCB. Histological analysis also demonstrated that MYT1L, which were abundantly expressed in normal neuron, were certainly present in tumor cells. These results suggest that oligodendroglioma, especially with 1p loss, has more or less neuronal characteristics although oligodendroglioma is thought to originate form glial lineage cell. With further pathological studies, those neuron-related genes might be good diagnostic markers for oligodendroglioma of better prognosis as well.

P072

LOSS OF HETEROZIGOSITY AT 1p-19q INDUCES A GLOBAL CHANGE IN OLIGODENDROGLIAL TUMOR GENE EXPRESSION.

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PURPOSE: Oligodendroglial tumors presenting loss of heterozigosity (LOH) at 1p and 19q have been shown to be sensitive to chemotherapy, thus making of 1p-19q status testing a key aspect in oligodendroglioma diagnosis and prognosis. Our aim was to develop a molecular signature identifying tumors bearing 1p-19q LOH. **METHODS:** Twenty-nine tumor samples (19 oligodendrogliomas, 10 oligoastrocytomas) were analyzed by microarrays in order to obtain the expression profile. Other genomic anomalies usually present in gliomas such as EGFR amplification, CDKN2A/ARF deletion, 10q LOH and TP53 mutation were also studied. **RESULTS:** Tumors with 1p-19q LOH over-expressed genes related to neurogenesis. Genes linked to immune response, proliferation and inflammation were over-expressed in the group with intact 1p-19q; this group could in turn be further divided in two subgroups: one over-expressing genes involved in immune response and inflammation which did not show major genetic aberrations other than TP53 mutation and EGFR trisomy in few cases, and another over-expressing genes related to immune response and proliferation which concentrated samples carrying several anomalies and presenting worse outcomes. This molecular signature was validated by analyzing a set of 10 tumor samples (3 oligodendrogliomas, 7 oligoastrocytomas); all 10 samples were correctly assigned. **CONCLUSION:** LOH at 1p-19q produces a global change in gene expression inducing a pro-neural status that results in restrictions to cell migration and proliferation. Tumors without LOH at 1p-19q exhibit opposite characteristics, explaining their more aggressive behavior.

P073

FUNCTIONAL ANALYSIS OF THE PROTEOME RELATED TO THE CHEMOTHERAPY SENSITIVITY IN ANAPLASTIC OLIGODENDROGLIOMA AND ANAPLASTIC OLIGOASTOROCYTOMA

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Malignant gliomas are generally resistant to all standard therapies. However, patients with anaplastic oligodendroglioma (AOG)/anaplastic oligoastorocytoma (AOA) with loss of heterozygosity on chromosome 1p and 19q (LOH+) frequently respond to chemotherapies. To date, the responsible molecular factors for this chemosensitivity have not been clarified in detail. In this study, we analyzed the specific proteins related to this chemosensitivity by using newly established proteomic strategies such as 2D-DIGE combined with phospho-specific staining and iTRAQ LC-shotgun methods. As a result, we identified 105 specific proteins differentially expressed in LOH- AOG/AOA, and 34 phosphorylated proteins highly modified in LOH- vs LOH+ tumors, including EGFR-MAPK, vascular PIFs, adhesion molecules, cell cycle regulator, CDK families, and cytoskeletal organizing factors. Among them, we focused on vimentins that were highly phosphorylated in LOH- samples (at least 15 vimentin modified spots were identified in the 2D). The specific increase of vimentin expression in LOH- tissues was confirmed by immunohistochemistry and wetern blotting. By 2D-Western blotting and MS analysis, we found that the specific phosphorylated vimentins had some cleavage forms and these short vimentins were statistically increased in 30 LOH-AOGs. Interestingly, the responsible enzymes for vimentin modifications, such as PAC, CaMK2, caspases, calpains, identified as up-regulated proteins in LOH- AOG in our proteomic study, were found on the ch1p, 19q or 10pq. We discuss the structure and function of vimentin and responsible enzymes related to AOG/AOA chemotherapy sensitivity in detail. The idea for cellular activity regulation of vimentin and its modification enzymes may hold some potentialities for a new strategy against AOG/AOA.

P074

ESTABLISH THE OVERALL PROTEINS REFERENCE DATABASE OF HUMAN NORMAL PITUITARY USING 2D-HPLC COMBINED WITH LTQ-ORBITRAP MS

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Objective: To establish the quantitative reference database of overall proteins expression of human normal pituitary. **Methods:** Three samples of human normal pituitary tissues were collected during the transphenoidal approach operations of pituitary microadenoma for enlarging resection, which was consented by the patients and approved by the Ethics Committee of Huashan Hospital, Fudan University. Total proteins were extracted and analyzed using 2D-HPLC combined with LTQ-Orbitrap MS proteomics technology to establish the quantitative overall proteins reference database of human normal pituitary. **Results:** The quantitative database containing 406 total proteins of human normal pituitary has been established successfully. The proteins by turns according to the amounts are type VI collagen 1.30%, fibrinogen 0.99%, vimentin 0.73%, prolactin 0.69%, ATP synthase and H+ transporting mitochondrial F1 complex 0.52%. The types of proteins by turns are the combining proteins 40.1%, the catalysis proteins 28.3% and the signal transducer proteins 9.8%. **Conclusion:** The quantitative overall proteins reference database of human normal pituitary has been precisely and efficiently established by the proteomics technology, which could settle the foundation for the studies of pituitary adenoma and other diseases related to the pituitary. The discoverable proteins such as cytoskeletal proteins, CA I and HSP 90 may provide suitable targets for the progressive research.

P075

CD52 PROTEIN MAY BE A POTENTIAL TUMOR MARKER OF HEMANGIOBLASTOMA IN CENTRAL NERVAL SYSTERM WITH CDNA MICROARRAY FILTRATION OF THE GENE EXPRESSION PROFILES

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[Abstract] **Objective** To analyze the gene expression profiles of central nerval systerm hemangioblastoma by using cDNA microarray and detect target genes for further study. **Methods** Two mixed samples from 5 cases of hemangioblastomas and 3 cases of normal cerebellum tissue were studied by means of cDNA microarray consisting of 14000 genes. **Results** There were 61 expressed genes which difference were more than 3 times between two samples including 10 up regulated and 51 down regulated genes. The results of cDNA microarray were repeated by RT-PCR and immunohistochemistry. Tumor suppressor genes, growth factor, cell signal conduction genes and so on were identified. Analysis with cDNA microarray, expression level of CD52 between HBs and normal cerebellar tissues were up-regulated for 3 times. With Western-Blot, expression level of CD52 in 4 HBs are obviously higher than normal cerebellar tissue. By CD52 immunohistochemistry stain, only stromal cells in 40 HBs showed positive reactions. By CD52 immunofluorescence stain in cultured cells of HBs , membrane and cytoplasm of stromal cells showed positive reaction. **Conclusion** The results of cDNA microarray in hemangioblastoma were reliable.Many genes would be involved in the pathogenesis of hemangioblastoma besides VHL gene. CD52 protein may be a tumor marker of HB. [Keywords] hemangioblastoma, gene expression, cDNA microarray

P076

GENOME-WIDE ANALYSIS OF GENE EXPRESSION DURING BRAIN METASTASIS FORMATION

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The brain is frequently affected by the spread of lung cancer, and hematogenous metastasis is a common route to brain metastasis. In the present study, we attempted to characterize genetically the dynamic changes occurring during brain metastasis formation by DNA microarray, and attempted to compare these findings with histological observations.

In histological observation, tumor cells were observed in capillary vessels at day 1 after injection. At day 3, the tumor cells had begun to proliferate, forming metastatic foci. At day 6, the metastatic foci showed an angiocentric pattern, and were designated perivascular proliferations.

Next, we performed a pairwise comparison of gene expression microarray data from day 1 to day 9 after injection. The first major change occurred between Phase Two and Phase Three. However, there were no distinct morphological changes at the point the second major change occurred. When hierarchical clustering was performed between different samples using the 867 genes that had been extracted, they could be classified into identical clusters for days 1 and 2, identical clusters for day 3 to day 5, and identical clusters for day 6 to day 9. For time course analysis of individual genes, we extracted 623 genes from the 5157 genes obtained by the pairwise comparison. By using the QT nonhierarchical clustering method, we identified 37 expression patterns. These 37 expression patterns can be separated into 8 clusters by using the k-means method.

The microarray results reported here strongly suggest that a large number of genes exhibit a spike pattern, which is tantamount to phase-specific expression.

P077

HYPOXIA PROMOTES EXPANSION OF THE CD133-POSITIVE GLIOMA STEM CELLS THROUGH ACTIVATION OF HIF-1α

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Hypoxia contributes to the progression of a variety of cancers by activating adaptive transcriptional programs that promote cell survival, motility, and tumor angiogenesis. Although the importance of hypoxia and subsequent HIF-1α activation in tumor angiogenesis is well known, their role in the regulation of glioma-derived stem cells is unclear. Here we show that hypoxia promotes the self-renewal capacity of CD133-positive human glioma-derived stem cells. Propagation of the glioma-derived cancer stem cells (CSC) in a hypoxic environment also led to the expansion of cells bearing CXCR4 (CD184), CD44-low and A2B5 surface markers. The enhanced self-renewal activity of the CD133-positive CSC in hypoxia was preceded by upregulation of HIF-1α. Hypoxia also promotes vascular endothelial growth factor production in CSCs. Knockdown of HIF-1α abrogated the hypoxia-mediated CD133-positive CSC expansion. HIF-1α protein expression and transactivity are due to the phosphorylation of Akt, extracellular signal-reglated kinase 1/2 (ERK1/2), p70S6 kinase, as indicated by application of these inhibitors, LY294002, PD98059 and rapamycin, suggesting that the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and ERK pathways, in a potentially independent and co-operative fashion, can modulate HIF-1α activation by hypoxia. Lastly, CSC propagated at hypoxia robustly retained the undifferentiated phenotype, while CSC cultured at normoxia did not. These results suggest that response to hypoxia by CSC involves activation of HIF-1α to enhance the self-renewal activity of the CD133-positive cells and inhibit induction of CSC differentiation. This study illustrates the importance of the tumor microenvironment in determining cellular behavior.

P078

EFFECTS OF HYPOXIA ON EXPRESSION OF A PANEL OF STEM CELL AND CHEMOSENSITIVITY MARKERS IN GLIOBLASTOMA CELL LINE-DERIVED SPHEROIDS

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Glioblastomas are the most frequent and malignant primary brain tumor. Tumor stem cells in these tumors have recently been suggested to possess innate resistance mechanisms against radiation and chemotherapy possibly explaining their high level of therapeutic resistance. Moreover, tumor hypoxia with oxygen tensions below 1-5% O2 has been attributed to play a crucial role in tumorigenesis and therapeutic resistance in glioblastoma. In this study the influence of hypoxia on the expression of a panel of stem cell and chemosensitivity markers was therefore investigated using glioma spheroids derived from the conventional glioblastoma cell line U87. The glioma spheroids were derived at normoxic (21% O2) and hypoxic (1% O2) culturing conditions in serum-free medium with EGF and bFGF. The entire immunohistochemical panel included hypoxia (HIF-1&alpha, HIF-2&alpha), proliferation (Ki-67) and stem cell (CD133, nestin, podoplanin, Bmi-1, Sox-2) markers as well as markers related to chemosensitivity (MGMT, MDR-1, TIMP-1, Lamp-1). Since spheroids derived in hypoxia were smaller than in normoxia, a set of experiments was included, in which the culturing time of hypoxic spheroids was extended to obtain equally sized spheroids. HIF-1&alpha was significantly increased in hypoxia, whereas the Ki-67 positive fraction of proliferative cells was significantly reduced. The expression of stem cell markers CD133, nestin, podoplanin and Bmi-1 was significantly increased in hypoxia similar to what was found for TIMP-1 and Lamp-1. Similar differences in the expression pattern was observed in equally sized normoxic and hypoxic spheroids, although more pronounced differences were found for podoplanin, nestin and TIMP-1 as well as for Ki-67. Hif-2&alpha, Sox-2, MGMT and MDR-1 were not detectable in U87 spheroids. In conclusion, the expression of tumor stem cell and chemosensitivity markers seems to depend on the oxygen tension suggesting that future development of therapeutic strategies targeting tumors stem cells should take this experimental design into account.

P079

CELL MOTILITY FEATURES ARE IDENTIFIED IN THE CD133-POSITIVE GLIOMA AND ENDOTHELIAL PROGENITOR CELLS

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Cell motility is required for gliomagenesis during invasion of glioma cells, migration of endothelial progenitor cells (EPCs) from the blood stream into tumor cell populations to form new blood vessels (vasculogenesis) and for navigation of local endothelial tip cells during sprouting angiogenesis. CD133-positive tumor stem cells are capable of tumor initiation and CD133-positive EPCs are a major determinant of nascent tumor neovascularization in experimental animals. Obviously, therapeutic interference with motility of both vascular cells as well as tumor cells within the CD133-positive population would be an important strategy in future anti-tumor therapy. These migrating cells usually escape from all conventional anti-tumor or anti-angiogenesis therapies. So far, no studies on the identification of motility-related cellular features in tumor cells and EPCs present in human tumor specimens have been undertaken. Glial tumor cells are notoriously invasive and also, in gliomas prominent neovascularization takes place. In the present study we sought motility-related cell features as described in cell cultures in glial tumor tissue obtained from surgery. To this aim, a cohort of samples of glioma were investigated by using confocal microscopy to double- and triple immunostained tissue specimens. Several functional stem cell and EPC surface markers were used for the investigation of the cell populations. We discovered a variety of plasma membrane protrusions which are known to be involved in cell motility in CD133-positive glioma cells and endothelial progenitor cells in the tumor samples. We conclude that the features which are characteristic for cell motility as observed in vitro are identifiable in native human glioma specimens. Subsequent studies are necessary to discover the trigger for the formation of these features and their protein composition, in order to develop strategies for therapeutic elimination of the migrating cells.

P080

GLIOBLASTOMA TUMOUR INITIATING CELLS DEMONSTRATE VEGF BUT NOT PDGF AUTOCRINE SIGNALLING

Cancelled

P081

ESTABLISHMENT OF ARTIFICIAL GLIOBLASTOMA INITIATING CELLS IN MOUSE

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Cancer initiating cells (CICs) in brain tumors, such as GBM, medulloblastoma, ependymoma and anaplastic oligoastrocytoma have been reported. CSCs are small fraction of cancer cells and CD133 is one of the markers of CICs in brain tumors. However even collecting CD133 expressing cells, bona fide CICs are still minor in this population. To progress the study of CICs on GBM, we tried to establish GBM initiating cells using mouse cells. Neurosphere cells (NSCs) were prepared from embryonic mouse brain at 14.5 dpc. Astrocytes (ASTs) and oligodendrocyte precursor cells (OPCs) were induced from the NSCs in suitable culture conditions and then purified them. NSCs, ASTs and OPCs were transfected with vector plasmid expressing oncogene X with GFP by electroporation. After passaging, GFP positive cells were collected with cell sorter to establish oncogene X expressing cell lines (NSCX, ASTX and OPCX). To characterize these cell lines, proliferation assays and soft agar assays were performed. The ability of proliferation was up-regulated in all three cell lines compared to individual control cell lines. Colonies were seen in soft agar culturing NSCX and OPCX, but not ASTX. Next to analyze tumor forming ability, these three cell lines were injected into nude mouse brain. In 1,000 cell injection, NSCX and OPCX could form GBMs and all mice died in a month but ASTX did not form tumor in the mouse brain. Moreover GBMs were detected in the mouse brain, even 10 cells injection of NSCX and OPCX, respectively. Three of eight mice injected 10.000 ASTX could initiate anaplastic astrocytomas more after one month. The cells in different lineages and/or stages of differentiation possess distinct transforming potential. NSCs and OPCs maintain the potential transforming to GBM initiating cells compared to ASTs in this model.

P082

CD133 ON STEM CELLS IS REQUIRED FOR APOPTOTIC INDUCTION IN GLIOMAS

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CD44 and CD133 enriched human umbilical cord blood stem cells (hUSC) were used for these studies, and a modified Boyden-s chamber for the co-culture experiments. Human glioma cells (SNB19 or U87) or xenografts (4910 or 5310) were cultured with hUSC, with or without CD133 (siRNA/CD133 antibodies), or with hUSC-conditioned media or serum-free media. 48h later the glioma cell layer was subjected to TUNEL assay and apoptotic induction observed in glioma hUSC cultures (70&plusmn3%) when compared to hUSC-conditioned media (3&plusmn1%) or with serum-free media (2&plusmn1%). Glioma cells cultured with CD133 siRNA/antibody pretreated with hUSC showed 42&plusmn5% (siRNA) 36&plusmn5% (antibody) apoptotic events. FACS analysis showed an increase in subG0/1 phase in SNB19 (83&plusmn3%) and U87 (87&plusmn4%) glioma cells and in 4910 (76&plusmn2%) and 5310 (73&plusmn5%) xenograft cells when cultured with hUSC indicative of DNA damage. Glioma cells cultured with CD133-siRNA pretreated with hUSC showed an increase of 23&plusmn5% (SNB19), 15&plusmn3% (U87), 22&plusmn2% (4910) and 26&plusmn4% (5310) in the sub G0/1 phase when compared to untreated cells. Similar results were observed when cultured with CD133 antibody pretreated with hUSC. Cleavage of caspase 8 was observed in glioma cells cultured with hUSC when compared to controls indicative of extrinsic apoptotic pathway. In vivo studies were done with nude mice implanted with $1x10^6$ SNB19 glioma cells followed by hUSC (&plusmnRNAi/antibody pretreatment) and showed the least tumor development in hUSC implanted mice (1.8&plusmn0.2mm^3) when compared to controls (15.2&plusmn0.5mm^3). Pretreatment of hUSC with CD133 siRNA/antibody showed a slight decrease in tumor development (8&plusmn2mm^3) when compared to controls. To determine the role of glioma CD133, we downregulated CD133 (siRNA/antibody) in gliomas, co-cultured with hUSC, and observed for apoptosis molecules. From western-blot analysis of caspase 8 and PARP, no contribution of CD133 to apoptosis was observed. Our results suggest that hUSC require CD133 for apoptotic induction in gliomas.

P083

TUMOR STEM-LIKE ASSOCIATED ANTIGENS AND THEIR IRRADIATIONS IMPROVE THE EFFECT OF DENDRITIC CELL-BASED VACCINATION AGAINST HUMAN GLIOMA

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Tumor stem-like cells (TSC) are becoming a novel target in cancer therapy,we compared the different effects of DC targeting of TSCs and of non-TSCs in immunogenecity and cytotoxicity in vitro.Two primary malignant glioma cell lines, SHG62 and SHG66 , as well as U87 cells were cultured in the DMEM contained serum (SCM) and in DMEM/F12 supplemented with EGF/bFGF but no serum (SFM). Immunocytochemistry showed that the two groups of glioma cells have different molecular and biological characteristics, and cells in SFM expressed higher levels of CD133 and manifested TSC features. Cells in SFM demonstrated a comparably higher potential for clone formation of 50.3±2.63%, while cells in SCM exhibited only 0.42±0.22% . Stem-like cells in SFM also expressed higher levels of HLA-A than differentiated cells. Autologous DCs from PBMCs were then used for immunotherapy on the tumor cells in vitro. Flowcytometric analysis determined that corresponding HLA-A, HLA-DR, CD80 and CD86 on DCs were up regulated after stimulated by different antigens. T cells were significantly multiplied by autologous DCs. DCs loaded with non stem-like associated antigens(non SAAs) killed 26% of differentiated glioma cells (GCs) and 9% of stem-like cells (SCs) respectively ($p<0.001$), whereas DCs loaded with SAAs eliminated 55% of GCs and 38% of SCs ($p<0.001$). Strikingly, antigens obtained by irradiating SCs(6 Gray) raised the cytotoxicity significantly to 75% (to GCs) and 59% (to SCs) ($p<0.05$). No difference of CTL killing ability was detected between targeted glioma cells before and after irradiation (2 Gray)($p>0.05$).These findings suggest that SAAs provide a higher protection against glioma and the irradiations seemed to improve the effect, and radiotherapy had no positive effect on immunotherapy, meanwhile SCs have a better ability of immune escape. These data offer a new design of clinical trials based on DC vaccination.

P084

IMMUNOSUPPRESSIVE B7-H1 PROTEIN IS EXPRESSED BY DIFFERENTIATED GLIOMA CELLS BUT NOT GLIOMA STEM CELLS

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Background: Transcript for the immunosuppressive T cell costimulatory molecule B7-H1 is constitutively expressed in many nucleated cells but protein expression is tightly regulated. B7-H1 protein expression can be increased in response to interferon-gamma. In glioblastomas, B7-H1 protein is constitutively expressed in tumors with PTEN deletion/mutation. Here, we explore B7-H1 expression in glioma stem cells and differentiated glioma cells. **Method:** Human glioma stem cell lines (BT12, BT25, BT30) were derived from fresh glioblastoma specimens by culturing single cell suspensions in minimally hormonally supplemented media in the presence of EGF and FGF. From these, differentiated glioma cells were created by culturing in the presence of 10% fetal calf serum. B7-H1 expression was determined in the presence or absence of interferon-gamma. PTEN, Akt, and phospho-Akt expression were also determined. Naive allogeneic T cells were co-cultured with glioma stem cells or differentiated cells and proliferation in the presence or absence of anti-B7-H1 antibodies was determined. **Results:** B7-H1 transcript was present in both glioma stem cell and differentiated cells but constitutive protein expression was only present in stem cells. Interferon-gamma minimally increased B7-H1 protein expression in stem cells but markedly increased it in differentiated cells. PTEN protein was detectable in all cells but phospho-Akt was increased compared to controls, suggesting PTEN function was impaired. More T cell proliferation was seen after co-culture with stem cells than differentiated cells but this could be reversed by blocking B7-H1. **Conclusions:** Immunosuppressive B7-H1 protein expression is limited in glioma stem cells but is upregulated in differentiated cells. This does not reflect differences in phospho-Akt and the mechanism regulating B7-H1 protein expression in these cells remains unknown. Glioma stem cells that do not express B7-H1 protein are an attractive target for immunotherapy.

P085

B7-H1 IS CORRELATED WITH THE MALIGNANCY GRADE OF HUMAN ASTROCYTIC TUMORS BUT IT IS NOT THE PRIVILEGE OF BRAIN TUMOR STEM-LIKE CELLS

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Human glioblastoma is notorious for its capacity to interfere with effective anti-tumor immune responses. B7-H1 is the third member of the B7 family that plays important roles in tumor immune evasion. Recent studies showed that brain tumor stem-like cells (TSCs) contributed to tumorigenesis and radioresistance. However, the relationship between B7-H1 and the clinical behavior of brain TSCs remain unclear. In the present study, we reported that B7-H1 was correlated with the malignancy grade of astrocytic tumors. There was significant up-regulation of B7-H1 at the growing edge of the tumors. Immunostaining indicated that B7-H1 was primarily expressed by Ki67 negative tumor cells. In vitro, tumors cultured under medium favoring the growth of neural stem cells were able to form spheres, along with expression of neural stem/progenitor cell markers. These cells were able to differentiate into different neural lineages when cultured in differentiation medium, indicating that these cells have TSCs characteristics. We also found that B7-H1 was expressed, but not exclusively on CD133+ stem cells. Interestingly, we found CD133- tumor cells also had the capacity to form brain tumor. Our data establish a correlation between the expression of negative costimulatory molecule B7-H1 and the malignancy grade of human gliomas, suggesting B7-H1 can be a novel tumor marker & target for therapy although it is not the privilege of brain tumor stem-like cells.

P086

EXPRESSION LEVEL OF STEM CELL MARKERS IS ASSOCIATED WITH THE SOURCE OF NEUROSPHERE MEDIA USED FOR GLIOBLASTOMA STEM CELL CULTURE.

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Glioblastoma multiforme (GBM) is a brain tumor with a very poor prognosis due to inevitable recurrence. A contingent of cells within the tumor, so-called stem-like tumor cells (STC), has been recently 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 considered to play a major role in the recurrence observed in GBM patients and are appealing targets for new therapeutic approaches. Most of STC studies are dependent of an in vitro step in which tumor cells are amplified in a serum-free medium in order to expand STC. In this work, we have amplified 5 tumor samples from GBM patients in two different neurosphere media containing EGF et bFGF: a serum-free medium (NS) previously described by Reynolds (Science, 1992) and a medium containing the commercially available B27 and N2 supplements (BN). Cell growth was faster in BN compared to NS culture and higher cell number was obtained with the BN medium. Phenotypic analyses show that cells derived from the BN cultures express higher levels of CD133 and to a lesser extent nestin, two neural stem cell markers. In contrast, A2B5 a progenitor marker, is expressed in a higher level on cells derived from NS cultures. Concerning the immunological characterization, cells derived from BN cultures express higher levels of HLA class I molecules, essential for specific recognition of tumor cells by cytotoxic T lymphocytes. Experiments of differentiation and injections in mice are currently in progress to test eventual functional differences between the neurosphere media. In conclusion, one should be aware of the use of specific media for generating STC from GBM samples due to their influence at least on the level of expression of the stem cell markers.

P087

AN EVALUATION OF THE NEURAL STEM CELL MARKER NG2 AS A BIOMARKER OF DISEASE IN PATIENTS WITH GLIOBLASTOMA

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IntroductionThe identification of cancer stem cells, including Glioblastoma (GBM), often utilises expression of CD133. However, an increasing body of evidence suggests that this is neither sensitive nor specific and alternative markers are urgently needed. We have investigated the expression and clinical relevance of markers expressed during neural development in GBM.MethodsUsing GBM as a model system we have derived tumour initiating stem-like cells (TICs) using our Cambridge Protocol1. TICs were sorted into NG2+/Olig2+ and NG2-/Olig2+ populations using FACS. Patterns of gene expression were examined in 39 GBM samples using Affymerix platforms and validated on independent data sets. Clinical data was obtained retrospectively from 8 GBM patients from whom TICs had been derived.ResultsNG2+/Olig2+ TICs can be derived from GBM under serum-free conditions and expression of both markers is common in GBM tumours (for more details see abstract by al Mayhani et al). Some TIC lines contain large numbers of NG2+ cells and some TIC lines contain low numbers of NG2+ cells. This pattern is representative of the parent tumour.The self-renewal rate varies between different tumour TIC lines from different patients.TIC lines containing lots of NG2+ cells (NG2hi) expand slowly. TIC lines containing low numbers of NG2+ cells (NG2lo) expand rapidly.NG2hi patients had reduced progression free survival compared to NG2lo patientsConclusionsTumour forming cancer stem-like cells are common in GBM. Cancer stem cells from different GBM patients behave differently.NG2 expression has potential as a biomarker of disease in GBM but a larger cohort of patients is needed to validate this hypothesis.

P088

GENE THERAPY USING TRAIL-SECRETING HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS AGAINST INTRACRANIAL GLIOMA

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Adenovirus-mediated gene therapies against brain tumors have been limited by the difficulty in tracking glioma cells infiltrating the brain parenchyma. Human umbilical cord blood-derived mesenchymal stem cells (UCB-MSC) are particularly attractive cells for clinical use in cell-basedtherapies. In the present study, we evaluated the tumor targeting properties and antitumor effects of UCB-MSCs as gene delivery vehicles for glioma therapy. We efficiently engineered UCB-MSCs to deliver a secretable trimeric form of tumor necrosis factor-related apoptosis-inducing ligand(stTRAIL) via adenoviral transduction mediated by cell-permeable peptides. We then confirmed the migratory capacity of engineered UCB-MSCs toward tumor cells by an in vitro migration assay and by in vivo injection of UCB-MSCs into the tumor mass or the opposite hemisphere of established human glioma in nude mice. Moreover, in vitro coculture, experiments on Transwell plates, and in vivo survival experiments showed that MSC-based stTRAIL gene delivery has more therapeutic efficacy compared with direct injection of adenovirus encoding the stTRAIL gene into a tumor mass. In vivo efficacy experiments showed that intratumoral injection of engineered UCB-MSCs (MSCs-stTRAIL) significantly inhibited tumor growth and prolonged the survival of glioma-bearing mice compared with controls. These results suggest that human UCB-MSCs have potential use as effective delivery vehicles for therapeutic genes in the treatment of intracranial glioma.

P089

BRAIN EASY ANALYSIS TOOL FOR 201TL SPECT IN BRAIN TUMORS

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Purpose: 201Thallium SPECT is a useful functional imaging modality for the diagnosis of malignancy and activity of brain tumors. The coregistration of functional SPECT images and high resolution anatomical MRI is considered to be a reasonable method to improve the low spatial resolution of SPECT. We used a newly developed software Brain Easy Analysis Tool for TI-SPECT (BEAT-TI) for the coregistration of TI-SPECT and MRI in patients with brain tumors. **Methods:** MRI was performed using the 1.5 Tesla clinical MRI scanner. TI-SPECT images were acquired 15 minutes (early) and 3 hours (delayed image) after the intravenous injection of 74 MBq of Thallium Chloride using multi-detector SPECT machine. Both the MRI and SPECT data of analyze format were transferred to the BEAT-TI program and then coregistered images were created. **Results:** The coregistered images were easily and promptly created. The image quality was sufficient. There were no large displacements of the MRI and SPECT images and no major artifacts. Small hot uptake in TI SPECT always coregistered with an enhanced tumor in MRI. **Conclusion:** BEAT-TI is a useful coregistration software program to evaluate brain tumors. It improve the spatial resolution of SPECT images and such coregistration images demonstrate a metabolic heterogeneity in tumors.

P090

123I-IMP SPECT IN BRAIN TUMOR: ANATOMICAL STANDARDIZED STATISTICAL MAPPING

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Introduction: 123I-iodoamphetamine Single Photon Emission Computed Tomography (IMP SPECT) is used to evaluate the cerebral blood flow. However, its application for patients with brain tumors has so far been rarely reported. Primary central nervous system lymphoma (PCNSL) is a rare tumor that shows a delayed IMP uptake. The relatively low spatial resolution of SPECT is a clinical problem to diagnose brain tumors. We examined anatomical standardized statistical mapping of 49 IMP SPECT in patients with brain tumor. **Method:** After the intravenous injection of 222MBq of I-IMP, early and delayed images were acquired using a multi-detector SPECT machine. **Results:** Sixteen patients showed a high uptake in the delayed IMP SPECT images. Other tumor or diseases did not show a high uptake of delayed IMP SPECT, so there was no false positive. Four patients with pathologically proven PCNSL showed no uptake in IMP SPECT. These tumors were too small to detect in IMP SPECT or IMP SPECT was taken after the steroid administration. However, statistical mapping revealed the IMP uptake in 2 of 4 patients. **Conclusion:** IMP SPECT is the sensitive examination for the diagnosis of PCNSL, although there is some false negative. Anatomical Standardized Statistical Mapping is useful to improve the diagnostic sensitivity, spatial resolution and accuracy of IMP SPECT.

P091

THE APPLICATION OF SUSCEPTIBILITY WEIGHTED MAGNETIC RESONANCE IMAGING (SWI) TO DIAGNOSIS AND TREATMENT OF MENINGIOMAS.

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[Purpose] To evaluate usefulness of Susceptibility magnetic resonance Imaging (SWI) for management of meningiomas. **[Methods]** Pathologically confirmed meninsiomas, 7 WHO grade 1, 2 atypical and 2 anaplastic, were included. Patients were evaluated by MRI preoperatively. SWI was compared with T2 and diffusion weighted images, and angiography and histological findings. **[Results]** Signal intensity of the tumor in SWI was similar with that in T2WI. But heterogeneity in the tumor was more clearly observed in SWI. Vascular structures in and around the tumor were displayed as no signal spots or curved-linear lines in SWI. Density and distribution of tumor vessels in SWI were well correlated with results of angiography. Signal intensity of atypical and anaplastic meingiomas in SWI were lower than that of grade 1 tumors. Meningiomas with higher MIB-1 staining indices showed lower signal intensity than those with lower MIB-1 indices. **[Conclusion]** SWI can provide more useful information for management of meningiomas such as vascularity and proliferation potential.

P092

CHOLINE-ENHANCED PROTON MRS OF BRAIN TUMORS

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OBJECTIVE The present study was done for evaluation of the possible influence of the oral administration of choline on metabolic characteristics of gliomas detected with proton magnetic resonance spectroscopy (MRS). **METHODS** Thirty patients (22 men and 8 women; mean age 38±15 years) with suspicious intracranial gliomas underwent single-voxel long-echo (TR: 2000 ms, TE: 136 ms, 128-256 acquisitions) MRS of the tumor, peritumoral brain tissue and distant normal-appearing white matter before and several hours (median, 3 hours; range, from 1.2 to 3.7 hours) after ingestion of choline with prescribed dose 50 mg/kg (median actual dose, 52 mg/kg; range, from 48 to 78 mg/kg). Investigations were done using 1.5 Tesla clinical MR imager. The volume of the rectangular MRS voxel was either 3.4 cc or 8 cc. At the time of both spectroscopic examinations similar voxels` positioning and size, and technical parameters of MRS were used. Surgery was done in 27 patients within 1 to 68 days thereafter. In all cases more, than 80% resection of the neoplasm was attained. **RESULTS** There were 12 low-grade gliomas and 15 high-grade gliomas. MIB-1 index varied from 0 to 51.7% (median, 13.8%). Statistical analysis did not disclose significant differences of any investigated metabolic parameter of the tumor, peritumoral brain tissue and distant normal-appearing white matter between two spectroscopic examinations. **CONCLUSION** Single-voxel MRS at 1.5 Tesla could not detect significant changes of the metabolic characteristics of gliomas, peritumoral brain tissue and distant normal-appearing white matter after oral administration of choline. Nevertheless, further search for possible modifiers of the metabolites` resonance intensity seems important, because their identification can potentially increase the diagnostic efficacy of the spectroscopic imaging.

P093

PREOPERATIVE EVALUATION BY PROTON MAGNETIC RESONANCE SPECTROSCOPY IN THE GRADING OF GLIOMAS

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Background: Advanced magnetic resonance (MR) techniques provide physiological and metabolic information that complements the anatomical information available from conventional MR imaging. The purpose of this study was to evaluate the clinical usefulness of proton MR spectroscopy (1H-MRS) in preoperative quantitative assessment of gliomas. Materials and **Methods:** Sixteen patients with histologically verified gliomas, comprising 7 cases with high-grade glioma (HGG) ((4 glioblastoma multiforme (GBM), 2 anaplastic oligodendroglioma (AO), 1 anaplastic astrocytoma (AA)) and 9 cases with low-grade glioma (LGG) ((8 fibrillary astrocytoma (FA) and 1 oligoastrocytoma (OA)) were evaluated using the 1H-MRS protocol following conventional MR imaging, diffusion-weighted imaging (DWI), and perfusion-weighted imaging (PWI) preoperatively. **Results:** HGG tended to demonstrate signal hyperintensity by DWI and higher relative cerebral blood volume (rCBV) by PWI. Increased ratios of choline (Cho) to N-acetylaspartate (NAA) (Cho/NAA) and Cho to creatine (Cr) (Cho/Cr) correlated highly with tumor malignancy. The presence of lipid was predominately detected in patients with HGG, though lactate was confirmed even by half of LGG. **Conclusion:** The combination of multiple MR parameters, based on DWI, PWI and 1H-MRS, appears valuable for preoperatively predicting the degree of malignancy in glioma.

P094

MODIFIED 11C-METHIONINE PET IMAGING TO DISTINGUISH RADIATION NECROSIS FROM RECURRENT GLIOMA

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^{11}C–Methionine (MET) –PET 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. MET accumulation in RN is mostly affected by the latter factors. To exclude the vascular factors, we made modified MET (mod–MET) PET images. We report the usefulness of these images for more precisely distinguishing RN from recurrence compared to MET–PET.

MET–PET and ^{11}C–Choline (CHO) –PET were examined simultaneously for each patient. Based on our previous studies, accumulation of CHO is thought to be mostly affected by the vascular factors. In the choroid plexuses of 30 normal adults, in which both MET and CHO were prominently accumulated only by vascular factors, there was a significant positive linear regression between MET–SUVmax and CHO–SUVmax. The vascular MET–SUV, which reflected only the vascular factors, could be obtained from the CHO–SUV using linear regression for 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. We studied histologically verified 13 RNs, 16 recurrent grade-3 gliomas (Gr.3) and 10 recurrent glioblastomas (Gr.4).

Mod–MET L/N ratio for RN, Gr.3 and Gr.4 were 3.33±1.37, 9.18±7.78 and 10.07±5.66, respectively. The mod–MET L/N ratio for RN was significantly lower than those for Gr.3 and Gr.4 (,P <.005). 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. The mod–MET–PET image is useful for distinguishing RN from recurrent gliomas.

P095

MATCHING ANALYSIS OF PET AND CHEMICAL SHIFT IMAGING ON PROTON MRS IN PATIENTS WITH SUPRATENTORIAL GLIOMA

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<Objective> The goal of this study is to collate the results of positron emission tomography (PET) and chemical shift imaging (CSI) on proton magnetic resonance spectroscopy in glioma patients. **<Materials and Methods>** Total 48 times of methionine (met) PET, fluorodeoxy glucose (FDG) PET and CSI (choline: cho, lactate: lac) were performed on the same day in 35 cases of supratentorial gliomas including 3 of astrocytomas (A), 4 of oligodendrogliomas (O), 3 of anaplastic astrocytomas (AA), 5 of anaplastic oligodendrogliomas (AO) and 15 of glioblastomas (GBM). PET results were qualitatively evaluated in 5 grades (0-4) and CSI results were in 2 grades (high signal: HS, low signal and below: LS). **<Results>** HS in cho was observed in A (2/4 cases), AA (2/3) and GBM (13/16). All cases of O and AO showed HS in cho. Cases showing HS in cho and high uptake (3,4) in met were in A (0/4 cases), AA (1/3) and GBM (12/16). They were much in the cases with high grade gliomas. In totality, 27 cases of them were significantly much than the 4 cases showing LS in cho and high uptake in met. Secondly, HS in lac was observed in A (0/4 cases), AA (2/3) and GBM (14/16). They were much in the cases with high grade gliomas In totality, 25 cases of them were significantly much than the 3 cases showing LS in lac and high uptake in met. Accordingly, it was demonstrated that the lesion showing high uptake of met tended to show HS in cho and lac. The concordance rate in met-cho and met-lac was approximately half, respectively. In contrast with met, FDG uptake did not correlate with HS of cho and lac. **<Conclusion>** CSI was secondarily useful for evaluating the degree of malignancy from the aspect of amino acid metabolism in tumors.

P096

AUGMENTING INTRAOPERATIVE MRI WITH PREOPERATIVE DTI AND FMRI BY SIMULATION OF BRAIN DEFORMATION USING MATHEMATICAL MODEL BASED ON THIN-PLATE SPLINES:A PRELIMINARY RESEARCH

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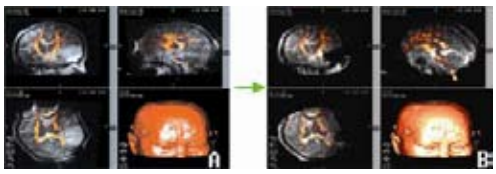
OBJECTIVE: Intraoperative magnetic resonance imaging(iMRI) integrated with diffusion tensor imaging(DTI) or blood oxygen level dependent(BOLD) proved to be effective in neurosurgical operations, but DTI or BOLD images was still provided before the surgery. Brain shift will certainly cause navigative errors. We used a mathematical model to adjust the this errors. **METHODS:** Pre-operative iMRI and intra-operative iMRI were used respectively for data fields, and a non-rigid calculation was applied for predicting the tendency of brain shift. When this calculated tendency was integrated into DTI or BOLD images, predictive DTI or BOLD images after brain shift were acquired. Then we integrated these compensated DTI or BOLD images into iMRI images to learn the relationships of the tumor and white matter fiber tracts or functional cortex after brain shift. **RESULTS:** The predictions of DTI and BOLD images shift were successfully computed. The real-measured brain shifts by iMRI were 0.5~9.8mm, the predicted brain shifts were 0.4~9.0mm. The slight extent of most of the predicted shift points was less than those in the real-measured points, however, the averages of predicted and real-measured points were statistically insignificant **CONCLUSIONS:** The mathematical model was effective and reliable in predicting the shift of DTI or BOLD images during neurosurgical operations.



P097

THE VALUE OF RCBV RATIO IN PERFUSION MR FOR DIFFERENTIATING RADIATION NECROSIS FROM TUMOR RECURRENCE IN HIGH GRADE GLIOMA; COMPARISON WITH 18F-FDG AND 11C-METHIONINE PET

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Object: The authors analyzed the characteristics of perfusion magnetic resonance images (MRI), 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) and 11C-Methinonine (MET) PET in order to evaluate the efficacy of the imaging modalities used to differentiate radiation necrosis from recurrence of high- grade glioma. **Materials and Methods:** Ten patients were evaluated with perfusion MRI, 11C-MET PET and /or 18F-FDG PET simultaneously for gadolinium- enhanced lesion on T1-weighted MRI during the follow-up period after radiation. Six cases of radiation necrosis and four cases of recurrence were confirmed by clinical course (n=7) or pathological examination (n=3). On perfusion MR images, the four regions of interest (ROIs), which measured 6*6mm, was drawn, and then the average values were calculated. A reference ROI of the same size was defined in the white matter of the contralateral hemisphere to obtain the lesion-to-normal brain ratio of rCBV (L/R ratio). After the coregistration of 18F-FDG and 11C-MET PET with MRI, and the maximum standard uptake values (SUVmax) of the lesion and contralateral cerebral hemisphere white matter were measured as a reference area for the Lmax/Rmax ratio. **Result:** Only the L/R ratio of rCBV was higher in the recurrence than necrosis group. (p=0.010) There was no difference in Lmax/Rmax ratio of 18F-FDG and 11C-MET PET between the groups. **Conclusion:** A quantitative ratio of rCBV ratio on perfusion MR is superior to the Lmax/Rmax ratio of 18F-FDG and 11C-MET PET in distinguishing recurrence of high-grade glioma from radiation necrosis.

P098

EVALUATION OF GLIOMA GRADING ON MR IMAGING: DIAGNOSTIC VALUE OF MINIMUM APPARENT DIFFUSION COEFFICIENT COMPARED WITH POSITRON-EMISSION TOMOGRAPHY

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Purpose: Apparent diffusion coefficient (ADC) calculations improve the diagnostic efficacy and the evaluation of glioma grading. Positron-emission tomography (PET) is another useful tool that provides valuable metabolic informations for gliomas. The aim of this study was to evaluate the advantages of ADC on MR imaging compared with PET for diagnoses of gliomas. **Materials and methods:** We performed MR imaging and ^{11}C-methionine (MET) PET for 71 newly diagnosed gliomas (35 grade II, 29 grade III and 7 grade IV). ADC maps were calculated from isotropic DWI, and images were obtained with a b-value of 0 s/mm^2. The minimum ADC for each tumor was determined by placing regions of interest (ROIs) using an Advantage Workstation. The ROI with the lowest ADC value was chosen from these ROIs as the minimum ADC for the tumor. PET examinations were performed to measure tumor / normal brain uptake ratios (T/N ratios). Each resected tumor was classified according to its histological diagnosis using the WHO classification and calculated Mib-1 labeling index by immunohistochemical examination. We investigated the correlations between the minimum ADCs, T/N ratios, tumor grades, and tumor proliferation activities. **Results:** The minimum ADCs of gliomas showed a significant inverse correlation with tumor proliferation activities ($r=-0.66$, $p<0.001$) and the MET T/N ratio showed a positive correlation with activities ($r= 0.64$, $p<0.001$). The mean minimum ADCs ($\times10^{-3}$mm^2/s) for the WHO grade II, grade III and grade IV gliomas were 1.058 ±0.203, 0.830 ±0.143 and 0.693 ±0.087, respectively. Significant differences were found between each glioma grade (grade II/grade III: $p<0.001$; grade III/grade IV: $p<0.001$). For MET T/N ratios, however, no significant differences were seen between the grade II and grade III gliomas. **Conclusion:** The minimum ADC can provide more valuable diagnostic information for the preoperative grading of WHO grade II and grade III gliomas than MET PET.

P099

GLIOBLASTOMA TREATED WITH POSTOPERATIVE RADIO-CHEMOTHERAPY: PROGNOSTIC VALUE OF APPARENT DIFFUSION COEFFICIENT AT MR IMAGING

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Purpose: To retrospectively evaluate whether the mean-, minimum-, and maximum apparent diffusion coefficient (ADC) of glioblastomas obtained from pretreatment MR images is of prognostic value in patients with glioblastoma. **Materials and Methods:** The the institutional review board approved our study and waived the requirement for informed patient consent. Between February 1998 and January 2006, 33 patients (24 males, 9 females; age range 10 - 76 years) with supratentorial glioblastoma underwent pretreatment magnetic resonance (MR) imaging. The values of the mean-, minimum-, and maximum ADC (ADC-mean, ADC-MIN, and ADC-MAX, respectively) of each tumor were preoperatively determined from several regions of interest defined in the tumors. After surgical intervention, all patients underwent irradiation and chemotherapy performed according to our hospital protocol. The patient age, symptom duration, Karnofsky performance scale score, extent of surgery, and ADC were assessed using factor analysis of overall survival. Prognostic factors were evaluated using Kaplan-Meier survival curves, the log-rank test, and multiple regression analysis with the Cox proportional hazards model. **Results**: Likelihood tests confirmed that ADC-MIN was the strongest among the 3 prognostic factors. Total surgical removal was the most important predictive factor for overall survival ($P < 0.01$). ADC-MIN was also statistically correlated with overall survival ($P < 0.05$) and could be used to classify patients into different prognostic groups. Interestingly, ADC-MIN was also the strongest prognostic factor ($P < 0.01$) in the group of patients in whom total tumor removal was not possible. **Conclusion:** The ADC-MIN value obtained from pretreatment MR images is a useful clinical prognostic biomarker in patients with glioblastoma.

P100

DIFFUSION-WEIGHTED IMAGING IN PATIENTS WITH GLIOBLASTOMA MULTIFORME TREATED WITH BEVACIZUMAB

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Purpose: Bevacizumab, a humanized monoclonal antibody directed against vascular endothelial growth factor, has shown effectiveness in relapsed glioblastoma multiforme. Conventional outcome measures (MacDonald criteria) have proved problematic in patients treated with antiangiogenic agents. Diffusion-weighted MRI (DW-MRI) has been shown to be useful in assessing tumor cellularity, grade, and early treatment response in a variety of brain tumors. This spurred our interest in studying DW-MRI in bevacizumab recipients. **Methods:** We performed a retrospective analysis of patients with relapsed malignant glioma treated with bevacizumab. Comprehensive MRI studies including diffusion-weighted imaging (DWI) and apparent diffusion coefficient maps (ADC) obtained at the time of disease progression, after initiation of bevacizumab treatment, and at the time of treatment failure were reviewed. Presence or absence of restricted water diffusion within enhancing and non-enhancing tumor areas were assessed at the time of best tumor response based on conventional criteria and thereafter until tumor progression. Kaplan-Meier survival curves were calculated. **Results:** 36 individuals with relapsed malignant glioma treated with bevacizumab between 12/05 and 12/07 were identified. In 34 patients who received more than 4 cycles, median time to treatment failure (TTF) and overall survival (OS) were 16 and 32 weeks, respectively. Seven patients were excluded due to insufficient clinical data. 29 patients met eligibility criteria. DW-MRI revealed areas of restricted water diffusion in non-enhancing tumor areas in 11 individuals. The analysis is ongoing. We will further assess the median time interval between initial occurrence of DWI/ADC abnormalities and TTF and correlate presence of diffusion abnormalities with TTF and OS. **Conclusion:** DW-MRI may be a sensitive tool to detect early tumor progression in patients treated with bevacizumab. Whether it may serve as a more accurate outcome measure than post-gadolinium T1-weighted sequences in this patient population will require prospective evaluation correlating DWI and ADC findings with performance status and survival.

P101

EVALUATION OF ANTI-ANGIOGENIC THERAPY RESPONSE IN PATIENTS WITH GBM; A HOMODYNAMIC RESPONSE IMAGING STUDY

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Aim: The aim of this study was to evaluate the sensitivity of a novel fMRI method, hemodynamic response imaging - HRI, to assess therapy response of patients with GBM who received antiangiogenic therapy. We have previously presented this method using hyperoxia and hypercapnia for the detection of vascular functionality and maturation. This method has been shown to facilitate detection of mature vessels resistant to anti-angiogenic therapy in animal models, and to differentiate between various tissue types with high sensitivity in humans1, 2. **Methods:** Three patients with recurrent GBM treated with a combination of anti VEGF and cytotoxic chemotherapy were scanned on a 3T GE magnet several times before and during the course of therapy with conventional MRI methods and the proposed HRI method, at intervals of 2-8 weeks (total of 10 MR examinations). For the HRI method, gradient-echo EPI sequence was used and two separated scans were performed, each using a block design paradigm with inhalation of either 95%O2+5%CO2 or 95%air+5%CO2 and 100% air. Statistical maps of the signal intensity changes were analyzed and realigned to the contrast enhanced 3D images using Matlab package SPM5. **Results:** At baseline scan (before the initiation of therapy), HRI maps demonstrate areas that response to O2 within the enhanced lesion and show no response to CO2. After two week along the antiangiogenic therapy, responses to CO2 become visible within the enhanced tumor area. This vascular response to CO2 might demonstrate a process of "vessel normalization" as a response to the antiangiogenic therapy. **Conclusion:** HRI is a novel non-invasive method for vascular assessment which provides additional information regarding vessel permeability and maturation. This method shows high sensitivity for evaluating therapy response and might have added value in clinical management.

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P102

PROGNOSTIC SIGNIFICANCE OF GROWTH KINETICS IN NEWLY DIAGNOSED GLIOBLASTOMA: A ROLE FOR PATIENT-SPECIFIC VIRTUAL CONTROLS

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A number of factors have been found to allow stratification of glioma patients according to probable survival times: 1) as to the tumor: type and grade, size and site, and 2) as to the patient: age and neurological functioning, and history of previous treatment (extent of surgery, x-irradiation, chemotherapy). None of these factors concerns the in vivo kinetics of the tumor itself. It is the purpose of this presentation to demonstrate statistically that two such factors, the net rates of proliferation and dispersal, offer independent additional prognostic information, more accurately predicting the duration of survival of the untreated glioblastoma. Our predictive bio-mathematical model for glioma growth and invasion (reviewed in [1]) allowed creation of virtual controls (VCs) for 32 newly diagnosed GBM patients by translating changes seen on serial pre-treatment magnetic resonance imaging (MRIs) into quantitative rates of net proliferation and invasion rates for each glioma. Univariate and multivariate analyses revealed that even when controlling for standard clinical parameters (e.g., age, RTOG RPA classification) the bio-mathematical model parameters quantifying net proliferation rate (&rho) of the glioma cells and the ratio of the net proliferation to the net invasion rate (&rho/D) were significantly associated with prognosis. To our knowledge, this is the first time in which routine clinical MRIs pre-treatment have been translated to prognostically significant measures of glioblastoma net cell proliferation and invasion rate. Such a novel tool for quantifying glioma kinetics has numerous potential applications focusing on the creation of VCs for each glioma against which treatment effects can be measured and in which novel treatment approaches can be optimized. 1. HLP Harpold, EC Alvord, Jr., KR Swanson: Visualizing Beyond the Tip of the Iceberg: The Evolution of Mathematical Modeling of Glioma Growth and Invasion. J Neuropath Exp Neurol, 66(1):1-9, 2007

P103

EFFECTIVENESS OF GOREI-SAN (WULING SAN) FOR ELIMINATING BRAIN EDEMA DUE TO MALIGNANT BRAIN TUMORS

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Objectives: Glyceol, isosorbide and steroids, which are covered by Japanese health insurance system, are widely used to eliminate brain edema produced by malignant brain tumors and to relieve headache and several focal neurological deficits. Their side effects, however, sometimes prevent them from long-term use. The authors have tried to reduce brain edema with one of the traditional oriental medical prescription, Gorei-San (Wuling San) , which promotes diuresis and eliminates dampness. This prescription is constituted of five kinds of herbs, such as Polyporus 3 g, Rhizoma Alismatis 4 g, Rhizoma Atractylodis 3 g, Poria 3 g, and Ramulus Cinnamomi 1.5 g. And it is known to suppress pathologically increased Aquaporin 4 which increases in various pathological conditions such as malignant brain tumor, trauma, cerebrovascular disease, and so on. **Methods:** Between October 2006, and December, 2008, Gorei-San were prescribed to 41 patients (49 courses) with malignant brain tumors (male 24, female 17, ages ranges between 24 to 83 years-old, mean 57.7). Headaches were complained of in 18 cases, and focal neurological deficits were complained of in another 33 cases. The efficacy were evaluated with improvement rate of symptoms and neurological deficits: excellent (Improvement rate more than 50 %), good (Improvement rate less than 50 %);, no effect, and deterioration. **Results:** excellent 13 (26.5%), good 23 (47.0%), no effect 13(26.5%). There were no deteriorated cases. No significant complications were recognized. **Conclusion:** Gorei-San (Wuling San) can be used as a substitute for glycerol, isosorbide and steroids to reduce mild brain edema.

P104

IMAGE GUIDED CONVECTION-ENHANCED DELIVERY OF NIMUSTINE HYDROCHLORIDE (ACNU) IN RECURRENT GLIOMA PATIENTS

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Object. Although Temozolomide improved the survival of patients suffering high-grade gliomas, efficacy of systemic chemotherapy is still not satisfactory. In this study, we investigated the safety of intra- or peri-tumoral convection-enhanced delivery of nimustine hydrochloride (ACNU) and feasibility of image guidance methods in patients with histologically confirmed recurrent malignant gliomas. **Methods.** Eight patients received a total of ten times infusion of intra- or peri-tumoral infusion of ACNU. In seven patients, nine times infusion was aided by image guidance using MRI. After determining the target with MRI, one to three infusion catheters were inserted stereotactically. After confirming the position of the catheters, ACNU solution (0.5-1.0 mg/ml) was infused via CED methods. For image guidance, 1uM meglumine gadoterate (Gd-DOTA) was co-infused with ACNU solution. MR image was acquired subsequently during the infusion to evaluate the distribution of infused drug. **Results.** Catheters were successfully inserted in all patients without any morbidity. No severe neurological deterioration or systemic toxicity was noted in any patients during and after infusion. MR image successfully detected the distribution of infused solution during infusion, though several problems including rapid clearance of Gd-DOTA were found. Four patients demonstrated stable disease and one patient partial response after the treatment. **Conclusion.** Intra- or peri-tumoral convection-enhanced delivery of nimustine hydrochloride (ACNU) can be performed safely with MR image guidance. Image guidance using co-infused Gd-DOTA visualized the infusion. Although there still are problems to be resolved, this strategy may improve the efficacy of chemotherapy against high-grade gliomas.

P105

CONVECTION ENHANCED DELIVERY OF NIMUSTINE HYDROCHLORIDE (ACNU) WITH SYSTEMIC ADMINISTRATION OF TEMOZOLOMIDE IN RECURRENT GLIOMA PATIENTS

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Object. Despite aggressive multimodal therapy, the prognosis of patients with high-grade glioma remains dismal. Temozolomide improves the survival of patients with high-grade glioma, median survival is still less than 15 months, and further exploration is needed in order to increase the efficacy of temozolomide-based regimens in the treatment of high-grade glioma. O6-methylguanine-DNA methyltransferase (MGMT) causes the chemoresistance of tumor cells to temozolomide. Co-systemic administration of temozolomide and other alkylating agents such as nimustine hydrodhloride (ACNU) might result in depletion of O6-methylguanine-DNA methyltransferase (MGMT), however enhance systemic side effect. Convection-enhanced delivery (CED) is a local infusion technique that delivers chemotherapeutic agents directly to the central nervous system, circumventing the blood-brain barrier and reducing systemic side effects. The abundant O6-alkylguanine produced by continuous infusion of ACNU using CED might deplete MGMT and enhance the efficacy of temozolomide. This study evaluated the safety and efficacy of combined-modality treatments using CED of ACNU with systemic administration of temozolomide. **Methods.** Three patients with progressive recurrent gliomas (two with glioblastoma, and one with diffuse astrocytoma) were treated with CED of ACNU (0.5mg/ml, day 1) with systemic administration of temozolomide (150-200mg/m^2, day 1-5). MR imaging was used to confirm accurate cannula placement and monitor drug distribution. **Results and conclusion.** Neither patients had clinical or imaging evidence of short- and long-term infusate-related toxicity. ACNU can be delivered safely and effectively to the human gliomas at therapeutic concentration and over clinically relevant volumes using convection enhanced delivery. All patients are still alive 1-5 months after CED treatment. While the efficacy of direct infusion of ACNU with oral administration of temozolomide in recurrent glioma cannot be determined until more patients are treated, all patients tolerated convective infusion of brain without irreversible toxicity. Patients with recurrent gliomas may benefit from this treatment paradigm.

P106

SURVIVAL OF PATIENTS WITH GLIOBLASTOMA MULTIFORME AT HARTFORD HOSPITAL

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Objective: Prognosis for patients (pts) with glioblastoma multiforme (GBM) remains poor, even with treatment with radiation (RT) and chemotherapy (CTX). This retrospective study evaluates survival for pts with GBM treated at Hartford Hospital over a ten years period. The purpose is to determine whether evolving treatment modalities influenced survival.**Design/ Methods:** From 1997 to 2007 181 pts with GBM were treated at Hartford Hospital. Median age was 59 years (24-87 years). There were 105 males and 76 females. All pts had surgery (32 biopsy, 123 subtotal resection, 26 gross total resection). 19 had second and 6 had third resection. 176 pts had RT, 24 pts had combination RT and temozolomide (TMZ). 83 pts had adjuvant CTX with BCNU, PCV, and starting in 1999 with TMZ. 42 pts were treated at recurrence with CPT-11 or Carboplatin and Etoposide, more recently CPT-11 and Bevacizumab. Survival data was analyzed by age and treatment modalities and compared with national SEER reported data and with 10 years survival reports in the literature. **Results:** Median survival for the whole group was 70 weeks (4- 719 weeks). 1 year, 2 years, 3 years, 4 years, 5 years and 10 years survival was 30%, 10%, 6%, 5%, 5% and 3.6% respectively, similar to SEER data. Survival was significantly better for pts younger than 45 years of age (40% at 1 year, 30% at 2 years, 23% at 3 years, 15% at 5 years and 10 years). Median survival was 70 weeks for pts who received adjuvant CTX (76 weeks with TMZ) vs. 40 w eeks with RT alone). CTX at recurrence further prolonged survival (150 weeks with CPT-11 or combination TMZ and 6 thioguanine). **Conclusions:** Our survival data compares favorably with the national SEER data and reports from other institutions. Multimodality therapy helps prolong survival for patients with GBM.

P107

SINGLE CENTER SURVIVAL OUTCOMES OF 167 GLIOBLASTOMA PATIENTS: BEFORE AND AFTER TEMOZOLOMIDE

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Purpose: Evidence based standard treatment of glioblastoma (GBM) is radiotherapy (RT)+concomitant and adjuvant temozolomide (TMZ), currently. In this study, we aimed to assess the value of addition of TMZ in a cohort of 167 GBM patients, treated in a single center consecutively.

Patients and Methods: Patients were treated between 1997 and 2008. Median age was 59 years (range 18-82) and male/female ratio 106/61. KPS score ranged between 30 and 100 (median 80). Median duration of symptoms was 1 month (1-84 months) and 19% had a history of convulsions. Multicentricity was present in 9% and the median tumor diameter was 4 cm (1.5-8 cm). Type of surgery was gross total resection in 49%, subtotal resection in 25% and stereotactic biopsy in 26%. All patients received median dose of 60 Gy RT (range 6-72 Gy). Concomitant and adjuvant TMZ was given in 76 patients (45%), after 2004. Overall survival (OS) curves were plotted by Kaplan-Meier method, and differences were calculated by log-rank test. Multivariate analysis of prognostic factors was performed by Cox regression analysis.

Results: Median OS was 12 months (%95 CI 10.18-13.82), and 2 year OS rate was 15% for entire cohort. In multivariate analysis, younger age (p=0.001), higher KPS score (p=0.006), longer history of symptoms (p=0.05), single lesion (p=0.029), type of surgery (p=0.005) and addition of TMZ (p<0.001) were observed to be predictive factors for higher OS rates. Patients treated with RT+TMZ had a median OS time of 16 months (%95 CI 13.94-18.06) compared to 9 months (%95 CI 6.61-11.39) with RT only (p<0.001). Two-year actuarial survival rates were 28% and 5%, respectively.

Conclusions: In the TMZ era, classical prognostic factors continue to be significant for survival. Out of clinical studies, TMZ addition to RT has increased the overall survival of GBM patients in this series very significantly.

P108

CLINICAL OUTCOMES OF ADULT SUPRATENTORIAL GRADE 3 MALIGNANT GLIOMAS IN A SINGLE INSTITUTION

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Objectives: We analyzed the clinical outcomes of adult supratentorial grade 3 malignant gliomas including anaplastic oligodendrgoglioma (AO), anaplastic oligoastrocytoma (AOA) and anaplastic astrocytoma (AA) and the relationship between their prognoses and 1p/19q LOH and MGMT (O6-methylguanine-DNA methyltransferase) promoter methylation status. **Methods:** Ninety newly diagnosed grade 3 malignant glioma patients were treated with in National Cancer Center Hospital in Japan from 1996 to 2008. **Results:** The average age of AO (n=10), AOA (n=18) and AA (n=62) were 47.8, 44.9 and 51.3 years old. RT plus ACNU (nitrosourea) or RT plus ACNU and VCR (vincristine) were used for AA or AO/AOA until 2007 as an initial therapy. Then ACNU or PAV (Procarbazine, ACNU, VCR) were used for AA or AO/AOA as a maintenance therapy for 2 years. RT plus TMZ (temozolomide) have been used for all grade 3 gliomas from 2007. Median survival time (MST) in AO, AOA and AA were NR (not reached), 54.5 months and 28.6 months, respectively. Progression free survival (PFS) of each patients are NR, 44.9 months and 15.0 months, respectively (p=0.08). **Discussion:** Malignant gliomas including oligodendroglial component have better prognoses than AA as reported so far. We are examining the genetical backgrounds of each tumor specimens and their influence for sensitivity of radiation therapy and chemotherapeutic agents.

P109

GLUCOCORTICOID-INDUCED BONE FRACTURE IN PROGRESSIVE GLIOMA PATIENTS

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[Objective] Glucocorticoid is necessary for progressive glioma patients with cerebral edema to improve their neurological symptoms and to keep performance status (PS). However, one of side effects of glucocorticoid is osteoporosis and bone fracture that worsen PS of glioma patients. Therefore, we examined the frequency of a bone fracture and its problems in glioma patients. **[Methods]** One hundred ninety five consecutive adult glioma patients (109 men and 86 women, mean age: 51.1 years old) were treated in Neurosurgery Division, National Cancer Center Hospital, Japan from 2002 till 2007. We reviewed their clinical records and picked up the patients with a bone fracture. Then, we examined the cause of fracture and duration and total dose of glucocorticoid in those patients. **[Results]** One hundred seventeen among 195 patients (60%) received glucocorticoid after they showed such neurological symptoms as hemiparesis because of a tumor progression or a cerebral edema. Eight (total 6.8%; 3 men (4.6%) and 5 women (9.8%)) among 195 patients had a fracture. Seven patients had a lumbar compression fracture and 3 patients (2.6%) had a femoral neck fracture. Two patients had fractures in both locations. Only one woman among 78 patients without use of glucocorticoid had a femoral neck fracture. Mean duration from use of glucocorticoids until fracture was 11.5 months (1-27 months). Average dose of oral glucocorticoid administered for the patients with fractures was 17.5mg (as prednisolone equivalent) at a fracture and average dose was 180 mg x months. All patients became disable to walk after a fracture. **[Conclusions]** Long-term application of glucocorticoid increased the risk of bone fracture in glioma patients. To increase quality of life in progressive glioma patients, we analyzed the relationship between glucocorticoid and fracture and discuss the prevention of fracture in glioma patients.

P110

NEWLY DIAGNOSED PREGNACY AND GLIOBLASTOMA MULTIFORME (GBM): AN ETHICAL COLLISION COURSE

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35 year old right handed female presented with an unprovoked seizure April 08. She was started on lamotrigine. Imaging studies at outside facility showed a right frontal lesion concerning for a stroke. Repeat MRI with perfusion studies at our institution 5 8 08 showed a lesion most consistent with a tumor. Routine pre-op pregnancy test was positive. Patient elected to maintain the pregnancy and proceed with the surgery. She underwent a craniotomy 5 12 08 and gross total resection of her lesion. Pathology revealed a glioblastoma multiforme, 1p19q intact and EGFR amplified. Her post-op beta human chorionic gonadotrophin was 800 mU/ml. Prognosis and treatment options were discussed. The patient wished to maintain her pregnancy and hence our recommendation was for radiation therapy with shielding without concomitant chemotherapy. Patient and husband refused any form of adjuvant treatment at that time. She was followed clinically with high risk obstetrics (OB). Mid July she presented with progressive headaches and left hemiplegia. MRI revealed a large cystic recurrence. She underwent a second craniotomy July 23 2008. Pathology again showed GBM. Seen by high risk OB and fetus was stable. Post operative she consented to radiation therapy with shielding and completed 60 Gy. She completed radiation therapy without specific complications and underwent a non contrasted MRI that showed minimal change from her preop MRI. She developed progressive headache and HELLP syndrome at 37 1/7th weeks and underwent a primary low transverse cesarean section. A healthy baby girl was delivered 12 5 08 with APGAR scores of 9 and 9 at one and five minutes. This presentation will explore the various ethical dilemmas faced during the care of the patient and fetus including surgery, radiation (shielding) and the role of chemotherapy.

P111

VNTRICULAR AND LEPTOMENINGEAL SEEDING IN A PATIENT WITH MEDULLARY ANAPLASTIC OLIGODENDROGLIOMA

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Intracranial metastasis originating from the glial tumors is rare. In a review of 116 cases of metastatic gliomas, the commonest metastasizing tumor was glioblastoma (41.4%) and oligodendrogliomas(ODGs) accounted for the lowest metastasizing rate(5.25%). We have treated a very unusual metastatic intraventricular and parenchymal metastases from a medullary anaplastic ODG(AODG). A 38-year-old woman was presented with the left facial pain and hemiparesis. She has been treated with steroids under the diagnosis of idiopathic pulmonary fibrosis for the past several years . She had facial hypesthesia on the left side with motor weakness of grade IV and hyperreflexia and pathologic reflex in the left extremities. Brain and Cervical MRI showed a discrete mass with a little enhancement in the left medulla oblongata extending down to C2 level. A midline suboccipital craniectomy with C1 laminectomy was done to excise the tumor partially. The pathology was turned out to be AODG. She was given conformal RT of 5,600 cGy to the medullary tumor followed by 12 cycles of Temozolomide(TMZ) to result in partial remission for 30 months. A routine follow up MRI revealed a well defined enhancing intraventricular mass with stable medullary tumor. Right frontal interhemispheric approach was done to remove the septal and intraventricular tumor totally. On the second postoperative day she had another emergency surgery to decompress venous infarct complicated by draining vein injury. Three months later she developed communicating hydrocephalus, for which she underwent a VP shunt. Six months after the ventricular tumor removal she developed generalized seizure showing bilateral medial temporal enhancing tumors. The medullary tumor remained stable. Postoperative WBRT of 6000cGy/6weeks was added. Even with active rehabilitation, her neurologic condition deteriorated gradually to be bed-ridden 38 months after the initial medullary surgery. We report this unusual upward intracranial metastases from a medullary AODG, even the original tumor remained stable.

P112

LONG-TERM OUTCOME OF LOW-GRADE GLIOMAS TREATED WITH RADIOTHERAPY-DEFERRING THERAPEUTIC POLICY

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Purpose: The therapeutic strategy for low-grade gliomas is still controversial especially for the use of radiotherapy. The purpose of this study is to know the long-term outcome of low-grade gliomas treated with a radiotherapy-deferring therapeutic policy. **Methods:** We have treated all low-grade gliomas with radiotherapy-deferring therapeutic policy, and analyzed 60 consecutive adult patients having diffuse astrocytoma (25 cases) or oligodendroglial tumors (35 cases). The treatment protocol was as follows: i) simple observation even after incomplete resection of tumors showing no evidences of progression, ii) salvage resection for the surgically-accessible recurrent tumors, iii) nitrosourea-based chemotherapy for progressive oligodendroglial tumors, and vi) radiotherapy for progressive astrocytoma. **Results:** The median follow-up period was 7.0 years and no patient was lost during the follow-up periods. All the patients with oligodendroglial tumors survived without receiving radiotherapy (10-year survival rate: 100 %), and the 5-year and 10-year progression free survival rates were 89.6% and 65.1 %, respectively. The patients in their sixties had a significantly higher recurrence rate, and two of them (29%) suffered from leukoencephalopathy following the chemotherapy alone. Among the patients with diffuse astrocytoma, 9 (36 %) subsequently received radiotherapy, and 7 of the 9 irradiated patients died. The 10-year survival rate of diffuse astrocytoma patients was 60.0 %. MIB-1 labeling index has predictive value for malignant transformation and subsequent shorter survival in diffuse astrocytoma. **Conclusions:** Our data suggest that low-grade oligodendroglial tumors can be successfully treated by the nitrosourea-based chemotherapy alone without applying radiotherapy.

P113

CONSIDERATIONS FOR DIAGNOSIS AND TREATMENT OF CLASSICAL GLIOMATOSIS CEREBRI

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Gliomatosis cerebri (GC) was included in the new WHO classification (4th edition) in the group of asrocytic tumors and the diagnostic criteria was also varied slightly. Because GC is a rare tumor, there were no large-sized studies and histological variation, such as astrocytoma, oligodendroglioma even glioblastoma, existed in the previous reports. For these reasons, we still could not establish an appropriate treatment. This time, we discuss the issue of making diagnosis and treatment of GC by means of clinical course and radiological findings of one case of classical GC we experienced. We present a 15-years-old man who had a classical GC. He complained neck pain and tinnitus of more than 1.5 year's duration, and blurred vision for a half year. These symptoms were getting worse but nobody could make the diagnosis of GC with a CT scan at the local hospital. MR imaging revealed a diffuse lesion spreading at each side of whole brain, especially brain stem and cerebellum, and no tumor mass could be detected on contrast enhanced MRI. Although ICP was elevated, lateral and third ventricles were enlarged and acquired Chiari malformation was shown. VP shunting improved his symptoms and histological examination of biopsy specimens revealed diffuse glioma. He received 60Gy irradiation to the whole brain and the lesion responses completely. Seven months after radiotherapy, he complained gait disturbance and sensory disturbance in his chest and MRI of the spine revealed a tumor spreading at entire spine. A spinal coed lesion responded partially with 35Gy irradiation, and then we started treatment with temozolomide administration.

P114

GROWTH RATES OF METASTATIC BRAIN TUMORS IN NON-SMALL CELL LUNG CANCER; ESTIMATION FROM MICRO-METASTASIS

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Among 1,372 lung cancer patients without brain metastasis who underwent resection of the lung cancer at our institute from 2001 to 2007, brain metastases developed in 72 patients (5.2%) during their hospital course. We hypothesized that there were micro-metastases in the brain at the time of lung surgery in these patients, even though there was no detectable brain metastases in the MRI. The purpose of this study was to evaluate the growth rates of metastatic brain tumors in this unique subset of patients, and to compare it with our previous report that calculated growth rate during chemotherapy. Among 72 patients, 22 patients with cystic or hemorrhagic metastases were excluded. 77 metastatic brain tumors in 50 patients were reviewed. 25 patients underwent adjuvant or neoadjuvant chemotherapy, however, for the rest of the patients, chemotherapy was not added after lung cancer surgery. Tumor volume was determined by using V-works software (v. 4.0) (Cybermed, Seoul, Korea) and T1 gadolinium enhanced MR images.The median tumor growth rate was 12.5 mm3/day (interquartile range, 5.7-32.6). There were no statistically significant differences between lung cancer stages, and the growth rates were similar regardless of chemotherapy. The growth rate reported here corresponded to that of our previous report (12.1 mm3/day). These findings may help optimize patient management during follow up.

P115

PROGNOSTIC FACTORS IN 57 PATIENTS WITH METASTASTIC BRAIN TUMORS FROM BREAST CANCER.

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Introduction: For neurosurgeon, it is difficult to analyze whole metastatic brain tumor patients from breast cancer who had neurosurgical therapy or medical therapy or radiation therapy. In this manuscript, 57 metastatic brain tumor patients from breast cancer who had not only neurosurgical treatment, but also medical treatment or radiation therapy, were reviewed and prognostic factors were analyzed by both univariate and multivariate analysis. **Patients:** 57 metastatic brain tumor patients from breast cancer were treated in our single hospital between January 2003 and October 2008. Median age is 56 years old. **Treatments:** 21 patients were undergone both surgery and whole brain radiotherapy. 5 patients were undergone surgery alone. 24 patients received radiotherapy alone. 7 patients received medical supports alone.Results: Median survival was 7.4 months. 1 year survival rate was 37%. Under univariate analysis, metastic status for other organs, positive rate of hormone receptors (ER, PR, HER2) were not significant as prognostic factors. Single brain metastasis($p=0.031$), resection($p=0.013$) and triple negative for hormone receptors($p<0.001$) were significant prognostic factors. Under multivariate analysis, resection($p=0.019$) and triple negative for hormone receptors($p=0.005$) were significant prognostic factors. **Conclusions:** Single brain metastasis, resection and HER2 positive were good prognostic factors. Triple negative for hormone receptors was poor prognostic factor.

P116

INTRAMEDULLARY SPINAL CORD METASTASIS IN BREAST CARCINOMA, CASE REPORT.

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Case report: 41-year-old premenopausal woman was presented with a locally advanced tumour of the left breast in 2001. Histopathological examination revealed an infiltrating ductal carcinoma. She underwent oncotherapy and surgical ovarectomy. She achieved complete remision. In August 2007, reccurency of the tumour was diagnosed in the left breast and a right axillary lymph nodes. She started hormonal therapy and underwent left breast ablation and bilateral axillary lymph nodes dissection followed by oncotherapy. This woman felt severe weakness of lower extremities in August 2008. A whole body PET/CT showed only tumour of the spine or spinal canal at level L1. MRI showed leptomeningeal infiltration of the spinal cord at L1 (medullary conus) and cauda equina. Laminectomy L1 was performed and partial resection of the intramedullary and cauda equina tumour was achieved. Histology revealed breast carcinoma metastasis. Postoperatively she underwent local radiotherapy. Her clinical status, especially right leg weakness and sensory loss in the lower extremities, immediately improved, but she still was not able to walk. The case report is documented by MRI, histological, immunohistochemical and cytogenetic examinations. Discussion: patients who have no evidence of widespread organ metastases or multiple intramedullary lesions and who have a life expectancy of at least a few months with tumours of nonlymphomas histology should be considered for tumour resection. Intramedullary spinal cord metastases are infrenquently observed. According to all cases previously published in the English literature, they clinically affects only 0.1-0.4% of all cancer patients. Lung cancer (54%) with breast carcinoma (11%)accounted for a majority of cases. We found only 7 cases of surgical resection (including 2 cases with localisation in medullary conus) of breast spinal cord metastases in the literature. To our knowledge, we report the first case of a succesful surgical treatment of metastatic leptomeningeal infiltration in medullary conus and cauda equina.

P117

SPINAL CORD METASTASIS OF ADENOCARCINOMA, CASE REPORT

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Introduction: Metastasis of adenocarcinoma to the spinal cord is a rare condition, that accounts in approximately 1-3% of spinal tumours. **Case report:** The authors present the case of a 74-year-old man who suddenly developed quadriparesis and was subsequently diagnosed with a tumour of the cervical spinal cord at level C4. The spinal tumour was removed surgically and a metastasis of an adenocarcinoma was shown histologically to originate from the colon. **Discussion:** Early diagnosis of spinal metastases by means of magnetic resonance imaging (MRI) and microsurgical resection can improve prognosis and neurological deficit of patients to certain extent. The available literature presents all together 71 cases of surgery, which is recommended in indicated patients, particularly in those with a potentially radio-resistant metastasis. The aim of surgery is the decompression of functional nerve tissue and histological verification of the tumour origin. Survival time of surgically treated patients is approximately twice longer that in those without surgery.The case report is well documented by preoperative and postoperative MRI, histological, immunohistochemical and genetic examination of the tumour.

P118

COMPLETE RESPONSE OF A LARGE RECURRENT METASTATIC BRAIN TUMOR TREATED BY CYBERKNIFE: A CASE REPORT AND REVIEW OF LITERATURE

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The CyberKnife(Accuray Incorporated, Sunnyvale, California, USA) is a frameless stereotactic radiosurgery system with real-time image-guidance to provide high-dose radiation with more accurate target localization and dose delivery. The CyberKnife has proven to play an important role in management of metastatic brain tumors, especially those that recurred following whole brain radiotherapy. Most metastatic brain tumors smaller than 1 cm3 have shown complete response after CyberKnife radiosurgery. However, a dramatic change in size of large metastatic brain tumors is rarely discussed in the literature. Here, we report a case of adenocarcinoma of the lung with brain metastases. Because the metastatic brain tumor initially measured about 3 cm in diameter, the patient had previously undergone two craniotomies followed by external beam radiation to the resection cavity. The tumor subsequently recurred and was treated with CyberKnife radiosurgery. Complete disappearance of the recurrent tumor by magnetic resonance imaging three months after radiosurgery was observed. The relevant literature is also reviewed.

P119

CONTINUOUS INTRATHECAL TREATMENT WITH METHOTREXATE VIA "VENTRICULAR PORT" FOR LEPTOMENINGEAL DISSEMINATION OF MALIGNANT TUMORS

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Recently, sustained-release cytosine arabinoside : DepoCyt, has been reported to be useful for the treatment of leptomeningeal dissemination of malignant tumors. But it is still off-label pharmaceutical in Japan. We succeeded at continuous intrathecal administration of arbitrary antineoplastic agents via "Ventricular port" . "Ventricular port"is a subcutaneous port (P-U Celsite port brachial ,Toray Medical Industries,Tokyo, Japan) connected to a ventricular catheter. The goal of the present study is to determine the effect and the safety of continuous intrathecal treatment(CIT) with methotrexate(MTX). Twenty patients with leptomeningeal dissemination (primary disease 10 cancers;6 gliomas and 4 lymphomas) were given 2 to 7 cycles of CIT with MTX 10 mg administered into the lateral ventricle for 5 consecutive days biweekly. The concentration of MTX in the lateral ventricle was 7 to 10 x 10-6 M from Day 1 to 4. Response to this therapy included 6 patients with complete remission, 7 with progressive disease, and 7 with stable disease. Kaplan-Meier analysis revealed a median overall survival of 8 months while the overall survival rate for leptomeningeal-specific death 13 months,and that for metastasis from cancer was 5 months,respectively. Complications of CIT with MTX were relatively low(<0.5%), and nausea and vomiting did not occur in any of the patients. In conclusion, CIT with 10 mg MTX via "Ventricular port" for 5 days improved the therapeutic effect and reduced the complications associated with treatment of leptomeningeal dissemination from malignant tumors.

P120

POPULATION BASED EPIDEMIOLY OF CENTRAL NERVOUS SYSTEM (CNS) MALIGNANCIES IN THE GIRONA PROVINCE (SPAIN). RESULTS OF AN 11 YEARS SURVEY (1994-2004).

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Background Epidemiological data incidence regarding survival of CNS malignancies are infrequently reported. We present an updated assessement of survival rates obtained from the Girona population-based cancer registry. **Patients and Methods** Analyses included all cases of primary CNS malignancies registered between 1994-2004. Meningeal and soft tissues tumours were not included. Pathological diagnoses were reviewed and grouped according to the last WHO classification of of CNS tumours (2007). Cases notified only by the death certificate [n= 27 (5.5%)] were excluded from the survival analysis. Overall survival was calculated from the date at diagnosis to the date of death or the end of the study (December 31st, 2005). Probability of survival was calculated according to Kaplan-Meier method. **Results** A total of 493 patients were registered during the 11 years survey study. Distribution by histology was: astrocytic tumours = 243 (49.3%); oligodendroglial and oligoastrocytic tumours = 17 (3.4%); ependimal tumours =13 (2.6%); embryonal tumours= 18 (3.7%) and CNS primary malignancies without histological confirmation = 202 (41.0%) The mean ages for embryonal tumours and CNS primaries without histological confirmation were respectively 18.2 yrs (±SD) and 66.3 yrs (±SD). A predominance of elderly patients was demonstrated. An increasing incidence of glioblastoma was also observed along the study period. Five-years overall survival rates were: astrocytic tumours=14.6%; oligodendroglial and oligoastrocitic tumours =35.7%; ependymal tumours=41.0%; embryonal tumours=32.4% and CNS malignancies without histological confirmation=7.5%. **Discussion** Our data showed a clear increased incidence of astrocytic tumours by age. Survival figures showed that ependymal and oligodendroglial tumours were the histological groups with a better survival rate. This is consistent with previously published data. The group of patients with CNS malignancies without histological confirmation had the worst survival figures. This finding is due to a more conservative attitude in the diagnosis and treatment of older patients.

P121

THE FIRST FINGERPRINTS OF NEUROONCOLOGY IN PREHISTORIC ERA; PREHISTORIC TRAPENATED SKULLS MAY BE THE FIRST ATTACT TO TREAT TUMORS IN NEUROONCOLOGY

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Prehistorical skull trepanations were discovered in some areas of the world. In this study we reported the operated skulls discovered in Samsun-Ikiztepe. These skulls were evaluated in the aspect of the possibility of the first effort to treat tumors in neurooncology. Four prehistoric skull skeletons were found in Samsun-Ikiztepe area. The craniectomy sites were located on the right parietal bone in two subjects. One of them had occipital craniectomy defect. In remaining one of them craniectomy was located on the superior sagittal suture line. In this study we described general charactheristics of prehistoric skull trepanation in the aspect of first fingerprints in neurooncology. Key words. Prehistoric skull skeletons, history of neurooncology.

P122

EXPANDED ENDONASAL RESECTION OF SUPRA- AND EXTRA-SELLAR LESIONS

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Introduction: The aim of this study was to report the results of a consecutive series of patients undergoing pituitary and non-pituitary surgery using a fully endoscopic endonasal approach and to evaluate the efficacy and safety of this procedure. **Methods:** For last14 months, we performed a fully endoscopic endonasal resection for 51 patients with pituitary and non-pituitary lesions in a single institute. Among them, we reviewed patients who underwent expanded endonasal resection for supra- and extra-sellar lesions. **Results:** Among 51 patients, a total of 21 patients had supra- and extra-sellar lesions including anterior cranial base meningiomas, giant pituitary adenomas, and craniopharyngiomas, etc. Their mean age was 51.5 years with a range of 27 to 84 years. There were 13 men and 8 women. Gross total resection was performed in 12 patients and subtotal resection in 9 patients. Postoperatively, CSF leakage requiring second revision operation developed in only one patient, vasospasm in one patient, and rebleeding requiring transcranial approach in one case. For even giant pituitary tumors, optic nerve and chiasm was not necessary to be intentionally visualized, because large pituitary tumors could be removed without interrupting the arachnoid membrane. **Conclusions:** Expanded endonasal resection for anterior cranial base lesions is still challengeable. Our series is too small to determine the clinical outcome and to compare its superiority to the conventional microscopic approaches. However, we believe that to share the preliminary experiences with each other institutes can be helpful to achieve the optimal surgical results in the treatment of anterior cranial base tumors.

P123

EXTENDED ENDOSCOPIC ENDONASAL TRANSSPHENOIDAL APPROACH FOR SKULL BASE LESION - AVAILABILITY OF MULTILAYERED RECONSTRUCTION METHOD -

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Background: Endoscopic surgery is suitable for transsphenoidal approach in the point which can be performed by bright vision and by minimum invasiveness. Using bilateral nostril approach, extended transsphenoidal surgery (E-ETSS) is able to be applicated easily. We examined the availability and issue of E-ETSS based on our experience. **Materials and methods:** Between 2004 and 2008, total 15 procedures of E-ETSS were performed in our institute. They included 8 meningiomas, 2 pituitary adenomas, 2 Rathke's cleft cysts, 2 chordomas and 1 craniopharyngioma. Operation was performed through bilateral nostrils using rigid endoscope with preparing wide mucosal flap from one septal mucosa Bone window was enlarged anteriorly to planum sphenoidale, laterally to expose the cavernous sinus and inferiorly to expose basilar artery depend on the lesion. Reconstruction of skull base after removal of the tumors were performed by multilayered method with PGA sheet and mucosal flap. **Results:** Gross total removal was achieved in all meningiomas, pituitary adenomas, and craniopharyngioma in the 11 frontal base lesion. Intracavernous extension of meningiomas and chordomas were partially removed. No cranial nerve injuries and visual disturbance was observed after surgery. CSF leakage was observed in one patient who was required surgical repair. Spinal drainage was performed in 10 of 11 frontal base lesion and continued for average 5.2 days. Excellent mucosal adhesion was observed within 3 months after the operation when we examined endoscopically. **Conclusion:** Extended endoscopic approach thorough bilateral nostrils and multilayered reconstruction of skull base with mucosal flap and PGA-sheet are safe and effective procedure.

P124

RESULTS OF EXTENDED TSA TO ANTERIOR SKULL BASE

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With development of navigation system and endoscopic surgery, the extension of TSA(transsphenoidal approach) was surprisingly increased with safety. We present the surgical results of extended TSA to anterior skull base including sellar, parasellar and clival lesion. We introduce several tactics of extended TSA for exposure and hemostasisand discuss about complications.From 2001 to 2007, we have done extended TSA on 24 patients. We analyzed extent of tumor removal and postoperative complications retrospectively. Patients were diagnosed pituitary adenoma(5), craniopharyngioma(2), meningioma(1), chordoma(9), chondrosarcoma(1), squamous cell carcinoma(1) and others(6). Cases of pituitary adenoma which showed large suprasella extension or cavernous sinus(CS) extension were included. Transseptal approaches were done only 2 cases in early period and the others were approached via endonasal paraseptal route. Trans-ethmoidal approach was combined in one pituitary adenoma extended to CS. Operations were done in microscopic view assisted endoscopic system. Total or radical removal rate was 88%(15/17). The group of subtotal removal were pituitary adenoma(1), meningioma(1), chordoma(1) patients. These three patients had previous operation on other institution. Squamous cell carcinoma was excluded analysis of removal rate because operation was scheduled biopsy. Others excluded in analysis of removal rate were non-tumorous condition which were CSK leakage(3), arachnoid cyst and rheumatoid arthritis(2) and their problems were resolved successfully after extended TSA. Complication rate was 17%(4/24). There were three CSF leak and one aggravation of visual compromise. One of CSF leak patients undergone reoperation and others were treated conservatively via lumbar drain. There were not ant complication related ICA.Extended TSA was useful and minimally invasive surgery on sellar and parasellar lesions. For safe and successful operation, precise acknowledges of surgical anatomy and technique of hemostasis and reconstruction were necessary. And addition of endoscopic surgical technique and equipment was needed. Navigation system was also useful.

P125

TWO-SURGEONS TECHNIQUE FOR INFRATEMPORAL FOSSA TUMOR; SIMULTANEOUS LATERAL TRANSZYGOMATIC AND ENDOSCOPIC ENDONASAL APPROACHES WITH TECHNICAL NOTE

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Although the advent and widespread development of endoscopic techniques have combined with transcranial microscopic surgery for skull base, its clinical adaptation for lesions around middle skull base has not been published to date. Endoscopic approach may have several advantages for the lesion in the infratemporal fossa (ITF), compared to traditional lateral approaches. However, the limitation of endoscopy is the inability to safely access to the rostral part of the ITF, especially in a case with extension into the middle skull base. To overcome this limitation, we used combined endoscopy and transcranial approaches for recurred carcinomas on the ITF.Two recurred carcinomas (adenoid cyst carcinoma, nasopharyngeal carcinoma) were mainly located on ITF with extension into middle skull base. Initially, endoscopic endonasal approach was used for debulking and peripheral demarcation of tumor. After deskeletalization of middle skull base via lateral transzygomatic approach, two surgeons could simultaneously access to the rostral part of the ITF and perform the gross total resection with preservation of the trigeminal nerve (V2 and V3). Despite the uncertain local control due to small number of cases and short follow-up duration, this combined approach may prove to be safe and effective under multidirectional visualization to the ITF and middle skull base.

P126

APPLICATION OF ENDOSCOPES IN MINIMALLY INVASIVE NEUROSURGERY

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Objective: The use of endoscopes in neurosurgery has made an unexpected progress during recent years. Advanced endoscopes have simultaneously stimulated the development and application of minimally invasive treatment concepts in many neurosurgical fields. Now they are considered as a well-established operative technique in skull base surgery. We will present our experiences of the application of endoscope in the skull base surgery. **Materials and Methods:** Various rigid and/or flexible endoscopes were used according to the indication and the planned operative approach. Neuroendoscopic interventions were applied for therapeutic interventions for more than 1000 skull base surgeries. Special monitor, instruments and Navigator were applied in some situations. **Results:** From 1997 to 2008, we have successfully performed more than 1000 endoscopic or endoscopy assisted microneurosurgical interventions, including 850 pituitary tumors, 30 skull base lesions, 30 acoustic neuromas, 20 intracranial aneurysms, 20 intracranial arachnoid cysts, 40 neurovascular compression syndromes, 50 hydrocephalus. No mortality was found. The morbidity rate is below 1% **Conclusion:** Neuroscopy has become an essential part of minimally invasive neurosurgery in skull base surgery. We feel that this technique will be more acceptable to treat a vast majority of skull base lesions with low morbidity and short hospital stay.

P127

CLINICAL CORRELATION BETWEEN PERITUMORAL EDEMA AND VARIOUS FACTORS IN MENINGIOMA.

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Introduction: Peritumoral edema in meningioma is important for clinical progress. The purpose of this study was to analysis the relationship between the clinical characteristics and the formation of peritumoral edema in meningiomas. **Methods:** From February 2005 to March 2008, we removed the meningioma from thirty patients whose mean age was 53.9 years old (29 - 73) and 1 : 1.5 (Male : Female). We checked edema index (peritumoral edema volume/tumor volume, EI=1; no peritumoral edema) in all patients. The patients were evaluated by MRI, MRS and pathology. We analyzed peritumoral edema according to patient sex, age, tumor size, peritumoral rim, pial blood supply, MR spectroscopy (NAA, tCho, tCr, Lip and Alanine) and pathology. **Results:** We evaluated various clinical factors with multivariate regression analysis. Pial blood supply was statistically significant (P = 0.035). However, sex, age, tumor size, peritumoral rim, MR spectroscopy and pathology were not significant. Especially, we analyzed between MR spectroscopic and peritumoral edema using various methods, but we could not find the correlation of that. **Conclusions:** Pial blood supply was associated with peritumoral edema, but sex, age, tumor size, peritumoral rim, MR spectroscopy and pathology were not associated. However, this result needs further study, because the number of patient was only thirty.

P128

MENINGIOMA OPERATIONS IN THE ELDERLY PATIENTS: EFFICIENT WAY TO INTERNAL DECOMPRESSION

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Objectives: Great care must be taken in the operations for the elderly because of their premorbid conditions. Blood loss and operation time should be minimized to get satisfactory results. We present the our surgical experiences for the meningioma in the elderly patients

Materials and Methods: The medical records of 10 patients older than 65 years old were reviewed retrospectively. All patients had neurological deficits and incidental meningiomas were excluded. In the large mass, two operators took participated in the internal decompression. One operator usually used ultrasonic aspirator to remove the mass and the other got the hemostasis. And then one of operators performed tumor-arachnoid dissection meticulously.

Results: The mean age was 68 (65-73) years old. Only 2 patients had not medical illness. The meningiomas are located in the convexity (4 cases), falx (4 cases), parasagittal (1 case), and lateral ventricle (1 case). The size of tumor was 47 ± 17 (25-72) mm. Only one case had the preoperative embolization. The operation time was about 5 hours. Four patients were transfused during the operation. The neurological deficits were completely recovered without any postoperative events.

Conclusions: Minimal blood loss and operation time is necessary for minimizing the operative morbidity. Two-operators internal decompression in the large meningioma may be helpful to get successful outcome.

P129

A 57-PATIENT EXPERIENCE ON THE SURGICAL STRATEGIES OF PETROCLIVAL MENINGIOMAS

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OBJECTIVE: To distinguish the possible risk factors in the surgical treatment of petroclival meningioma and define optimal surgical strategies concerning about the balance between postoperative quality of life and tumor progression/recurrence rate.

METHODS: We recruited 57 patients with petroclival meningioma who received operations in our hospital during 2002~2006. There were 14 male and 43 female patients with a mean age of 50.5 years, of which 53 patients (93%) were primary cases. The primary outcomes including postoperative neurological deficits, modified Rankin scale (MRS) score and recurrence rate were evaluated and the possible risk factors were examined. The mean follow-up time was 34 months (range, 10-67 months).

RESULTS: Gross total resection was achieved in 58% of patients. One patient died in the peroperative period because of intracranial hemorrhage. 67% patients experienced new postoperative neurological deficits and 26% patients had a higher MRS score when assessed at follow-up. Postoperative complications were observed in 24 patients. Postoperative radiation or γ-knife therapy was adapted to 19 of the 24 patients who had residual tumors and no progression was found. Radiographic recurrence occurred in 12.3% of patients at a mean follow-up of 42 months. Tumor adhesion, hypervascularity and engulfment of neurovascular structures were three risk factors to increased MRS score (p=0.0002; p=0.0051; 0.0009) and furthermore adhesion to adjacent structures obviously affected the extent of resection (p=0.0029). The risk of postoperative cranial nerve deficit increased with tumor engulfment of neurovascular structures (p=0.0004) and fibrous tumor consistency showed a tendency (p=0.0622).

CONCLUSION: Intraoperatively defined tumor characteristics played a critical role in identifying the subset of patients with an increased risk of postoperative morbidity and an unfavorable postoperative MRS score. Despite of a conservative attitude in the operation, an individual strategy of treatment based on the careful preoperative evaluation could help to improve the quality of life.

P130

RETROSPECTIVE ANALYSIS OF THE POST-OPERATIVE OUTCOME OF PETROCLIVAL MENINGIOMAS

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Introduction: The purpose of this retrospective study was to analyze the outcome and recurrence of petroclival meningiomas. **Methods:** A total of 571 meningiomas were operated upon between Jan.1994 and Dec.2002. Of these, 35 were true petroclival meningiomas that were amendable for follow-up including regular ambulatory visits and a questionnaire. **Results:** The median age of the 27 women and 8 men was 55.4 years. The patients most commonly presented with headaches, trigeminal neuralgiform pain, dizziness, ataxia, unsteadiness, and hearing loss. The median tumor volume was 62 cm3. Histology was mostly meningothelial (16) and transitional (9). Intraoperative radicality according to Simpson was 14 grade 2, 6 grade 3, and 12 grade 4. Four pts. underwent two-staged operations. There was no perioperative death. Six pts. suffered from liquorrhea treated with lumbar drainage or open revision. One post-operative VP-shunt placement, one tracheostomy , one facial nerve reconstruction were necessary. Four patients received radiation therapy for recurrence/progression. Post-OP morbiditiy was improved with regards to hearing loss, trigeminal-type pain, eye movement tempotrarily worsened. Operative radicality was associated with location, medial structure involvement, and size. **Conclusion:** Petroclival meningiomas represent an entity that has a higher than usual treatment related morbidity than meningiomas in other tumor locations.

P131

ADVANTAGES OF THE PTERIONAL APPROACH FOR OLFACTORY GROOVE AND TUBERCULUM SELLAE MENINGIOMAS

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Objective There is much controversy regarding the surgical approach for olfactory groove and tuberculum sellae meningiomas. There is a need for a retrospective review concerning the approach for such tumors in reducing the symptoms, morbidity and mortality.The author is reporting on the advantages of the pterional approach for such cases(5cases of Tuberculum sellae meningiomas, 3cases of Olfactory groove meningiomas and one case of planum sphenoidale meningioma). **Methods** During a period of 5 years(2003 to 2008) there were 9 cases approached by the pterional approach with microsurgical tumor resection(5cases of Tuberculum sellae meningiomas, 3cases of Olfactory groove meningiomas and one case of planum sphenoidale meningioma)The diameter of the tumor ranged from 3.0 to 7.3cm presenting with visual disturbance, anosmia, headache, mental dysfunctions and seizures. **Results** Total removal(Simpson Grade I-II) was possible in all cases with intact parenchyma on postoperative MRIs. There was one death unrelated to surgery(sepsis secondary to pseudomembranous enterocolitis). In all, except for anosmia, the symptoms were improved **Conclusion** The main advantages of the pterional approach is that the main tumor feeders(ethmoidal arteries) can be devascularized early in the dissection, with the dissection of the sylvian fissure a very good orientation of the sellar area can be realized, tumor excision can be done with minimal retraction, contralateral olfactory nerve can be preserved in cases of minimal invasion, and the frontal ascending veins can be preserved to prevent venous infarction postoperativelySo we are of the opinion that the pterional approach is a superior approach compared to other approaches to olfactory groove meningiomas or tuberculum sellae meningiomas

P132

MENINGIOMA ARISING FROM THE LATERAL RECESS OF 4TH VENTRICLE

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Seung-Min Kim, Ki-Seok ParkDepartment of Neurosurgery, Eulji University College of Medicine, Daejon, KoreaThe primary occurrences of meningiomas without attachment of the dura are rare. Clinical considerations and pathophysiologic mechanisms about theses tumors have not been sufficiently explored, and a complete classification has not been accomplished. A 59-year-old female presented with headache and dizziness for 1 year. Neurologic deficits were not found on admission. Magnetic resonance imaging revealed a 52x28 mm mass lesion without dural attachment located in the Lt. lateral recess of the 4th ventricle. The tumor was dumbbell-shape, iso-intense on T1-weighted and heterogenous on T2-weighted images, and became homogenously well enhanced with gadolinium. We removed the tumor via Lt. far-lateral transcondylar approach. Care was taken not to injure the lower cranial nerves and the tumor was totally removed except for a tiny piece which invaded the brain stem. Histologic examination indicated that the lesion was a mixed pattern meningioma of meningothelial and fibroblastic type.We present case of meningioma on lateral recess which should be considered as one type of meningiomas without attachment of the dura

P133

SURGICAL MANAGEMENT FOR TRIGEMINAL SCHWANNOMA

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Surgery has historically been the mainstay of treatment of trigeminal schwannoma. With gross total resection, cure can be achieved. However, before the era of microsurgery, gross total resection was achieved in only 50% of patients, and often with high morbidity and mortality. The advance of microsurgical techniques and skull-base approaches has greatly enhanced the surgical management of these tumors, and outcomes have improved markedly. This report documents 57 cases of histologically verified schwannomas that arose from the trigeminal nerve and were treated surgically in our clinic between December 2002 and May 2008. The patients were 36 women and 21 men of age 45.8 years (range, 17~81 years). The tumor was located mainly in the middle fossa (type A) in six cases, in the middle and posterior fossa (type C) in 36 cases, mainly in the posterior fossa (type B) in 15 cases. Total excision was achieved in 35 cases, subtotal excision was achived in 21 cases, partial cytoreductive excision was achived in 1 case, and there was no mortality in the series. In the latter two groups, all 22 patients underwent planned stereotactic radiosurgery for residual tumor. Outcome after surgery was gratifying. The mean follow-up time for this study was 42 months (range 7~72 months). 52 of the 57 patients who had their tumors surgically excised have no aggravation of clinical symptoms and showed no MR imaging evidence of recurrent disease during follow-up. 38 of them have obvious amelioration of clinical symptoms. Our results indicate that surgical resection to the best of the operator's ability via skull-base approaches, combined with stereotactic radiosurgery for residual tumor, was the secure and efficient treatment for trigeminal schwannoma.

P134

LATERAL OR ANTEROLATERAL APPROACH FOR LARGE DUMBBELL-SHAPED CERVICAL NEURINOMA

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[Abstract] **Objective:** Generally, the two-stage operation (combined posterior and anterior approach) for the large dumbbell-shaped cervical neurinoma is widely accepted by the most of neurosurgeons. The purpose of the research is whether the tumors can be resected with one-stage operation without corpectomy and fusion of the vertebral bodies. **Methodes:** Datas from seven cases of patients (female 3, male 4) with large dumbbell-shaped cervical neurinoma from February 2006 to November 2008 were analyzed retrospectively. There was tumor in the enlarged cervical intervertebral foramen on MRI of the patients. The tumors of the patients were resected through the enlarged cervical intervertebral foramen by the lateral or anterolateral approach. The partial removal of the intracanal tumor of two patients was carried out using posterior approach some time ago in other hospitals. **Results:** Three patients were treated with the lateral approach for the tumor locating above C3 level. The tumors of the other patients were resected by the anterolateral approach without corpectomy and fusion of the vertebral bodies. Total removal of the tumor was obtained with one-stage operation in all seven patients. Six of the seven patients showed excellent neurological improvement. One patient had slightly muscle weakness of the left shoulder remained up to six months after surgery. **Conclusion:** Total removal of the large dumbbell-shaped cervical neurinoma can be obtained with one-stage operation by the lateral or anterolateral approach. The approaches can expose tumors through the enlarged cervical intervertebral foramen and require no laminectomy, discectomy or interbody fusion, which may frequently produce spinal deformity. **[key words]:** Lateral approach, Anterolateral approach Dumbbell-shaped neurinoma, Intervertebral foramen

P135

CLINICAL COURSE OF PIUITARY ADENOMA AFTER APOPLEXY

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Object: To elucidate the characteristics of pituitary apoplexy, the authors retrospectively reviewed the clinical course of pituitary adenomas after apoplexy, which were remnant after surgical resection or managed conservatively in a single institution.**Material and method:** We collected the clinical and radiological data of twenty-eight patients (15 males and 13 females) who were managed for pituitary apoplexy from January1997 to May 2007. Nineteen of 28 patients were operated and 9 tumors were resected subtotally. And 9 patients were managed conservatively in the absence of altered mentality and progressive visual deterioration. Totally, the authors reviewed clinical and radiological characteristics of these 18 tumors. On radiological and pathological examination, the cause of apoplexy was the intratumoral hemorrhage in all cases. **Result:** Seven (39%) of eighteen tumors disappeared completely during the follow-up period and 8 tumors (44%) were decreased in size spontaneously. Two residual tumors regrew increased in size at 55 and 66 months after surgery. One tumor managed conservatively was increased in size due to the resolution of hemorrhagic content without clinical deterioration. Twelve patients were suffered with visual disturbance and vision was recovered in 10 patients. On last clinical follow-up, 9 (50%) patients needed the hormone replace therapy due to the panhypopituitarism. Eight patients complained the diplopia or ptosis and these symptoms resolved spontaneously. **Conclusion:** We showed the spontaneous shrinkage of pituitary adenoma after apoplexy and improved vision and 3rd nerve palsy during the follow-up period. Considering radiologic, visual and endocrinological outcomes, complete surgical resection of apoplexic pituitary adenoma is not mandatory. And conservative management should be considered in the absence of rapid clinical deterioration.

P136

EFFICACY AND SAFETY OF CABERGOLINE AS A FIRST LINE TREATMENT FOR INVASIVE GIANT MALE PROLACTINOMA

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Cabergoline is known to be effective in the treatment of micro- and macro-prolactinoma. We investigated the efficacy and safety of cabergoline in the treatment of invasive giant prolactinoma. Ten male patients with invasive giant prolactinomas participated in this study and their average age was 37.2 years. Before treatment, mean serum prolactin level was 11,426 ng/ml (range: 1,450-33,200 ng/ml) and mean maximum tumor diameter was 50 mm (range: 40-77 mm). They received cabergoline treatment (0.5-3 mg/week) without surgery and radiotherapy. More than 97.7 % decrease in serum prolactin levels was achieved in all patents except one at 3 months after initiation of cabergoline treatment. At the last follow-up (average treatment duration: 19 mo), they showed average 99 % decrease in serum prolactin levels, five of them having normal serum prolactin levels. In all patients, cabergoline treatment caused a significant reduction in tumor size (85 &plusmn 13 %; range: 57-99 %) at first MRI follow-up conducted between 3 to 12 months of cabergoline treatment. A greater reduction in tumor size was achieved by cabergolin treatment of more than 12 months, compared to that of less than 12 months (97 &plusmn 2 % vs. 85 &plusmn 13 %, p< 0.05). An excellent and rapid reduction in both serum prolactin levels and tumor sizes was induced by a low dose of cabergoline in the most of patients with giant prolactinoma. Therefore, cabergoline represents an effective and well-tolerated first-line therapy for invasive giant prolactinoma in male patients.

P137

COMPARISON OF LATERAL AND SUPERIOR WALLS OF THE PITUITARY FOSSA WITH CLINICAL EMPHASIS ON PITUITARY ADENOMA EXTENTION: CADAVERIC-ANATOMIC STUDY

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Pituitary adenomas extend to the suprasellar region via diaphragmal passage and extend to the cavernous sinus via the medial wall. Better understanding of the dynamics of suprasellar and parasellar extension of sellar region pathologies requires microanatomical comparison of diaphragma sellae and the medial wall of the cavernous sinus. This study provides the first detailed quantitative assessment between diaphragma sellae and medial wall of the cavernous sinus. Microanatomical details and histopathological examinations of the diaphragma sellae and medial wall"s of the cavernous sinus were studied in sphenoid block samples obtained from adult cadavers, and the thicknesses of the diaphragma sellae and the medial wall of the cavernous sinus were measured. Mean thickness of the diaphragma sellae was 216.73 ± 51.26 μm in the center and 367.33 ± 133.66 μm in the periphery. Mean thickness of the lower third of the medial wall was 161.53 ± 53.86 µm and that of the upper third was 278.46 ± 162.79 μm. Difference between the thicknesses of the upper and lower thirds was significant (P<0.001). When the central thickness of the diaphragma sellae and lower third of the medial wall were compared, a significant difference was found (P<0.01). Our measurements suggest that when the diaphragma sellae is thick and the stalk opening is complete, the thickness of the lower third of the medial wall could be the major determinant of parasellar extention.

P138

METASTATIC ACTH PRODUCING PITUITARY CARCINOMA (PCA) TREATED WITH TEMOZOLOMIDE(T): CASE REPORT AND REVIEW OF THE LITERATURE

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PCa is a rare tumor defined by the presence of metastatic disease. It is poorly responsive to treatment. The majority are functionning and frequently produce ACTH. This is the first reported case of an ACTH producing tumor treated with T. It is also unusual in that the patient experienced a flare response either to treatment or treatment induced hemorrhage with a transient but symptomatic increase in ACTH production immediately after treatment with T. A 59yo WF underwent STR of a pituitary adenoma followed by post op XRT in 1997. In 2005 she presented with back pain and Cushings disease. Total free urinary cortisol, serum cortisol and ACTH were elevated and accompanied by severe hypokalemia. An extramedullary intradural tumor at L2-3 was resected. Pathology was identical to the pituitary tumor. IHC was strongly positive for ACTH. Post op XRT was given. The Cushings resolved. In 2008 she presented with diplopia and right third nerve palsy caused by a right parasellar mass encasing the carotid artery. MGMT immunoexpression in the spinal tumor was low. T was administered at 150mg/ m2 days 1-5 and was complicated by severe myelosuppression and hemorrhage into the mass. Hypokalemia and elevated ACTH followed but resolved after her second cycle. After 8 cycles, her diplopia and palsy have resolved. Six other pt(5M/1F)with PCa treated with T have been reported. 4 produced prolactin, 1 luteinizing hormone and 1 was silent. Two had MGMT testing which correlated with response. 5/6 responded to T.Responses were often prolonged. None had a flare in hormone production. Hemorrhage/necrosis were documented in one.This pt lends support for the use of T in this rare group of pts.

P139

ISOLATED OCULAR PARESIS IN THE PITUITARY ADENOMA: REVIEW OF 4 CASES

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Objectives: Neuro-ophthalmological consequences of the pituitary apoplexy are included in loss of visual acuity, defect of visual fields, and ocular paresis. Isolated ocular paresis, the clinical symptom of ophthalmoplegia without visual symptoms, is rare. We are to present the characteristics of the pituitary apoplexy with the isolated ocular paresis.

Materials and Methods: We reviewed retrospectively 32 cases of the pituitary adenomas with surgical resection. Four cases (2 male and 2 female) were presented with isolated ocular paresis. The neuro-ophthalomogical, radiological and hormonal characteristics were investigated.

Results: In the clinical symptoms, all the cases had headaches 1-3 day(s) before onset of ophthalmoplegia. One had the left oculomotor nerve palsy, one was the right abducens nerve palsy, and the others were the right total ophthalmoplegia. The involvements of the oculomotor nerve were shown pupil-sparing ophthalmoplegia. In the hormonal studies, one was Cushing's disease, one acromegaly, and the others non-functioning adenomas. In the radiological findings, intrasellar hemorrhages and invasion or compression into the cavernous sinus of the affected side were shown. No suprasellar extensions enough to compress the optic nerve were found. The tumors were removed totally (2 cases) or subtotally (2 cases) by the trans-sphenoidal approach in elective surgeries(mean intervals from ocular paresis to operation: 9.3 days) and the only one case(acromegaly) was explored for the cavernous sinus. The symptoms were gradually improved few days after operation and the abducens palsy was the last to recover. The coagulative necrosis and hemorrhage was found in the pathology of all the cases.

Conclusions: Isolated ocular paresis is one of presenting symptoms of pituitary apoplexy. The outcome of ocular paresis seems to be good with the fashion of elective surgery.

P140

THE EFFECTIVE TREATMENT OF CRANIOPHARYNGIOMA USING HIGHLY CONFORMAL HYPOFRACTIONATED STEREOTACTIC IRRADIATION

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Objective: The development of an effective dose protocol for the treatment of craniopharygioma through planned dose escalation and follow-up analysis. **Methods:** A total of 15 patients with recurrent or post-operative residual craniopharyngioma were treated with Novalis shaped beam radiation. Three fractionated stereotactic radiation protocols were used with effective biological effective doses ranging from 50Gy to 140Gy. Group A comprised three patients who were treated with aproximately 50Gy of BED (range 39.9~53.2). Group B was made up of seven patients and were irradiated with about 66.5Gy of BED(range 61.2~66.5). The third group, group C was made up of 5 patients and were irradiated with more than 80Gy of BED(range 80~140). The equivalent single doses being 10.7 Gy for group A and 12.6 Gy and 15.3 Gy for groups B and C respectively. MRI and ophthalmological evaluation was conducted during follow-up. **Results:** Local tumor control was achieved for all members of group C and for the majority of patients in group B(71%). However all patients in group A showed increased tumor volumes with a median period of 20 months for regrowth. No further visual impairment was recorded for any of the groups while three patients in group C demonstrated improvements in their visual field. **Conclusion:** Effective local control of craniopharygioma was achieved using highly targeted hypofractionated irradiation with a dynamic conformal beam and intensity modulation. No reduction in visual function was recorded in any of the patients despite the relatively high BEDs used (3 to 4Gy daily fraction sizes). Additional long term follow-up is necessary to ascertain the optimal minimum dose and the ultimate efficacy of this approach.

P141

LYMPHOCYTIC HYPOPHYSITIS WITH REFERENCE TO MR IMAGING

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Purpose: Lymphocytic hypophysitis (LH) is a very rare disease but now being recognized more frequently as a cause of pituitary mass or pituitary insufficiency. LH occurs almost exclusively in women during pregnancy or postpartum, but may occur unrelated to pregnancy. The authors report the clinical experience and unique imaging characteristics of LH. **Methods:** Seven cases (M/F=4/3, mean age=42.3 (rage 27-61) years) were included this study. Two out of three female patients were related to pregnancy. Common presenting symptoms were visual disturbances by pituitary mass and polyuria/polydipsia in three patients respectively. None of the patients were related to autoimmune disorders. **Results:** Six patients were pathologically confirmed with trans-sphenoidal route. Biopsy was performed in four patients and mass decompression was performed in two patients with significant visual disturbances. The three patients with diabetes insipidus showed no improvement with steroids therapy. The other two patients with mass decompression showed hypopituitarism and required hormone replacement. All cases showed a thickening of pituitary stalk/infundibulum and a strong enhancement of basal hypothalamus on MR with Gd-DTPA. Five cases revealed a symmetrical triangular shaped diffuse enlargement of pituitary gland and an enhancement of diaphragma sellae of adjacent dura mater. **Conclusions:** LH showed heterogeneity in presenting symptoms and treatment outcome. It was difficult to differentiate LH from other pituitary diseases before tissue diagnosis. Preoperative presumption should be attempted with characteristic MR findings and endocrinological work-up.

P142

CENTRAL NEUROCYTOMA PRESENTING WITH INTRAVENTRICULAR HEMORRHAGE

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Introduction: Central neurocytomas are rare neoplasms that usually occur in young adults and the most commonly arise in the lateral ventricles near the foramen of Monro. Although intracranial hemorrhage is a well-recognized complication in several types of neuroepithelial tumors, intratumoral hemorrhage in central neurocytoma is not generally acknowledged. We report a case of central neurocytoma with sudden-onset of symptoms due to intratumoral hemorrhage. **Method and result:** A 47-year-old male patient was transferred to our hospital from a nearby local clinic due to sudden onset of severe headache and vomitting. On admission, he had progressive disturbance of consciousness, but there were no focal neurological deficits. The computed tomography scan showed hydrocephalus and a huge mass with heterogeneous density involving left lateral ventricles near the foramen of Monro. On magnetic resonance imaging (MRI), the mass appeared grossly isointense on the T1-weighted image and hypointense on the T2-weighted image. A well-demarcated central hypointensity was noted on T2-weighted image. After contrast medium injection, the MRI showed no enhancement of the lesion. The tumor was subtotally resected by a frontal transcortical approach. Pathological examinations confirmed a neurocytoma. **Discussion:** Spontaneous hemorrhage occurs very rare in primary brain tumors. As tumors of neuroepithelial origin, glioblastoma, astrocytoma, oligodendroglioma, and medulloblastoma are more susceptible to producing intratumoral hemorrhage. There are few cases of central neurocytomas presented by intraventricular hemorrhage in the literature. Hemorrhagic neurocytomas may present with an acute onset of symptoms such as is generally observed in stroke patients. The cause of bleeding in neurocytomas is unclear. Numerous thin-walled tumor vessels may be responsible for the bleeding. **Conclusion:** Central neurocytoma have a favorable prognosis and for this reason it is important to consider neurocytoma in the differential diagnosis of neoplasms in the lateral ventricles near the foramen of Monro that present with intraventricular hemorrhage.

P143

CLINICAL TREATMENT OF INTRACRANIAL MALIGNANT NERVE SHEATH TUMORS

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Objective To understand more about intracranial malignant nerve sheath tumors (MNST) by analyzing the clinical data of a group of patients with this kind of rare tumor, in order to improve treatment effects. **Methods** From January 1994 to November 2006, 11 cases of intracranial MNST were diagnosed pathologically and treated at our department. Their clinical data were retrospectively analyzed. **Results** There were 7 male and 4 female patients ranging in age from 3 to 51 years. Most of them had cranial neurological disorder, and main clinical manifestations included local swell and their previous symptom recurrence. Three subgroups were categorized by their origins: one was from cranial nerve root, the other was from unspecified central nerve root, and the last one had malignant transformation after recurrence. 5 cases had total removal of tumors, 3 cases subtotal removal, and 3 partial removal. Short-term effects were good. 3 cases underwent radiotherapy. Though these patients recovered quickly after operations, 3 cases survived longer than 5 years, and the longest life span was more than 10 years. **Conclusion** Such a tumor often occurs in children or middle-aged people. The illness course is short in most patients. Clinical manifestations are mainly neurological disorders of the site where the tumor is located on. The short-term curative effects are satisfied. The complete resection and radiotherapy can promote the 5-year survival rate.

P144

A RARE TUMOR-LIKE LESION OF PERIPHERAL NERVE SHEATH IN AN UNUSUAL SITE. NEUROMUSCULAR CHORISTOMA OF INTRAORBITAL RETROBULBAR CONUS. A CASE REPORT AND LITERATURE REVIEW.

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OBJECTIVE: Neuromuscular choristoma (NMC), are rare benign tumors of the peripheral nerves. Histopathological characteristics of this unusual lesion well described, but neuroimaging findings have not been well descriped. We report first intraconally located NMC that affecting the oculomotor nerve, with histopathologic and radiological characteristics. **CLINICAL PRESENTATION:** A 10 years old girl presented with sudden onset left temporal and retro-orbital pain. Magnetic resonance imaging scans demonstrated a small, retro-orbital, intraconal solid lesion that have capsule formation. Our first provisional diagnosis was dermoid cyst in an atypical site. **INTERVENTION:** The tumor could be resected subtotally. Post-operatively, the patient became pain-free but she had ipsilateral ptosis and upward movement failure. Histologically, the lesion consisted of well differentiated striated muscle fibers intermingled with mature nerve elements consistent with the NMC. **CONCLUSION:** NMC may need histological confirmation for diagnosis if they occur in the intracranial space. Pre-operative diagnosis with neuroimaging would be changed management strategy of treatment. This lesion is the fist NMC lesion which intraconally located. It is very rare, but should be considered in the differential diagnosis of intraorbital tumors.

P145

CLINICAL EXPERIENCES OF INTRACRANIAL HEMANGIOBLASTOMA

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Introduction: Intracranial hemangioblastoma is benign vascular tumors arising either sporadically or as a manifestation of von Hippel-Lindau (VHL) disease, a hereditary cancer syndrome. We analysed a series of patients with intracranial hemangioblastoma to review our strategy of treatment. **Materials and Methods:** Consecutive patients with intracranial hemangioblastoma who underwent surgery and then adjuvant therapy if needs, at our institute were enrolled. Eighteen patients (14 female and 4 male patients) underwent 22 operations for removal of 20 intracranial hemangioblastoma (age at surgery 45 years, follow-up duration 37 months). Serial clinical characteristics, radiologic studies and medical records were analyzed. **Results:** Twelve patients (66%) were sporadic hemangioblastoma and six patients (34%) were von Hippel-Lindau disease. Symptoms and signs included headache (60%), ataxia (66%), gait disturbance (20%), and hydrocephalus (47%). Total resection was achieved in 16 cases(73%). 16 patients underwent total removal of tumor were more likely to have good clinical outcome than 6 patients whose tumor were partially removed. Adjuvant radiosurgery was performed to 6 patients whose tumor partially removed. 4 patients of them showed good tumor control afrer radiosurgery. 2 cystic hemangioblastomas were aggravated in the size of cystic portion, so revision were performed. Preoperative tumor embolization was performed in two patients. Preoperative hydrocephalus resolved after tumor removal in 6 cases (100%) without cerebrospinal fluid diversion One VHL patient was expired due to pheochromocytoma. **Conclusion:** Symptoms and signs caused by intracranial hemangioblastoma can be treated safely and effectively with surgical resection. Cerebrospinal fluid diversion is rarely necessary after complete tumor removal in patients with preoperative hydrocephalus. Tumor recurrence is avoided by meticulous extracapsular resection. Radiosurgery may be adequate adjuvant therapy for remnant or recurrence intracranial hemangioblastoma except cystic hemangioblastomas.

P146

SURGICAL MANAGEMENT OF CNS HEMANGIOBLASTOMAS IN VON HIPPEL-LINDAU DISEASE

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Central nervous system (CNS) hemangioblastoma (HB) is the most frequent tumor in von Hippel-Lindau disease (VHL). The tumor occurs in cerebellum, brain stem, and spinal cord. However, management of CNS HBs in VHL has been controversial. Here, we discuss the management of CNS HBs in VHL. Surgically treated 59 CNS HBs (cerebellar 42, spinal 17) in 33 VHL patients from 1992 to 2008 were retrospectively examined in diagnosis and surgical treatment. Diagnosis of CNS HBs depended on symptoms, neurological examination, laboratory data, CT, MRI, and gene diagnosis. Symptoms in cerebellar HB were faintness, gait disturbance, nausea, and headache while those in spinal ones were pain, numbness, and weakness in extremities. Neurological examination revealed cerebellar dysfunction for cerebellar HB and regional hypesthesia or muscular weakness for spinal one. Laboratory data often reveal polycythemia due to secretion of erythropoietin from the tumor. Gene diagnosis for VHL gene mostly revealed germline mutation in VHL gene. Treatment for CNS HBs in cerebellum as well as spinal cord after symptomatic was surgical resection in the first choice. Surgical outcomes was mostly excellent or good except for large tumors (cerebellar HB above 4cm, spinal HB above 2 cm). In the second choice, stereotactic irradiation did not always control the tumor. VHL patients bearing CNS hemangioblastoma mostly developed other CNS ones in a different site. In conclusion, surgical resection is the first-choice treatment for CNS HB in VHL, and it should be done at appropriate timing without neurological deterioration.

P147

CLINICAL DIAGNOSIS AND TREATMENT OF HYPOTHALAMIC HAMARTOMA IN CHILDREN

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Objective To discuss the clinical features, diagnosis and treatment of hypothalamic hamartoma. **Methods** The clinical data of 4 patients treated in our department from January 1998 to December 2007 was retrospectively analyzed. Results There were 2 male and 2 female patients ranging in age from 6 to 14 years. 2 of them had initial symptom of precocious puberty, while the other two had clinical manifestation of hypoevolutism and visual disorder. But none of them presented with gelastic seizures. Good results were achieved with surgical resection in all the patients. Neuronal structure and gliosis were found by pathology. **Conclusion** Hypothalamic hamartoma can be treaed by surgical resection. Drug therapy may be useful for precocious puberty.

P148

CLINICAL DIAGNOSIS AND TREATMENT OF THIRD VENTRICLE CAVERNOUS ANGIOMA

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Objective To explore more about third ventricle cavernous angioma by analyzing the clinical data of 4 patients in order to improve treatment effects of this rare disease. **Methods** From 2001 to 2007, 4 cases of third ventricle cavernous angioma were diagnosed pathologically and treated at our department. Their clinical data were retrospectively analyzed. **Results** There were 3 male and 1 female patients ranging in age from 21 to 44 years. The initial symptom of all of them was headache and dizziness. None of them presented with polydipsia and polyuria. The physical examination of them was negative. All of them accepted CT scan and MR imaging. Among them, 4 cases had total removal of tumors, 1 case partial removal. Short-term effects were good. **Conclusion** Cavernous angioma rarely occurs in third ventricle. Clinical manifestations are not specific, only headache or dizziness. CT scan and MR imaging are the key methods to diagnose this disease. Surgical resection can achieve good curative effect.

P149

MULTIFOCAL, PROTEINACEOUS CNS DEPOSITS MIMICKING NEOPLASIA: REPORT OF AN UNUSUAL CEREBRAL AND CEREBELLAR AMYLOIDOMA WITH LONG LASTING CLINICAL HISTORY

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Aim: A rare, multifocal pathological tissue alteration is presented because it may represent diagnostic challenges. Detailed imaging features are compared to histomorphological and immunohistochemical (IHC) characteristics. **Methods:** State-of-the-art neuroimaging (MR, MRS, PET/CT) is not pathognomonic, biopsy material is necessary for establishing the proper diagnosis. The latter is based upon traditional and immunomorphology, histochemical stains for amyloid (Congo red, CR) and polarized light microscopy. **Case report:** 32-y-old female patient with headaches dating back to her early childhood had had her first MR in 2003, which showed several intracerebral abnormalities with variable calcification. She had no epileptic seizures, only mild perioral hypaesthesia. Eventually increasingly marked tremor developed. Repeated MR studies prior to biopsy showed increasing number of foci in both hemispheres; low T1W and variable T2W signal intensity was seen, only one deposit showed intensive contrast enhancement. Tissue obtained by craniotomy was paucicellular, almost avascular; the amorphous, often concretion-filled masses induced low intensity inflammation with scattered foreign body type giant cells. Flocculent or laminated, frequently partially calcified deposits displayed characteristic birefringence proving beta-pleated sheath structure. The congophilic structures were often concentric with a central, lamellated and calcified core. Deposition of the von Kossa +, intensely PAS+ (digestion resistant) material induced marked macrophage accumulation (CD68, vimentin +). Mib-1 LI was less than 1%. Only a few, thin walled blood vessels were seen (CD34). The neuropil around the affected foci was rich in reactive, densely arborized astrocytes. The multinucleated giant cells were also CD68 +. The process is independent from congophilic angiopathy and/or plasma cell neoplasia. **Conclusions:** Differential diagnoses of irregular, multifocal lesions, mimicking tumor, particularly in women, include proteinaceous deposits with or without calcification. Our case had no relationship with systemic or dural lymphoma or plasma cell dyscrasia. Currently no active treatment is possible, systemic amyloidosis must be excluded.

P150

PRIMARY BURKITT LYMPHOMA OF CNS IN AN IMMUNOCOMPETENT OLD WOMAN: A RARE CASE REPORT AND REVIEW LITERATURES

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Primary CNS Burkitt lymphoma is rare and often occurs in patients with immune deficient state, especially in acquired immunodeficiency syndrome (AIDS). Herein, we presented an immunocompetent 77-year-old female, who was health in the past, complained of general malaise and dizziness 3 days prior to admission. Computed tomographic scan revealed a butter-flied shape mass located in the pericallosal white matter, extending to the left basal ganglion and peduncle. The mass appeared as homogenous-enhancement after contrast injection. Magnetic resonance images exhibited a huge, enhancing and infiltrative tumor involving the genu to splenium of corpus callosum, bilateral radiata, bilateral corticospinal tract, hippocampi, posterior limb of internal capsule and left cerebral peduncle and ventrolateral pons, causing prominent vasogenic edema of bilateral parietal, left frontal and temporal lobes. She underwent stereotactic biopsy of the brain lesion and the pathology confirmed the diagnosis of Burkitt lymphoma. Postoperatviely, intrathecal chemotherapy and palliative radiotherapy were administrated and the patients expired half a year later because of systemic sepsis. The relevant literature is also reviewed.

P151

PRIMARY LEPTOMENINGEAL MELANOMATOSIS IN A 31-YEAR-OLD WOMAN

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The majority of the central nervous system melanomas are metastatic in origin, and primary cerebral melanoma or melanomatosis is rare. We have treated a very unusual primary cerebral melanomatosis, which presented with repeated bouts of intracerebral hemorrhages. A 31-year-old woman was presented with parietal ICH. she had been healthy till two days prior to admission, when she complained right hemiplegia. At admission, she was alert with grade 3 motor deficits on the right side. Brain CT showed a left parietal ICH. Brain MRI showed an acute left parietal hematoma with two small round enhancing nodules and well enhancing meninges. Surgery was undergone to find black leptomeninges overlying the hematoma. After drainage of the hematoma partial removal of the fragile and vascular tumor was done. Postoperatively, her motor function improved. Pathologic diagnosis was melanoma. After surgery, she underwent conformal radiation therapy(RT) to the enhancing leptomeninges and the tumor followed by 6 cycles of DVC(Dacarbazine, Vinblastine, Cisplatin) chemotherapy. Her neurologic status remained normal with no tumor recurrence on MRI follow up until 10 months after surgery, when she revisited with sudden right hemiplegia and facial palsy. Brain CT & MRI showed another left parietal ICH with new leptomeningeal tumor. Second surgery was undergone to remove the ICH and the tumor to result in neurologic improvement. On routine follow up two new tumors occurred in the falx cerebri, for which surgery was done followed by gamma knife radiosurgery(GKRS). Two months after the GKRS, she presented again with stuporous mentality. Brain MRI showed diffuse basal meningeal seedings and ventricular seedings and. She died of tumor progression 18 months after the first clinical presentation. Here, we present a rare case of primary leptomeningeal melanomatosis showing characteristic MR findings and clinical courses with pertinent literature review.

P152

PRIMARY INTRACEREBRAL MALIGNANT FIBROUS HISTIOCYTOMA, CASE REPORT

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Malignant fibrous histiocytoma (MFH) is a soft tissue sarcoma arising from the extremities and retroperitoneum in older adults. Primary intracranial MFH is extremely rare. A 29-year-old woman presented with headache showing three large masses in the right frontal lobe, left parietal and right occipital lobe on brain MRI scan. She had undergone extended total gastrectomy with splenectomy followed by 6 cycles of chemotherapy(5-FU, Pirubicin, Cisplatin) because of advanced gastric cancer three years previously. PET scan revealed no hot spot elsewhere. Under the impression of the multiple metastases the three masses were totally removed at a single session. Postoperative MRI scans confirmed complete removal of the masses. Pathology was confirmed to be typical MFH, for which 5000 cGy/5weeks WBRT was done followed by chemotherapy.Two months later, she presented with tonsilar exophytic mass and underwent tonsillectomy to get the same pathologic diagnosis of the brain. Then, another 6 cycles of chemotherapy with vincristine, ifosfamide and etoposide(VIP) was followed. Ten months after the brain surgery she presented again with severe headache with left hemiparesis showing huge recurrent tumor at the right frontal lobe with massive peritumoral edema. Two months after the second surgery gamma knife radiosurgery was undergone to the marginal enhancing nodule on routine follow up scan. Three months later, another metastasis was discovered at the mediastinum and anterior chest region to be treated by local radiotherapy. Under the basis of PET-CT scan pre- and postoperatively of the intracranial MFH, we concluded this case to be primary cerebral MFH metastasized to the tonsil and the mediastinum. We report this rare primary MFH with pertinent literature review.

P153

INCIDENTALLY DISCOVERED CLIVAL PSEUDOMENINGOCELE : A CASE REPORT

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Clival pseudomeningocele is an extremly rare lesion. Commonly, pseudomeningocele may result after trauma, brain surgery, spine surgery, or brachial plexus avulsion injury. A 21-year-old man with headache was found to have a clival pseudomeningocele. He had not specific past medical history, for instance, trauma or surgery. At first, this lesion was misunderstood to clival chordoma or chondrosarcoma, due to Magnetic resonance(MR), 3-dimensional computerized tomography(CT) finding. At operation, by a extended transsphenoidal approach, clival pseudomeningocele was diagnosed grossly, and confirmed by pathologic finding. This is the first case of clival pseudomeningocele in an adult, incidentally. Clival pseudomeningocele should be taken into consideration in patient with clival chordoma or chondrosarcoma.

P154

MALIGNANT BRAIN TUMOR IN A PATIENT WITH AIDS

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Purpose: Toxoplasmosis and lymphoma are frequently occurring intracranial mass lesions in AIDS patients. The authors would like to report 2 cases of malignant brain tumors developed in AIDS patients, our experiences in their treatment in consideration of related articles. **Method:** A female patient of 31 year old presented with headache and right side weakness.Another male patient of 58 years old presented with left side weakness. Their HIV antibody quantification at admission showed positive and a more specific examination proved them AIDS. Brain MRI revealed a 22x18x20mm sized mass at left frontal lobe with peripheral rim enhancement pattern and peritumoral edema. The other MRI showed multiple rim enhanced masses at right frontal and temporal lobes and left cerebellum and pons. **Results:** Navigationguided biopsy was performed to make the diagnosis and GMB and primary CNS lymphoma was confirmed through pathologic report. CCRT for the case 1 patient and temozolomide chemotherapy following radiation of 30 Gy for the case 2 patient were applied respectively. **Conclusion:** It is difficult in terms of treatment in AIDS patients because active management of the tumor is necessary as well as the AIDS itself. Recently developed medications for AIDS allowed positive treatment measures. Temozolomide is recommended for limited cases of CNS lymphomas.

P155

GLIOBLASTOMA DEVELOPED IN A CURED PEDIATRIC CNS LEUKEMIA

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Purpose: It have been known that CNS invasion of ALL is less than 10%. The authors would like to report a cured ALL with CNS leukemia patient through chemotherapy and WBRT, who developed glioblastoma at 6 years after RT. **Method:** A 17 year old female patient presented with 3 weeks of progressive headache and right hemiparesis Gr. III. Brain MRI showed a 58x56x52mm sized cystic mass at left frontal lobe which enhanced peripherally. She had started chemotherapy for ALL(type L1) 10 years ago, undergone concomitant chemotherapy and 20 Gy of RT for CNS leukemia 6 years ago and was decided cured 5 years ago. **Result:** The mass was removed under functional neuronavigation technique and globlastoma was confirmed through pathologic report. After surgery, right hemiparesis was gained to Gr. IV and adjuvant CCRT was applied. **Conclusion:** The authors experienced a globlastoma suspected its origin as previous radiation, developed in 6 years after cured pediatric CNS leukemia.

P156

A PATIENT WITH GLIOBLASTOMA MULTIFORME AND A LEFT CERVICAL MASS- A CASE REPORT

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A Patient with Glioblastoma Multiforme and a Left Cervical Mass- A CASE REPORT ABSTRACTGlioblastoma multiforme (GBM) is a highly malignant primary brain tumor in which the reported frequency of extracranial metastasis is only 0.44%. We report a patient with lymph node metastasis from a left parieto-occipital GBM 30 months from diagnosis. This is the first documented report of extracranial metastasis from a GBM in the Philippines. The patient is a 51 year-old, Male with GBM, left parieto-occipital area post gross total excision followed by concomitant radiotherapy and Temozolomide and adjuvant Temozolomide for 6 months. 11 months after diagnosis, he was enrolled in a Clinical Trial of Immunotherapy Peptide Vaccine and received a total of 3 doses of the said vaccine but went off study because of a presumed recurrence. Another craniotomy was performed and excision of the mass was done, and patient continued to receive Temozolomide on a metronomic schedule. He remained well with a KPS of 100 until 20 months post diagnosis when he developed another recurrence and again had a craniotomy followed by Tarceeva and carboplatin chemotherapy. He again remained well for 10 months until 30 months postdiagnosis when a left cervical mass was noted. No other extracranial lesion was seen on PET CT Scan. A biopsy of the mass showed sheets and nodules of neoplastic cells with pleomorphic hyperchromatic nuclei and clear cytoplasm separated by dense to fibrous stroma. Areas of necrosis were seen with neoplastic cells palisading around them. Immuno-histochemical staining showed positive immunoreactivity for glial fibrillary acid protein (GFAP), CK, CK 7, S100 and Vimentin. Shortly thereafter, a recurrence of the intracranial mass was noted on routine follow-up MRI. Prolonged survival and multiple craniotomies may increase the risk of occurrences of extracranial **GBMKeywords:** Glioblastoma Multiforme, Extracranial metastasis

P157

GLIOBLASTOMA MULTIFORME IN FASCIA LATA

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Glioblastoma multiforme (GBM) is a malign and highly aggressive entity that rarely shows extracranial and extraneural invasion. In the literature approximately 250 extracranial metastases that of 8 cases with cutaneous-subcutaneus metastases were published during the last 70 years. The dura defect can be occurred after primary tumor surgery. A 51 year-old man with a metastatic mass developed in the graft donor area of tensor fascia lata tendon due to the reconstruction of dura is presented in this report. According to the excisional biopsy result; the developed mass is defined as gliosarcoma showing the exact characteristics of the primary tumor. The authors report that the contaminated surgery tools and instruments can bring on the fact of distant spread of tumoral cells. Depending on this fact, the renewal of the surgery tools and instruments and the irrigation of the surgery area after primary tumor resection is emphasized.Key words: glioblastoma multiforme, subcutaneous metastases, metastases in the donor area, fascia lata metastases

P158

INTRAPARENCHYMAL GERMINOMA

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Purpose: Germinomas are known to occur at pineal gland or suprasellar region. We would like to report a case of a patient who underwent operation with an impression of PNET which turned out to be intraparenchymal germinoma. **Method:** A 10 year old male patient presented with 2 weeks of headache, vomiting and left side weakness. On brain MRI, a 56x48x42mm sized mass at right basal ganglia, accompanying multiple cysts which enhanced peripherally was shown. Results: A transcortical approach through superior temporal gyrus was performed and the soft mass which bled at multiple stages was removed. Pathologic examination confirmed the tumor as germinoma and adjuvant chemotherapy was applied. Left hemiparesis was improved after surgery and no further neurologic deterioration was noted. **Conclusion :** We experienced a good treatment results in management of a very rare case of pure intraparenchymal germinama which occurred in pediatric patients through surgery, chemotherapy and radiotherapy.

P159

EARLY DETECTION OF THE VENOUS THROMBOEMBOLISM IN GLIOMA PATIENT

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Purpose: The purposes of this study are to clarify the incidence of venous thromboembolism (VTE) in patient with glioma and to investigate the efficacy of quantitative d-dimer test and venous echogram with Doppler ultrasonography (USG) for early detection of deep vein thrombosis to prevent the pulmonary embolism (PE). Clinical Materials and **Methods:** During the period from June 2007 to December 2008, 90 patients were treated for glioma in our institution. Histologically, 42 cases were diagnosed as WHO grade IV, 23 cases were grade III, 18 cases were grade II and 7 cases were grade I. In all patients, d-dimer was measured once a week and USG was followed every two weeks since admission.**Results:** Of these patients, 12 patients were diagnosed as VTE (13.3%) and among them, 3 cases presented PE so that anticoagulant was started (3.3%). The maximum d-dimer value in patient with VTE was significantly higher than those without VTE (mean 14.6μg/l vs 4.19μg/l). USG showed thrombus in soleus vein to popliteal vein in all VTE cases. Histologically, high grade tumor was a significant risk for VTE occurrence (Grade I and II : Grade III and IV=0% : 21.4%). Patients with hemiparesis or low performance status after operation were prone to VTE occurrence. **Conclusions:** Our prospectively collected data showed that VTE incidence in glioma patient is much higher than we have expected. Continuous quantitative d-dimer measurement is useful for early detection of VTE for appropriate therapy.

P160

2 CASES OF EXTRADURAL GLIOBLASTOMA MULTIFORME PRESENTING AS TRIGEMINAL NEURALGIA; PATTERNS OF MOLECULAR TARGETS OF INVASIVENESS.

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Background: Glioblastoma Multiforme, a highly invasive tumour, is the most common primary brain tumour. Despite the aggressive local growth pattern, extradural extension and metastasis is rare, usually occurring in patients with prolonged survival. The molecular characteristics and mechanism of dissemination in a GBM that spreads beyond the central nervous system is not fully understood.

Method: We describe two cases of extradural Glioblastoma Multiforme (GBM) presenting as trigeminal neuralgia. Both followed aggressive disease courses. One case metastasised to liver and bone. The second case was refractory to multiple lines of therapy. We have compared the histopathology of these 2 cases with controls. Morphology and immunohistochemistry panels were examined. The immunohistochemistry panel included stains previously explored in the literature as possible molecular targets for invasiveness of GBM. The controls consisted of tissue from temporal lobe GBMs demonstrating typical pathological features of WHO grade 4 astrocytoma. The panel included pEGFR, pAkt, pMAPK, p53, c-MET, PTEN, Tenascin C, E-cadherin, N-cadherin, MMP2, and MMP9.

Results: The initial radiological diagnosis in both patients was thought to be a benign process due to the extradural extension along the trigeminal nerve. From time of pathological diagnosis, the patients had survivals of 10 months and 15 months. Both underwent debulking surgery, local radiotherapy and at least 2 lines of chemotherapy. The 2 tumours were morphologically similar. The results from the panel of immunohistochemistry staining are pending, but will be included in the final discussion.

Conclusion: We report two cases of extradural GBM presenting unusually as trigeminal neuralgia. Both patients had refractory disease, resulting in a decreased survival. Morphologically these tumours were similar. Assessing these tumours for molecular targets of invasiveness, may assist in identifying those at risk of extradural extension and a more aggressive clinical course.

P161

PONTINE CRYPTOCOCAL GRANULOMA IN A HEALTHY, NON-IMMUNOCOMPROMISED PATIENT-CASE REPORT-

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We report a very rare case of the brain stem cryptococcoma in a previously healthy, non-immunocompromised adult without previous evidences of CNS infection. The patient is a 47-year-old man with no significant medical history presented with a month history of progressively worsening headaches and nausea. He had several episodes of vomiting without fever for several days until the day of admission. He had no predisposing factor of immunocompromised condition such as diabetes mellitus, organ transplantation, HIV infection and chemotherapy. The patient showed an alert mentality with no neurologic deficits. There were no evidences of infection on physical examination. An Gd-enhanced MRI scan of the brain demonstrated a homogeneous enhancing mass with central necrosis in the left pons with adjacent edema. MRI images demonstrated a heterogenously low or iso-signal intensity on diffusion-weighted images and a increased cerebral blood perfusion. PET scans of the whole body including a brain demonstrated a hot uptake of FDG on brain stem and didn't show any evidences of systemic metastasis.. We thought this lesion the tumorous conditions such as high grade glioma than nontumorous condition including granuloma and performed the excision of mass. The mass was removed totally via the lateral suboccipital craniotomy using neuronavigation system and SSEP monitoring. The final report of pathology revealed the Cryptococcus neoformans. The CSF study and HIV test via lumbar tap were negative. The patient recovered without any neurologic deficits. The patient had been treated initially with two antifungal agent such as Amphotericine B and flucytosine for 4 weeks .The clinical course of CNS cryptococcosis is highly variable, relating in part to underlying medical conditions. If untreated, it is invariably fatal. Early diagnosis and initiation of treatment such as the surgical strategy and antifungal therapy is essential.

P162

SURGERY FOR INTRAMEDULLARY SPINAL TUMORS

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Prognositic analysis of 220 patients with intramedullary spinal cord ependymomas
Objective: To assess the factors relative to the surgical prognosis of intramedullary spinal cord ependymomas. **Methods:** 220 patients harbored intramedullary spinal cord ependymomas were treated surgically in Beijing Tiantan Hospital from 2000 to 2007. We followed them on telephone and analyzed their JOA score preoperatively and postoperatively about age, gender, length of tumor and spinal cord function by Spss 11.5 software. **Results:** Age, gender and length of tumor had no significant influence on prognosis. Through multiple linear regression analysis and Logistic regression, we concluded that the postoprative JOA score highly relatived to the preoperative neurological status and other factors. **Conclusion:** Determinant factors of prognosis for intramedullary spinal cord ependymomas are preoperative neurological status, extent of removal of the lesion. ***KEY WORDS:*** intramedullary spinal ependymomas, surgery

P163

THE RATIONAL NEUROSURGICAL TREATMENT OF PINEAL REGION TUMORS

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h pineal region tumors were treated by microsurgery from June, 1993 to July, 2008. Infratentorial supracerbellar approach (Krause approcach) was adopted in 63 cases, and occipital transtentorial approach (Poppen approcah) in 13 cases; Parieto-occipital transtentorial approach (Brunner-Dandy approach) in 10 cases respectively. The Krause approach was performed when tumor is under moderate size and mainly inferior to the tentorial incisure; the Brunner-Dandy approach was selected when tumor is large or mainly superior to tentorial notch or invaded to the corpus callosum or growth laterally; the Brunner-Dandy approach was elected when tumor is mainly within the third ventricle. The tumors were resected totally in 82% cases and no mortality concerned with surgery occur in the study. No-Germinoma Germ cell Tumor recurred in 8 cased and tumor metastasized localy in 1 cases, among these, 7 cased died within 1 year , whether the shunt for hydrocephalus seconday to the pineal region tumors were needed, the time of shunt, the choice of shunt style were discussed. We think contimuous CSF external drainage, before and after tumor remove, was suital for exposing tumor, reducing the operative complication and unnecessary CSF shunt. The ICP mornitor care have a significant value in judge of the patency of CSF circulation postoperatively. The ventriculoper itoneal shunt is superior to the ventriculoatrial shunt. It is very importance to measure the tumor marker such as HCG in serum routintly. The functional tumor for positive tumor marker is differ to differ to other ones in poor effect of radiotherapy and recurrence of metastasis. Prophylactic spinal radiation must be performed for the functional germ-cell tumors(GCT). The tumor tissue must be examed carefully in different site of tumor sample to prevent ignorance of combined malignant GCT. When the tumor marker was positive in germinoma diagnosed initially. Comparison between the tumor remove followed by radiation and CSF shunt followed by radiation, no significant difference were made in survive rate of short term follow-up. In the view of operative morbidity, the shunt followed by radiation is superior to the tumor remove followed by radiation in pure germinoma. According to the character of patient clinical course, neuroradiological image and tumor marker measure in serum. The malignant GCT, which is 10-20 year-older, male and prediagnosis course within 1 month, is differ to the benign tumor, which is 30-40 year older , no difference in sex and prediagnosis within 2 months to 6 months. Considering of the safity of tumor remove. The patients whose tumor feature is uncertain and unimproved after the control of increasing ICP, and serum tumor marker positive should be performed direat tumor removal. Malignant tumor should be undergone radiotherapy or/and chemotherapy deponding on the tumor pathological diagnosis. **Key word:** Pineal region tumors; Operative approach; Shunt HCG; Radiotherapy

P164

CONSERVATIVE MANAGEMENT OF PSEUDOMENINGOCEAL FORMATION AFTER POSTERIOR FOSSA TUMOR SURGERY IN CHILDREN

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Introduction This is the report of two cases of children, who had complication of pseudomeningocele after resection of posterior fossa tumor, medulloblastoma, and had cured with conservative management not with exploration and reclosure. **Case Report** Case 1 6 years old, male, who had symptoms of headache and vomiting, had findings which suspecting medulloblastoma at brain magnetic resonance imaging(MRI). Near total resection with craniotomy had performed. After 14 days from operation(OP), the wound site bulging and increasing fluid collection were identified and pseudomenigocele was confirmed with computed tomography (CT) scan and MRI. But with a week after removal of LD, only with compression bandage, the bulging of wound was improved progressively. Case 2 13 months old, male, had hydrocephalus and findings of which suspecting medulloblastoma at brain magnetic resonance imaging(bMRI). Total resection with cranietomy and external ventricular catheter(EVD) insertion had performed. Several days after EVD removal, wound bulging was identified and pseudomenigocele with progressed hydrocephalus was confirmed with CT scan. Ventriculo-peritoneal shunt OP had taken with compression bandage application. Now, 14th day after V-P shunt OP, wound bulging was disappeared. **Conclusion** The rate of pseudomeningoceles after posterior fossa tumor surgery in this study was consistent with that report, with an incidence of 23% when clinically diagnosed and 30% if the radiologically diagnosed cases only are included. So, many maneuvers and techniques was suggested, but in adults and children who kept of pseudominigocele and CSF leakage were concerned revision OP. But, revision OP may have anesthesia and OP complication rather than conservative management, revision OP should not be considered impatiently at children cases.

P165

POSTERIOR FOSSA TUMORS WITH HYDROCEPHALUS: OUTCOME OF SURGERY WITHOUT CSF DIVERSION

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ABSTRACT The authors reviewed 72 patients with posterior fossa tumors and hydrocephalus, treated at a large tertiary hospital between 2006 and 2007, to determine clinical outcome and factors that predispose to subsequent need for post-operative CSF shunting, after surgery without CSF diversion. A retrospective review of prospectively collected clinical, radiologic and operative data was conducted for all patients treated surgically with suboccipital craniectomy/craniotomy and tumor excision without pre- or intra-operative external ventricular drainage and ventriculoperitoneal shunting. The factors evaluated included age, tumor location, extent of tumor resection, and tumor type. The low postoperative shunt insertion rate (5%) and good clinical outcome in our series led us to believe that routine pre- or intra-operative CSF diversion is not entirely justified. Factors such as age, tumor type, tumor location and extent of excision, which showed a statistically significant association with the postoperative shunt requirement in our study, should be considered when the decision regarding CSF diversion is made.

P166

ATYPICAL MININGIOMA IN FRENCH WEST INDIES

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Introduction
Meningiomas are the most common intracranial primary neoplasm in adults. Annual incidence of meningiomas is approximately 6 for 100 000habitants in the medical literature. We noticed a rate abnormally increased especially atypical meningiomas in Martinique(French west Indies), we analysed retrospectively the patients admitted and operated for a meningioma in our department during a period of 6 years from 2003 till 2008 .

Material and Method
The study was performed in department of neurosurgery at General hospital of Fort de France in Martinique(French west Indies). This regional unit provides neurosurgical service for a population of 600 000(400 000 in Martinique and 200 000 in French Guyana)
369 Patients have been admitted in our department for a meningiomas during a period of 6 years: January 2003 till December 2008 .The incidence of meningioma is 10.25 per year.11 patients were not operated for diverse reasons and they were excluded of study.358 patients underwent surgery.

Results
We found 62 atypical meningioma (Grade II according to the WHO classification) among 358 patients(17.3%).We had 38 female and 24 male with an average of 54.6 year.47 of patients had at first side an atypical meningioma and 15 had an atypical tumour at the time of the recurrence. 41 patients underwent two interventions and 13 three interventions .8 patients died after their exit of the service for diverse reasons. When surgery was macroscopically complete(Simpson grade 1-3), for the atypical miningiomas the recurrence arose within 4 in 48 months with a average period of 40 months. In the patients who underwent a radiotherapy after one or several operations, the recurrence arose on average after 7,63 years. We found no previous history of radiotherapy for atypical miningioma.

Discussion
More detailed studies are necessary to clarify causes and possible genetic and environmental factors of this high frequency of miningioma especially atypical miningioma in Martinique.

P167

ATYPICAL RATHKE'S CLEFT CYST WITH CALCIFICATION

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Rathke's cleft cysts should be differentiated from craniopharyngiomas because of different treatments. We report a rare case of Rathke's cleft cyst in a 14-year-old boy, presenting with headache, hormonal imbalance and conscious change. Computed tomographic scans showed round calcification on the wall of the cyst. Magnetic resonance images revealed a large cystic intra- and supra-sellar lesion with a relatively large solid part extending to the roof of the third ventricle. He underwent placement of an external ventricular drain emergently for acute hydrocephalus and then the tumor was excised via the subfrontal approach. Following surgery, the boy had a good recovery except diabetes insipidus. This case illustrates that calcification of the intra- and supra-sellar cyst with large solid portion does not always suggest craniopharyngioma.

P168

THE CORRELATION AND PROGNOSTIC SIGNIFICANCE OF MGMT PROMOTER METHYLATION AND THE MGMT PROTEIN IN GLIOBLASTOMAS

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Objective: The aim of this study was to evaluate the correlation and prognostic significance of MGMT promoter methylation and protein expression in patients with glioblastoma.**Methods:** Eighty-three patients with glioblastoma underwent surgery followed by radiotherapy and temodal chemotherapy between October 2000 and June 2008. To investigate the correlation between MGMT methylation and MGMT expression, methylation-specific PCR (MSP) and immunohistochemical staining (IHC) was performed. To analyze the correlation between MGMT methylation and MGMT expression according to location, biopsies were obtained from 37 different sites within the tumors in 12 patients. Age, gender, KPS, extent of removal, chemotherapeutic methods, MGMT promoter methylation and protein expression were analyzed as prognostic factors. **Results:** The total median survival was 15.8 months (min 12.6, max 19.1). The results of MSP were the same at various sites in 12 patients. A correlation between MSP and IHC was observed in 50 percent of the patients. Out of 73 patients, negative MGMT expression was detected in 70.5 percent of 44 patients with MGMT promoter methylation, and positive expression was observed in 55.2 percent of the 29 patients with unmethylated promoters. Multivariate analysis revealed that the extent of removal ($P<0.001$) and the combination of MGMT promoter methylation and negative MGMT expression (median survival: 20.06 months, $P<0.006$) were significantly associated with longer survival. **Conclusions:** We suggested the feasibility of using MSP combined with IHC as a prognostic factor. The results of the present study suggest that MGMT promoter methylation in combination with negative MGMT expression might be a good prognostic factor in patients with glioblastoma.

P169

RELEVANCE OF MSP ASSAY FOR THE DETECTION OF MGMT PROMOTER HYPERMETHYLATION IN GLIOBLASTOMAS

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O^6-Methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation has recently emerged as a powerful determinant of chemotherapy sensitivity in glioblastomas. To adapt such an important epigenetic biomarker to routine application in the clinical setting, we validated the conventionally used methylation-specific polymerase chain reaction (MSP) assay for its relevance in the determination of *MGMT* methylation status. *MGMT* promoter hypermethylation analysis employing MSP was performed on 25 primary glioblastoma samples and 7 cell lines, and compared with the more robust direct promoter sequencing that profiled the methylation status of 27 CpG sites within the *MGMT* promoter. In addition, the MGMT expression at the protein level was evaluated in the primary tumor samples using immunohistochemistry and in the cell lines using Western blotting analysis. Our MSP analyses yielded reproducible results, which were identical to the bisulfite sequencing data in all except one primary tumor that was negative on MSP. A poor correlation existed between the immunohistochemical staining results and the methylation status of the *MGMT* promoter in primary glioblastoma samples. In all of the cell lines with loss of MGMT expression, signals of methylated DNA were detected by MSP. Our data support the feasibility and reliability of MSP analysis, which could be routinely implemented in the diagnostic setting.

P170

CLINICAL SIGNIFICANCE OF POLYMORPHISM OF DNA REPAIR GENE MGMT IN GLIOBLASTOMAS

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(Purpose) Inactivation of O6-methylguanine DNA methyl-transferase (MGMT) gene by promoter hypermethylation is a major determinant of sensitivity to alkylating agents in chemotherapy of malignant glioma, and is correlated with survival. Since single nucleotide polymorphisms (SNPs) may influence the methylation status of gene promoters, here we evaluated five MGMT SNPs in relation to promoter methylation and expression of the MGMT gene in patients with glioblastomas. We also examined the influence of the SNPs on prognosis.**(Materials and methods)** In 25 patients with glioblastomas, admitted between 2002 and 2007, MGMT promoter methylation status was assessed by methylation-specific polymerase chain reaction (MSP) and direct promoter sequencing. Gene expression was determined by immunohistochemistry. MGMT SNPs were evaluated by real-time PCR. **(Results)** Leu53Leu (CTC/CTT) and Leu84Phe (CTT/TTT) were found in 9 of 25 patients (36%) with glioblastomas, and were linked in all those patients. The minor allelic frequency of each SNP in patients with glioblastomas was higher than that of a control population. Leu84Phe was detected in 4 of 8 patients (50%) with MGMT promoter hypermethylation and in 5 of 17 patients (23.5%) without hypermethylation, but the difference was not statistically significant ($p=0.394$). The presence of MGMT SNPs was not associated with MGMT expression. These SNPs did not appear to be related to prognosis. **(Conclusion)** Our data suggest that MGMT SNPs might not affect MGMT promoter methylation and expression in patients with glioblastomas, although the sample number was small.

P171

IMMUNOHISTOCHEMICAL ASSESSMENT OF O6-METHYLGUANINE DNA METHYLTRANFERASE FOR GLIOBLASTOMAS: A REAPPRAISAL

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Background: Preusser and Stupp et al. reported the unreliability of O6- methylguanine DNA methyltransferase (MGMT) immunostaining as a clinical biomarker for glioblastomas (GBM). **Purpose:** To demonstrate the prognostic significance of MGMT immunohistochemisty for GBM, we retrospectively stained 15 surgical specimen of newly diagnosed GBM taken from patients who went on to receive at least 3 courses of maintenance therapy with temozolomide (TMZ) after induction therapy. **Method:** Paraffin embedded surgical tissue of GBM was stained with MGMT using the ABC method. Endogeneous peroxidase was blocked with 0.3% hydrogen peroxide in methanol and sodium azide. Antigen retrieval was achieved with autoclave processing. MGMT positivity was assessed at the site of greatest MGMT positivity, and subdivided into three groups: MGMT positivity of 0-10% ((-) group), 11-50% ((+) group), and 51-100% ((++) group). Only MGMT positive tumor cells were counted; MGMT positive vascular endothelial cells, oligodendrocytes, and lymphocytes were discarded. **Results:** The mean follow up period was 16 months (6 to 16 months). 2 cases were MGMT (-), 9 cases were (+), and 4 cases (++). 6 month progression free survival was 100%, 67%, and 0% in the 3 groups, respectively. Kaplan-Meier curves also showed the 3 groups to have different outcomes. **Conclusion:** With careful staining and interpretation of MGMT positivity in GBM specimen, we were able to successfully divide patients into 3 groups with different outcomes. MGMT immunostaining should be reconsidered as a useful biomarker in GBM patients.

P172

MGMT METHYLATION STATUS MAY PREDICT SURVIVAL IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA (GBM)

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Purpose: In patients with GBM <70 years old, methylation of the MGMT gene promoter is associated with prolonged survival and response to temozolomide. We evaluated if this held true for patients >=70. **Methods:** We reviewed MGMT promoter methylation status in patients >=70 years with newly diagnosed GBM seen at our institution from 1998-2008. Patients were included if tumor tissue was available for MGMT testing. MGMT analysis was performed by extracting tumor genomic DNA for bisulfite conversion. MGMT methylation specific PCR (MSP) was then performed using PCR primer sets specific for methylated and unmethylated MGMT promoter sequence (EZ DNA Methylation Gold-Kit, Zymo Research). **Results:** Thirty-five patients were included in the analysis. Twenty-one patients (60%) were ME while 14 (40%) were UN. The table below summarizes the characteristics of the study population. Patients were treated with multimodality treatment (radiation + chemotherapy), radiation alone, or chemotherapy alone. **Conclusions:** MGMT methylation is associated with prolonged PFS and OS in elderly patients with GBM. Knowledge of MGMT status may help improve prognostication in this patient population.

	All	ME	UN
Number of patients	35	21	14
Extent of surgery:			
STR	22	13	9
GTR	13	8	5
KPS	80 (50-100)	90 (60-100)	60 (50-80)
Age	74 (70-83)	74 (70-78)	74 (70-83)
MMSE	28 (19-30)	28 (25-30)	28 (19-30)
Gender (men)	22	11	11
mOS (months)	10.6	14.5	6.9 (logrank p=.0042)
mPFS (months)	7.7	11.7	3.5 (logrank p=.0127)
First Line Therapy:			
Multimodality therapy	17	12	5
Chemotherapy or RT alone	12	5	7
Unknown/none	6	4	2

P173

METHYLATION-SENSITIVE HIGH RESOLUTION MELTING ANALYSIS :A NEW QUANTITATIVE ASSESSMENT OF MGMT PROMOTER METHYLATION IN GLIOMAS

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<Objectives> The methylation status of the MGMT (O6-methylguanine-DNA methyltransferase) gene has been shown to be a predictive marker in malignant gliomas treated with temozolomide. Methylation-specific PCR (MSP) is widely used method for the detection of the *MGMT* methylation. Despite its widespread use, MSP has several disadvantage. False positives can arise if primers are badly designed or used at too low a temperature. 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 moreover estimate quantitatively the extent of methylation in tumors. **<Materials & Methods>** We used genomic DNA derived from high-grade glioma samples and univeral 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 acquire 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 *MGMT* methylation levels of glioma samples. **<Results and Conclusions>** 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. Gliomas with *MGMT* methylation less than 45 % showed significantly short progression-free survival. Methylation-sensitive HRM is the rapid and useful method for predicting the effect of Temozolomide in glioma therapy.

P174

MGMT TESTINGS FOR TEMOZOLOMIDE EFFICACY ON GLIOBLASTOMA

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Background: *O*^{6}-methylguanine-DNA methyltransferase (MGMT) is implicated in resistance to temozolomide (TMZ), a standard chemotherapeutic agent against glioblastoma (GBM). MGMT status has been shown to correlate with survival of patients with GBM. We evaluated the relationship between promoter methylation status and protein expression of the MGMT gene in patients with GBM who were treated with TMZ, and also analyzed their prognostic value regarding response to TMZ and survival. **Methods:** Among the TMZ-treated patients with GBM from September 2003 to January 2008, frozen tumor tissues were available for analysis in 33 cases. Among them, 22 patients who were treated with TMZ only at tumor progression were subjected to survival analysis. TMZ was given daily orally 75mg/m^2 during radiotherapy, or administered 150-200mg/m^2 on days 1 to 5, repeated every 28 days. MGMT protein expression was examined by Western blotting, and promoter methylation status by the methylation-specific PCR (MSP) and pyrosequencing. **Results:** MGMT promoter methylation was detected in 14/33 cases (42.4%), and significantly correlated with low MGMT protein expression ($p<0.001$). Upon TMZ treatment for recurrent GBM, patients with low MGMT protein expression as well as those with methylated MGMT promoter had a significantly improved progression-free survival (PFS) and overall survival (OS) compared to those with either high expression or unmethylated promoter, respectively. Both low MGMT expression ($p = 0.02$) and promoter methylation ($p = 0.04$) persisted as significant independent favorable prognostic factors for PFS, while low MGMT expression remained significant for OS ($p = 0.004$). **Conclusions:** The two MGMT assays showed close association to each other, and were suggested to be important prognostic factors for patients treated with TMZ. Further quantitative analysis of MGMT promoter methylation is underway using pyrosequencing.

P175

A NOVEL AND SPECIFIC INHIBITOR OF MGMT TRANSCRIPTION INCREASES THE SENSITIVITY OF GLIOMA CELL LINES TO TEMOZOLOMIDE TREATMENT.

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Introduction: Alkylating agents are the mainstay of chemotherapy for gliomas. An important mechanism of resistance to alkylating agents is mediated by the DNA repair enzyme O6-methylguanine methyltransferase (MGMT), the only gene known to be critical to direct reversal repair of the effects of alkylating agents on DNA. We previously demonstrated (Lavon I. et al. Cancer Res;67(18):8952, 2007) that MGMT transcription is dependent on binding of NF-kappaB to specific kappaB sites within MGMT promoter. **Objective:** To reduce MGMT expression and sensitize resistant gliomas to alkylating therapy by a conditioning regimen that use decoy oligonucleotides that distinctively interfere with NF-kappaB binding to MGMT promoter. **Methods:** The specific interference with the binding of NF-kappaB to MGMT promoter was accomplished by prototypes of locked nucleic acid (LNA) modified-oligonucleotides (LMO) corresponding to the sequence of the active NF-kappaB site within MGMT promoter. Specific and nonspecific LMO were liposomaly introduced (Lipofectamine 2000, Invitrogen) to various glioma cell lines. Twenty four hours later the cells were subjected to increasing doses of temozolomide, and then followed by cell viability tests. **Results:** We demonstrated that conditioning treatment with 2nm of specific LMO sensitized the cells to temozolomide and enhanced cell mortality by approximately 300% (from 60% live cells following temozolomide treatment to only 22% of live cells after temozolomide that was anteceded by LMO conditioning). Furthermore, we showed that LMO by itself was not toxic to the cells as treatment with a nonspecific LMO did not induce cell death. Thus, the observed cytotoxic effect was related to the targeted activity of the specific LMO. **Conclusions:** We demonstrated that, in-vitro, specific MGMT transcription inhibitors effectively overcome glioma cell chemoresistance. These findings may lead to a novel therapeutic approach that utilizes a conditioning regimen of modified oligonucleotides given to chemoresistant glioma in order to sensitize the tumor to alkylating agents.

P176

MECHANISM OF THALIDOMIDE TO ENHANCE THE CYTOTOXICITY OF TEMOZOLOMIDE IN U251-MG GLIOMA CELLS IN VITRO

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Background: Chemotherapy is the key part of the treatment for gliomas. Temozolomide, a novel oral cytotoxin inducing autophagy, has been used with Thalidomide, the anti-angiogenic agent. Although a promising result is shown, but the synergistic mechanism is poorly documented. To provide an optimal strategy for combined treatment of the two drugs, we investigated the synergistic mechanism of Thalidomide possibly to enhance the cytotoxicity of Temozolomide in vitro. **Methods:** Human malignant glioma cells U251-MG were cultured and divided in four groups with different treatment for 3 days: Temozolomide group (100uM), Thalidomide group (100ug/L), Temozolomide plus Thalidomide group (100uM Temozolomide with Thalidomide 100ug/L) and control group. MTT assay was used to evaluate the cell viability. Cell cycle was analyzed with flow cytometry. Acridine orange and monodansylcadaverine were adopted to label autophagosomes and flow cytometry was applied for quantification of autophagosomes. The expression of autophagy-associated protein was detected by Western Blotting. **Results:** Proliferation of tumor cell was obviously suppressed by Temozolomide with Thalidomide treatment than either drug used alone (P=0.000 for each day). The combination treatment induced cell cycle arrest at G0/G1 phase. Thalidomide promoted the autophagy induced by Temozolomide. The autophagy-associated proteins --- Microtubule Associated Protein 1 Light Chain 3 (MAP1LC3) and Belin1 were more significantly up-regulated by Temozolomide with Thalidomide treatment than Temozolomide used alone (MAP1LC3 P=0.000; Beclin1 P=0.004). The expression level of phosphatase and tensin homolog deleted on chromosome ten (PTEN), which promoted autophagy, was elevated by in Temozolomide with Thalidomide group (P =0.002), while the expression level of phosphorylated-AKT in the same group was decreased significantly (P=0.000). **Conclusions:** Thalidomide enhances the cytotoxicity of Temozolomide by promoting the autophagy induced by Temozolomide. The mechanism might be related to the blockake of PI3K/AKT/mTOR signalling pathway which antagonized autophagy, contributing to the up-regulation of PTEN by Thalidomide.

P177

NEURAL DIFFERENTIATION MARKERS EXPRESSION IN HUMAN GLIOBLASTOMAS MAY PREDICT THEIR RESPONSE TO CHEMOTHERAPY

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Introduction: Glioblastoma multiforme (GBM) often represents a chemoresistant tumour. Authors decided to analyse the immunophenotype of GBM cells and correlated their immunophenotypic characteristics with sensitivity to chemotherapy. **Materials and methods:** The expression of selected differentiation markers including A2B5, CD34, CD45, CD56, CD117, CD133, EGFR, GFAP, Her-2/neu, LIFR, nestin, NGFR, Pgp and vimentin was analysed by flow cytometry in eleven GBM patients. The sensitivity of tumour cells to a panel of chemotherapeutic agents was tested by the MTT assay. **Results:** All tumours were positive for A2B5, CD56, nestin and vimentin. CD133, EGFR, LIFR, NGFR and Pgp were expressed only by minor tumour cell subpopulations. CD34, CD45, CD117, GFAP and Her-2/neu were constantly negative. Direct correlations were found between the immunophenotypic markers and chemosensitivity: A2B5 vs. lomustine (r^2=0.642, p=0.033), CD56 vs. cisplatin (r^2=0.745, p=0.013), %Pgp+ vs. vincristine (r^2=0.846, p=0.008), and %NGFR+ vs. daunorubicine (r^2=0.672, p=0.047) and topotecan (r2=0.792, p=0.011). In contrast, inverse correlations were observed between: EGFR vs. paclitaxel (r^2=-8722;0.676, p=0.046), CD133 vs. dacarbazine (r^2=-8722;0.636, p=0.048) and LIFR vs. daunorubicine (r^2=-8722;0.878, p=0.004). Significant associations were also found between sensitivity to different chemotherapeutic agents and some immunophenotypic markers. **Conclusions:** Histopathologically identical GBM tumours displayed a marked immunophenotypic heterogeneity. The expression of A2B5, CD56, NGFR and Pgp appeared to be associated with chemoresistance whereas CD133, EGFR and LIFR expression was characteristic of chemosensitive tumours. The flowcytometric analyses of GBMs may predict chemoresponsiveness and help to identify patients who could benefit from chemotherapy.Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract No. APVT-20-032504, APVV-20-052005 and VEGA grant 1/3361/06.

P178

EXTENDED USE OF TEMOZOLOMIDE BASED ON O6-METHYLGUANINE-DNAMETHYLTRANSFERASE (MGMT) EXPRESSION PATTERN FOR GLIOMA PATIENTS: EXPERIENCE OF 17 CASES

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The 6-cycle of temozolomide (TMZ) have being used as a standard chemotherapy. We have treated 17 patients with high-grade gliomas (HGGs) or unfavorable low-grade gliomas (LGGs) with TMZ for more than 6 cycles. The regimen of TMZ was selected based on MGMT expression of the tumor. Patients with MGMT-negative tumors had received the standard dosing regimen (200 mg/m2 for 5 days every 28 days). While patients with MGMT-positive tumors were treated with 3-weeks-on/1-week-off schedule, or cisplatin plus TMZ (cisplatin 75 mg/m2 on Day 1 and 2, TMZ 200 mg/m2 on Day 2 to 6, every 28 days). About 174 cycles of TMZ were administered to the 17 patients. The TMZ cycles administered per patient ranged from 7 to 24 (median = 8 cycles). Myelosuppression including lymphopenia and leukopenia were the most common high grade toxicities (grade 3), both occurring in 11.8 % (2 / 17) of patients. The most frequent toxicities were fatigue (76.5 %), lymphopenia (58.8 %), alopecia (58.8 %), constipation (41.2 %), leukopenia, nausea, depression and vomiting (all with 35.3 %). The median progress free survival (PFS) was 29.9 months. Six month PFS and 12 month PFS were 100% and 73% respectively. Two patients received gross total tumor resection are still no evidence of disease now. One patient (5.9 %) demonstrated a complete response (CR), 12 patients (70.6 %) a minor response (MR), and 2 patients (11.8 %) stable disease (SD). The overall response rate was 76.5 % (95% CI, 50-90 %) and the disease control rate was 88.2% (95% CI, 64-98 %). Extended use of TMZ based on MGMT expression pattern is safe to glioma patients. Extended use may improve response rates and PFSs compared to conventional regimen.Key words: chemotherapy, glioma, temozolomide

P179

PYSOSEQUENCING: A GOOD OPTION FOR MGMT ANALYSIS.

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MGMT is currently recognized as a major mechanism of resistance to alkylating agents in glioblastoma (GBM) patients, but so far there is no consensus about the best technique for MGMT analysis. In a previous study, we compared five techniques (qualitative and semi-quantitative MS-PCR, pyrosequencing, MGMT RNA level and immunohistochemistry) on a retrospective series of 81 newly diagnosed GBM patients treated with a standard therapeutic schedule involving TMZ. The best predictive value for overall survival was obtained by pyrosequencing (PyroMarkTM MGMT kit - Biotage) with a cut-off of 8% (median of the population). We analyse here the analytical performance of this technique, a critical point if such a technique is to be used in a clinical setting. To assess repeatability and reproducibility, 2 primary cell lines (obtained in the laboratory), the U251 cell line and a universal methylated DNA (100% methylated) were tested at least 5 times in single or separate runs. For linearity, sequential mixing of the 100% and 0% samples were tested 5 times in a single run. The limit of quantification was defined as the lower concentration detected with a coefficient variation (CV) over 15%. Mean levels of methylation were 9, 73, 90 and 97% for GB3, GBM2, U251 and universal methylated DNA, respectively, with repeatabilities and reproducibilities below 10%, 9%, 5% and 3%. A good linearity was observed for each CpG site tested, as well as for the mean of methylation. A CV below 15% was obtained for the lowest sample tested (5% for a theoretical result of 2.5%), indicating a limit of quantification between 0 and 5%. Pyrosequencing appears as a very robust technique for analysing MGMT status. It is currently compared to other promising approaches such as MethyLight and MS-HRM in a French multicentre study.

P180

ANGIOGENIC POTENTIAL OF LOW GRADE OLIGODENDROGLIOMAS IS AN IMPORTANT DETERMINANT OF PROGNOSIS

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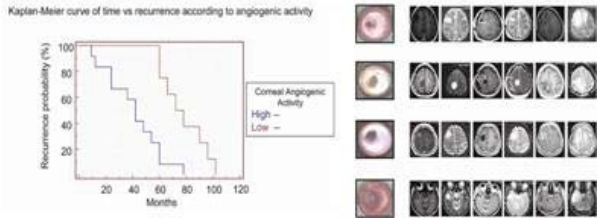
Angiogenic potentials of oligodendroglioma WHO grade 2 tumors were studied by rat cornea angiogenesis model and compared the relationships between MRI contrast enhancement, 1p/19q loss of heterozygosity (LOH) and time to tumor recurrence.Twenty oligodendroglioma WHO grade-2 cases had been randomly chosen, 10 of those were enhancing with contrast in preoperative MRI, while 10 were not. All of those had total excision verified by postoperative 24 hour MRI and none had an adjuvant treatment unless a recurrence was noted. The tumor samples were inoculated into rat corneas and observed and graded. 1p/19q LOH study was performed by fluorescent in-situ hybridization.The time to tumor recurrence of those cases were correlated with contrast enhancement and angiogenic grades, but 1p/19q LOH. The angiogenic potentials of tumors were significantly different between those having a time to recurrence >60 months and those having =60 months. 1p/19q LOH was not a determinant of angiogenesis.Though all tumors were oligodendroglioma WHO grade 2 their angiogenic potentials were heterogenous and angiogenic potentials seemed to have an important role on time to recurrence and thus survival. This study, for the first time, tests the angiogenic activity of low grade oligodendrogliomas and examines its possible role in prognosis.



P181

IDENTIFICATION OF A NOVEL SMALL MOLECULE HIF-1α TRANSLATION INHIBITOR

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Purpose: Hypoxia inducible factor-1 (HIF-1) is the central mediator of the cellular response to low oxygen and functions as a transcription factor for a broad range of genes that provide adaptive responses to oxygen deprivation. HIF-1 is over-expressed in cancer and has become an important therapeutic target in solid tumors. In this study, a novel HIF-1α inhibitor was identifiedand its molecular mechanism was investigated. **Methods:** Using a HIF-responsive reporter cell-based assay, a 10,000-membered natural product-like chemical compound library was screened to identify novel HIF-1 inhibitors. This led us to discover KC7F2, a lead compound with a central structure of cystamine. The effects of KC7F2 on HIF-1 transcription, translation and protein degradation processes were analyzed. **Results:** KC7F2 markedly inhibited HIF-mediated transcription in cells derived from different tumor types, including glioma, breast and prostate cancers. KC7F2 prevented the activation of HIF-target genes such as Carbonic Anhydrase IX, Matrix Metalloproteinase 2 (MMP2), Endothelin 1 and Enolase 1. Investigation of the mechanism of action of KC7F2 showed that it worked through the down-regulation of HIF-1α protein synthesis, an effect accompanied by the suppression of the phosphorylation of eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) and p70 S6 kinase (S6K), key regulators of HIF-1α protein synthesis. **Conclusion:** These results show that KC7F2 is a potent HIF-1 pathway inhibitor and that its potential as a cancer therapy agent warrants further study.

P182

PHASE I STUDY OF VANDETANIB, IMATINIB MESYLATE AND HYDROXYUREA FOR RECURRENT MALIGNANT GLIOMA

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Background: Malignant glioma (MG) is characterized by frequent aberrant activation of EGFR, VEGFR and PDGFR. We determined the MTD and DLT of vandetanib (V), a once-daily, oral selective inhibitor of VEGFR and EGFR when combined with imatinib mesylate (IM), a multi-kinase inhibitor including PDGFR, and hydroxyurea (H). **Methods:** Adult recurrent MG patients with ≤ 3 prior recurrences, KPS ≥ 60% and adequate organ function were stratified based on concurrent enzyme-inducing anticonvulsant use (EIAC). Both strata were independently escalated using a "3+3" design. V was increased by 100 mg in successive cohorts beginning at 100 mg and 200 mg for patients not on and on EIAC, respectively, with established dose levels of IM and H. Evaluations were after every other 28-day cycle. Pharmacokinetics of V and IM were obtained on days 1 and 28 of cycle 1. **Results:** Twenty-six patients (grade 4 MG, n=20; grade 3 MG, n=6) enrolled. Only 1 DLT (reversible grade 4 transaminase elevation; dose level 1) occurred among 22 non-EIAC patients. The MTD of V for patients on EIAC is 200 mg/day due to 2 of 3 patients developing grade 3 thrombocytopenia at the 300 mg/day dose level. Evidence of therapeutic benefit to date includes 1 partial response and 15 patients (58%) with stable disease for at least 4 weeks, including 4 patients for ≥ 4 months. **Conclusions:** V, IM and H is well tolerated in recurrent MG patients. An update of outcome, toxicity and pharmacokinetic analyses will be presented.

P183

A PHASE I STUDY OF SUNITINIB PLUS IRINOTECAN IN THE TREATMENT OF PATIENTS WITH RECURRENT MALIGNANT GLIOMA

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Background: Malignant glioma (MG) are highly angiogenic due to overexpression of VEGF/VEGFR. The current study was designed to determine the MTD and DLT of sunitinib (S), a once-daily, oral selective inhibitor of VEGFR when combined with irinotecan (I), a topoisomerase-1 inhibitor among recurrent MG patients. **Methods:** We employed a "3+3" dose escalation design to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of S, administered once daily for the first 28 days of each 42 day cycle, with I, administered every 2 weeks. The initial S and I doses were 25 mg/day and 75 mg/m^2. Key eligibility criteria included KPS ≥ 70%, adequate organ function and no concurrent CYP3A-inducing anti-epileptics. Pharmacokinetic studies for S are obtained during cycle 1 among 6 additional patients treated at the MTD. **Results:** Eleven patients (grade 4 MG, n=6; grade 3 MG, n=5) have enrolled. No DLTs were observed in cohort 1, but 2 patients experienced hematologic DLT (grade 3 thrombocytopenia, n=2; grade 4 neutropenia, n=1) in cohort 2. Therefore the MTD for this regimen is 25 mg of S plus 75 mg/m^2 of I. Evidence of therapeutic benefit to date includes 8 patients (73%) with stable disease including 3 who continue on therapy. **Conclusions:** Combination of sunitinib plus irinotecan is well tolerated in recurrent MG patients at the defined MTD dose level. Accrual to the PK dose expansion cohort continues.

P184

A PHASE IB TRIAL OF CEDIRANIB IN ADDITION TO STANDARD TEMOZOLOMIDE AND RADIATION THERAPY IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA

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Background: Pre-clinical evidence suggests the effects of radiation therapy (RT) and chemotherapy may be potentiated by inhibitors of angiogenesis. This is a phase Ib study to evaluate the safety and toxicity of the combination of cediranib, a potent pan-VEGF receptor tyrosine kinase inhibitor, standard RT and temozolomide (TMZ) in patients with newly diagnosed GBM who were not taking enzyme-inducing anti-epileptic drugs (EIAED).

Methods: 14-21 days after undergoing tumor biopsy or resection 6 patients with histologically confirmed GBM were treated with concurrent TMZ (75mg/m2/day), RT (60 Gy) and cediranib (dose level 1 = 20mg/day; dose level 2 = 30mg/day) followed by post-radiation TMZ (150-200 mg/m2 on days 1-5 of each 28 day cycle) and cediranib (45mg/day) for up to 6 monthly cycles.

Results: Six patients were treated (cediranib 20mg = 3 patients; cediranib 30mg = 3 patients). No patients experienced a DLT, establishing the maximum tolerated dose (MTD) for cediranib in combination with RT and TMZ as 30mg/day in this patient population. Expected toxicities of hypertension, fatigue, and palmar/plantar erythema were observed. During combined cediranib and TMZ therapy in the post-radiation setting, 1 patient discontinued cediranib because of toxicity (grade 3 transaminase elevation) and 1 patient required dose reduction to 15mg/day due to grade 3 proteinuria. During the course of cediranib treatment, no intratumoral or intracerebral hemorrhages were detected and no cases of wound infection or wound dehiscence were observed. All patients are alive with a median follow-up of 156 days.

Conclusions: The MTD for cediranib given in combination with standard RT and TMZ for newly diagnosed GBM patients not on EIAEDs is 30mg/day, and the therapy is well tolerated. The phase II component of this trial will include correlative biomarker and MRI studies and is underway.

P185

THE POTENTIAL BENEFIT OF NEOADJUVANT AND EXTENDED-ADJUVANT TEMOZOLAMIDE WITH THE STUPP-REGIMEN IN THE TREATMENT OF GLIOBLASTOMA

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In adults, glioblastoma (GBM) is the most common primary cerebral neoplasm and is associated with a poor prognosis. However encouragingly, post–operative concomitant temozolamide (TMZ) – radiotherapy (RT) has led to improved 2, 3 and 4–year survival rates of approximately 37, 25 and 12 % respectively.

Purpose – Evaluation of the potential benefit of combined neoadjuvant TMZ/concomitant TMZ–RT / extended–adjuvant TMZ in the treatment of good performance status patients with GBM.

Methods – Thirty two consecutive patients with supra–tentorial GBM (WHO Grade 4) and KPS of 90 – 100% received the combined regimen. Craniotomy and maximal tumour debulking had been performed in 27, the remainder underwent biopsy only. The median age was 53 (18 – 69) years. Patients were predominantly male (28). All received 1 cycle neoadjuvant TMZ (150 mg /m^2 po daily × 5d) prior to commencing standard Stupp–regimen concomitant TMZ–RT (60 Gy 6 MV X–rays TD/ 30 fractions / 42 d; TMZ 75 mg/ m^2 po daily). Extended–adjuvant TMZ (*vide supra*) was given at 28 day intervals subject to satisfactory blood count, 4 weeks following completion of RT. On average, patients received 18 cycles extended–adjuvant TMZ (7 – 31), which was well tolerated with negligible haematological toxicity or deterioration in QOL. Survival data was analysed using the Kaplan–Meier lifetable method in order to estimate the survival probability over a 36 month period.

Results – For all patients, the estimated survival rate at 12, 18, 24 and 36 months was 87.5, 78.1, 68.8 and 68.8 % respectively. Of the majority subgroup that underwent craniotomy/debulking, survival was not significantly better (70.4 % at 36 months).

Conclusion – The addition of neoadjuvant TMZ and extended–adjuvant TMZ to standard concomitant TMZ–RT proved well tolerated and has appeared to confer a significant survival benefit, with an estimated 3–year survival rate in the order of 70 %.

P186

DOSE DENSE ONE WEEK ON/ ONE WEEK OFF TEMOZOLOMIDE IN RECURRENT GLIOMA.

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Background.
Only few studies examined the effect of dose dense temozolomide (TMZ) in recurrent glioma. We investigated the efficacy and tolerability of one week on/one week off TMZ (ddTMZ) regimen in patients with a recurrent glioblastoma or other heavily pretreated recurrent glioma.

Material and methods.
In a retrospective study we evaluated a cohort of patients treated with ddTMZ in our center between 2005 and 2008 for progression of a glioblastoma during or after chemo-irradiation with TMZ or a recurrence of another type of glioma after radiotherapy and at least one line of chemotherapy. All evaluated patients received TMZ at 150 mg/m^2/d (days 1 through 7 and 15 through 21 on a 28-day basis) with individual dose adjustments according to hematologic toxicity. Response was assessed with MRI using Macdonalds criteria, complete and partial responses were considered objective responses. Primary endpoint was progression free survival (PFS). Totoxicity was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE; version 3.0).

Results.
Thirty-seven patients were included. 16 patients had a progressive glioblastoma after or during chemo-irradiation with TMZ. Twenty-one patients had a recurrence of an originally low-grade or anaplastic glioma after radiation. All these 21 patients received one line or two lines of prior chemotherapy before the start of ddTMZ. The median number of cycles of ddTMZ was 4 (range 1-12). Six patients had to stop chemotherapy because of toxicity; two because of grade 4 thrombocytopenia, 1 because of persistent grade 2 thrombocytopenia, 1 because of elevated transaminases and 2 because of fatigue. The 6 months PFS was 37%, the median overall survival was 9 months. An objective response was obtained in 6 of the 35 patients (17%) evaluable for response. Two of the 16 patients with a progressive glioblastoma had a complete or partial responseand the 6 months PFS was 29%.

Conclusion.
This study indicates that ddTMZ is safe and effective in recurrent glioma, despite previous TMZ and/or nitrosurea chemotherapy.

P187

CLINICAL TRIAL WITH TEMOZOLOMIDE IN AN ALTERNATING WEEKLY REGIMEN AGAINST RECURRENT MALIGNANT GLIOMAS-A PRELIMINARY REPORT

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Wick et al. reported therapeutic data of temozolomide in an alternating weekly regimen against recurrent gliomas. They used temozolomide at 150mg/m^2 Days 1 through 7 and 15 through 21 every 4 week. Although progression free survival rate at 6 months was 43.8%, grade 4 lymphopenia still developed in 12% of patients. Also, their dose is beyond the limitation set by the Ministry of Health, Labour and Welfare of Japan. Therefore, we reduced the dose to the half. Five patients with recurrent malignant gliomas (4 glioblastomas and 1 anaplastic astrocytoma) initially treated with surgical resection and radio-chemotherapy (60Gy (2Gy x 30 fr.) to extended local field plus concomitant temozolomide (75mg/m^2 x 42 days)) were treated with temozolomide in alternating weekly regimen (at 75mg/m^2 Days 1 through 7 and 15 through 21 every 4 weeks). One patient (20%) has responded to the regimen although the mean and longest follow up period is 2 months and 4 months, respectively, so far. Grade 4 lymphopenia or myelosuppression developed in none of patients. We will discuss a response rate and toxicity of this regimen.

P188

SAFETY AND EFFICACY OF NEAR-CONTINUOUS DOSE-DENSE TEMOZOLOMIDE (TEGWONDO) FOR RECURRENT GLIOMAS

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Background: We report on safety and efficacy of a rechallenge of recurrent gliomas with dose-dense 21/28-day or near-continuous 5/7-day ("Tegwondo") temozolomide (TMZ). **Methods:** 56 recurrent glioma patients received over 300 cycles of TMZ, initially at 100mg/m^2 with individual dose adaptation: 21 pts. received 148 cycles day 1-21/28 (18 WHO IV [GBM], 3 WHO III). 35 patients with recurrent or progressive glioma received 186 cycles of near-continous TMZ day 1-5/7, initially at 100mg/m^2 (17 GBM, 8 WHO 8, 6 low-grade). All malignant gliomas were pretreated with temozolomide. **Results:** Toxicity was: hematotoxicity grade 3/4 (asymptomatic): 2/21 pts. (21/28-day, 10%) and 6/35 pts. (5/7-day, 17%); nonhematological 3/4: 4/21 Pts. (21/28, 19%), 0/19 (5/7, 0%). Only in 2/56 patients (one 21/28, one 5/7), haematological toxicity required treatment (platelet transfusion, G-CSF). GBM-pts. (n=35) had a mean age of 55y, median KPS was 65%. Efficacy was: 4 CR (11%), 2 PR (6%), 11 SD (31%) and 18 PD (51%) at >3 months. Progression-free survival at 6 months was 37%, at 12 months 9% and at 24 months 6% (2 pat.). Survival after relapse was 30.6 weeks. First exemplary tests during the 5/7-day regimen indicate that MGMT in the peripheral blood is permanently depleted despite the 2-day pause of TMZ application. **Conclusions:** In line with our previous reports, we demonstrate that rechallenge of recurrent gliomas with dose-dense temozolomide starting at 100 mg/m^2 and individual dose adaptation is feasible and active also in patients with critical blood counts and unfavourable prognosis. Near-continuous application day 1-5/7 allows best for an individual dose adaptation and seems to permanently deplete the alkylation-resistance factor MGMT. These updated results support our previous experience that near-continous TMZ ("Tegwondo") (TEmozolomide Glioma WOrkiNgday DOse-dense regimen) has activity and may overcome MGMT-mediated resistance to chemotherapy.

P189

PHARMACEUTICAL CONTROL OF TEMOZOLOMIDE CHEMOTHERAPY WITH ORIGINAL LEAFLETS AND ADMINISTRATION HISTORY DATABASE

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Background
Temozolomide (TMZ) is a new oral cytotoxic chemotherapeutic agent for gliomas. The usage and dosage are different between standard induction course combined with radiotherapy and maintenance course of five days every 28 days. Therefore, monitoring and assessment of prescription contents are important to keep validity in dosage and schedule. On the other hand, TMZ has many hematologic and non-hematologic adverse effects. Several reports showed lethal Pneumocystis carinii infection in patients with severe lymphocytopenia caused by TMZ. The observance of treatment schedules including TMZ, 5-HT_3 antagonist, and antibacterial medicine regimens are of importance to achieve maximum therapeutic effects and also to reduce side effects.

Purpose and methods
We constructed an administration history database using a commercial software (Microsoft Office Access) and routinely checked each dosage and treatment schedule. We also made leaflets of TMZ for patients to support their start and maintenance of the treatment. Adverse events were analyzed in 53 patients (twenty-two patients treated with induction course and thirty-one patients treated with maintenance course) according to CTCAE grading system.

Results
Prescriptions were monitored and assessed in 81 patients. Administration deviations were reported to prescribing doctors and regulated in 2 patients by our database monitoring. Support with our original leaflets were performed for 23 patients in ward; it helped especially children with safe administration of TMZ. Grade 3 or 4 lymphocytopenia were observed in 12 patients without noticeable decrease in white blood count or clinical manifestations. No patients suffered from pneumonia during investigation.

Conclusion
Our new administration history database was simple and useful to monitor treatment schedules and doses. Our pharmaceutical service with original leaflets helped patients and families to understand drugs and treatment schedule. Lymphocyte count must be followed up in patients during treatment with TMZ.

P190

SAFETY AND EFFICACY OF TEMOZOLOMIDE TREATMENT WITH NEWLY DIAGNOSED GLIOBLASTOMA IN JAPAN. A MULTICENTER PHASE II CLINICAL STUDY

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Objective: An open-labled phase II study was conducted to evaluate the safety and efficacy of temozolomide (TMZ) in Japanese patients with newly-diagnosed glioblastoma. **Methods:** Thirty patients with supratentorial glioblastoma (26-69 years old, median: 55.5) were enrolled post-operatively from Oct. 2005 to Nov. 2006. They were fourteen males, and sixteen females. Tumors were resected totally in 7, partially in 21, and biopsied in 2. According to the therapeutic regimen of Stupp et al. (EORTC study, N Engl J Med 2005), the patients received daily administration of TMZ concomitantly with radiotherapy (RT) followed by TMZ monotherapy (5 on/23 off) up to 12 months after the beginning of RT. For prophylaxis of pneumocystis pneumonia (PCP), oral administration of 1g trimethoprim-sulfamethoxazole 3 times a week or another alternative was required during the RT period. **Results:** Seven patients did not progress to the TMZ monotherapy phase, 4 due to disease progression and 3 due to grade 3-4 adverse events. Of the remaining 23 patients, 9 completed this treatment and 14 discontinued treatment, 11 due to disease progression, 2 due to other malignancies, and 1 due to pulmonary embolism. The tumor reduction effect in 19 patients with measurable lesion was CR in 3, PR in 6, NC in 6, and PD in 4, and the response rate was 47%, with a median response period of 10.5 months. The main adverse events with Grade 3&4 were neutropenia(17%), lymphocytepenia(7%), and constipation(7%). No PCP was observed. Median progression-free survival was 8.3 months, and median overall survival was 18.1 months. **Conclusions:** Concomitant therapy with TMZ and RT followed by TMZ monotherapy was also effective in Japanese patients with newly-diagnosed glioblastoma. The safety profile in this trial was similar to that observed in the EORTC Phase III study.

P191

RETROSPECTIVE ANALYSIS OF GLIOBLASTOMA (GBM) PATIENTS TREATED WITH TEMOZOLOMIDE (TMZ)-RADIOTHERAPY FOLLOWED BY TMZ FOR 2 YEARS OR UNTIL PROGRESSION: SURVIVAL ANALYSIS

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The standard treatment of newly diagnosed GBM is TMZ followed by adjuvant TMZ for six months. The optimal duration of maintenance therapy with TMZ is controversial.We evaluated in a retrospective analysis the 12 and 24 month overall survival and progression free survival in GBM patients treated with resection or biopsy followed by chemo-radiotherapy followed by TMZ (150 to 200 mg/m2/day for 5 days each 28) for two years or until disease progression or unacceptable toxicity.We also evaluated if age (< 40 vs. > 40), gender, KPS (< 70 vs. > 70), and type of resection (partial -PR, complete -CR and biopsy) impacted survival. Fifty-six patients with primary GBM treated in our institution between May 2005 and September 2008 were included and stratified according the study parameters.The median age was 59 years, range (35-75), 62% were males. Ninety two percent had undergone debulking surgery. MGMT promoter gen metilathion status was done in 25/56 (15/25 were metilathed), and EGFR amplification in 39 of 56. (26/39 were amplified).The median follow-up was 11 month, range (5-44). PFS was 32% at 12 months; the two-years survival rate was 30%. Interruptions due to toxicity occurred in 7% of patients during induction and 95% completed both radiation and TMZ therapy as planned. Nine (16%) patients could not start maintenance because progression of disease or severe toxicity.Concomitant treatment with radiotherapy plus temozolomide resulted in grade 3/4 hematological toxic effects in 10,7% of patients. During the maintenance phase with TMZ 6,4% of patients experienced hematological toxicity (grade 3/4).These results suggest that long-term treatment with TMZ is feasible and well tolerated. The KPS >70 and age < 40 was related significantly to survival (p=0.004 and p=0.05 respectively). Update results will be presented at the meeting.

P192

PSEUDPROGRESSION OR TUMOR PROGRESSION IN GLIOMAS: WHAT KINDS OF DIAGNOSTIC TOOLS ARE MORE RELIABLE?

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Differentiation of peudoprogression from tumor progression remains a challenging diagnostic problem. Conventional MR imaging findings are considered to be inadequate for reliably distinguishing peudoprogression from tumor progression in patients with glioma.. This study was undertaken to determine diagnostic value for peudoprogression in 15 patients with peudoprogression who were underwent surgery for glioma during last 10 years. All patients had previously undergone tumor resection followed by radiotherapy. The most histological type was glioblastoma in 12 patients (63%). There were 10 males and 5 females (ratio female: male 2:1) ranging in age from 31 to 85 years(mean 47.8. years). The clinical symptoms of the patients with peudoprogression was motor weakness in 8. headache in 5, mental change in 3, generalized seizure in 2 patients. 18-FDG PET was done in 9 patients who have high uptake in only 5 patients. The sensitivity of a combination of MR spectroscopy with perfusion imaging for peudoprogression was better than conventional contrast-enhanced imaging and /or 18-FDG-PET. Three patients were treated conservatively with steroid medication and other 12 patients were treated by decompressive removal of necrotic mass after confirmed diagnosis by stereotaxic biopsy. Follow-up examination showed 12 patients alive with median survival of 56.3months. Three patents were died of severe brain swelling and systemic complications. The survival following stereotactic biopsy was markedly increased in patients suffering from radiation effect compared with those harboring recurrent malignant glioma. In patients with previously irradiated gliomas in whom were clinically or radiographically suspected, MR spectroscope and perfusion MRI might be more reliable diagnostic tool to differentiate peudoprogression from tumor recurrence. In addition, the decompressive removal of necrotic mass could improve survival rates.

P193

HYPOFRACTIONATED HIGH-DOSE IRRADIATION BY IMRT CONCURRENT WITH PROPHYLACTIC INTRATHECAL CHEMOTHERAPY FOR THE TREATMENT OF GLIOBLASTOMA

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Backgrounds: For the better survival of pts with glioblastoma (GBM), we initiated hypofractionated high-dose irradiation (HdI; 68Gy/8F) by IMRT to control regional tumor, and prophylactic intrathecal administration of thiotepa (piT) to prevent cerebrospinal fluid dissemination (CSFd). The aim of this analysis is to evaluate the clinical significance of these treatments. **Methods:** Histologically confirmed GBMs without CSFd at diagnosis were enrolled. Pts were classified into three groups owing to the post-surgical treatment as follows: Group A pts (n=62) were treated by conventional radiotherapy (60Gy/30F) alone, Group B pts (n=28) were by HdI alone and Group C pts (n=30) were by HdI concurrent with piT. The patterns of recurrence and overall survival (OS) were compared in these three groups. **Results:** The dominant pattern of recurrence was local in all groups. HdI decreased the local failure from 71 to 41% (p=0.003), but increased the CSFd from 8 to 37%, resulted in the similar total recurrence rates (Group A: 79%, Group B: 78%). However, in Group C, CSFd was decreased to 13% while keeping the low ratio of local failure (30%), and total recurrence rate was decreased to 43% (p=0.029 vs. Group B). The median OS was 12.4 months in Group A, 17.3 months in Group B and 37.4 months in Group C. There was no significant difference of OS between Group A and B (p=0.409), but Group C showed significantly longer OS than Group A (p=0.005) and B (p=0.010). Radiation injury was the cause of deterioration in 50% of the pts who lived more than 2 years, although in only 5% of pts died within 2 years. **Conclusions:** HdI concurrent with piT showed significant effect on both the local control and prevention of CSFd, resulted in good survival of pts with GBM. However, radiation injury was a problem in long survivor.

P194

A PILOT SAFETY TRIAL OF HYPOFRACTIONATED RADIATION THERAPY PLUS CONCOMITANT AND ADJUVANT TEMOZOLOMIDE FOR ADULTS WITH GLIOBLASTOMA MULTIFORME

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Purpose To prospectively evaluate the impact of hypofractionated radiotherapy (RT), confirmed by randomized clinical trial with no differences in survival compared with standard radiotherapy and meaningful neurocognitive toxicity profile in older patients with glioblastoma multiforme (GBM), in adults with newly-diagnosed GBM when given concurrently with temozolomide (TMZ). **Patients and Methods** Twenty adults with newly-diagnosed GBM received shorter-courses RT (45 Gy in 15 fractions over 3 weeks) concurrently with TMZ given for 3 weeks followed by a 28 days rest period and continued TMZ at 150-200 mg/m2/d on days 1-5 until tumor progression or unacceptable toxicity. Primary objectives were to determine progression-free survival (PFS) at 6 months for abbreviated RT plus TMZ treatment. Secondary objectives were to assess health-related quality of life (HRQOL), early progression, and seizure control. HRQOL was assessed using Karnofsky performance status (KPS) and Functional Assessment of Cancer Therapy-Brain (FACT-Br) Subscale. **Results.** Twenty patients were enrolled, of all were treated and evaluable for both PFS and HRQOL. The 6-month PFS measured from start of treatment was 70%. KPS score and FACT-Br Subscale have a good correlation but there were not statistically significant between the pre- and post-treatment. Early progression occurred in 5 patients (25%), and seizure relapse was observed in two (10%) patients. **Conclusions.** Hypofractionated RT concurrently with TMZ has superior impacts on PFS compared with a historical control. Decreased in seizure frequency, same impacts on early progression, and reduced treatment time offers advantage for adults with GBM.

P195

UPFRONT CHEMOTHERAPY FOR GLIOMAS DELETING 1P AND 19Q FOLLOWED BY SECOND-LOOK REMOVAL AND/OR RADIOTHERAPY AT MAXIMUM RESPONSE

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Background: Oligodendroglial tumors losing 1p and 19q are associated with increased response to chemotherapy and survival. In low-grade gliomas, presence of the codeletion correlates with those favorable clinical parameters regardless of histology (Neurology 68:1831-6, 2007; J Clin Oncol 24:4758-63, 2006). **Methods:** Gliomas with 1p and 19q codeletion have been treated by upfront chemotherapy regardless of histology (Keio protocol Feb/08). At maximum response, patients are re-evaluated to see if effective removal is possible, and second look-removal is recommended if near total removal seems amenable. Grade 3 tumors are irradiated after max. response or after second look-removal if performed. **Results:** Eighteen gliomas with the codeletion have been treated (8 PR/MR, 8SD, 2NE). Two tumors recurred after partial response (AO at 12 months; OD at 17 months). However, tumor recurrence occurred within the area that was to be resected if second look-removal were planned. Near total removal was performed in two tumors after tumor shrinkage by upfront chemotherapy for which effective removal was not initially possible. In an AO, radiotherapy was given after max. response. **Conclusion:** Upfront chemotherapy for gliomas deleting 1p and 19q followed by second-look removal and/or radiotherapy seems beneficial to improve survival times of patients with those tumors.

P196

BENEFIT OF CHEMOTHERPY IN ANAPLASTIC ASTROCYTOMAS

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Object: To evaluate the role of chemotherapy in the management of anaplastic astrocytomas (AAs), the authors retrospectively reviewed the survival outcome according to the treatment regimens in a single institution.**Patients and Methods:** Eighty six patients (39 men and 47 women) with newly diagnosed AAs between 1990 and 2004 were enrolled in this study. All lesions were proven pathologically and 47 lesions were resected surgically. The mean age was 40.9 years (range: 18 ~70). Thirty one (36.0%, median age; 42 years) of all patients were treated with only conventional radiotherapy (CRT) post-operatively (CRT Group) and 25 patients (29.1%, median age; 37 years) with the CRT followed by adjuvant chemotherapy with PCV (procarbazine, lomustine and vincristine) regimen (PCV group) Thirty patients (34.9%, median age; 40.5 years) were managed postoperatively with nimustine-cisplatin followed by CRT (ACNU-CDDP group). The survival time as analyzed according to the performance status, degree of surgical resection and post-operative management strategy with log rank test. Toxicity associated with treatments was also analyzed. **Results:** The median survival was 72.0, 30.0, and 14.0 months in PCV, ACNU-CDDP and CRT group, respectively. (95% CI= 52.0-91.9; 21.4-38.6; 7.8-20.2) The PCV group showed the longer survival time than both ACNU-CDDP and CRT group. (p=0.045; p=0.002) And neoadjuvant nimustine-cisplatin was beneficial than only CRT(p=0.039) In the multivariate analysis, good performance status, large degree of surgical resection (than biopsy only), adjuvant or neo-adjuvant chemotherapy (ACNU/CDDP-CRT or CRT-PCB than CRT only) were positive prognostic factors. In terms of complications related to treatments, PCV group showed the low incidence than ACNU-CDDP group, also. **Conclusion:** Adjuvant or neo-adjuvant application of chemotherapy to radiation therapy showed benefit compared with RT in prolonging the survival in anaplastic astrocytomas patients. PCV after CRT seems to be better than other strategies, considering the survival benefit and low toxicity profile.

P197

THERAPY-RELATED MYELODYSPLASTIC SYNDROME FOLLOWING TREATMENT FOR MALIGNANT GLIOMA

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Objective: Therapy-related myelodysplastic syndrome (t-MDS) is a rare complication of cancer treatment. The prognosis is very poor. We present three cases of myelodysplastic syndrome (MDS) following treatment for malignant glioma. **Clinical presentation:** Case 1: A 2-year-old boy was diagnosed with anaplastic astrocytoma after gross total removal of the tumor. The cumulative doses of chemotherapy were 1100 mg MCNU and 590 x 106 IU interferon-beta. Gamma knife therapy was done for the recurrent tumor. T-MDS was diagnosed 12 years after the initial treatment. The patient is alive without recurrent tumor. Case 2: A 10-year-old girl was diagnosed with anaplastic astrocytoma after biopsy. Craniospinal radiation and chemotherapy were performed. The cumulative doses of chemotherapy were 1495 mg MCNU, 915 x 106 IU interferon-beta, 200 mg cisplatin and 100 mg etoposide. Gamma knife therapy was done for the recurrent tumor. t-MDS was diagnosed 15 years after the initial treatment. The patient is alive without recurrent tumor. Case 3: A 45-year-old man was diagnosed with anaplastic oligoastrocytoma after partial removal of the tumor. He received chemotherapy and irradiation. For the recurrent tumor, partial removal was performed. The cumulative doses of chemotherapy were 2400 mg MCNU, 562 mg ACNU, 16 mg vincristine, and 5600 mg procarbazine. t-MDS was diagnosed 7 years after the initial treatment. He died although he received bone marrow transplantation. **Conclusions:** t-MDS may be a complication of chemotherapy for patients with brain tumor. Therefore, they should be carefully followed up by regular physical examination and complete blood counts.

P198

TENIPOSIDE AND NIMUSTINE REGIMEN FOR MALIGNANT GLIOMAS WITH O6-METHYLGUANINE-DNA METHYLTRANSFERASE (MGMT) NEGATIVE EXPRESSION: EXPERIENCE OF 18 CASES

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[ABSTRACT] BACKGROUND and OBJECTIVE: The present study was to elucidate the efficiency and side effects of teniposide(VM-26) and nimustine(ACNU) regimen for high grade gliomas(HGGs) with O6-methylguanine-DNA methyltransferase(MGMT) negative expression. **METHODS:** Eighteen HGG patients received previous radiotherapy were enrolled in this study. The expression of MGMT protein was detected by immunohistochemistry. The regimen consisted of ACNU 2-3 mg/kg administered once and VM-26 300mg/m2, which was divided for 1-3 days intravenous administration with every 6~8 weeks a cyc1e. **RESULTS:** Seventy cycles were admitted and included for evaluation, with the cycle range of 2-6(median 3.9) for each patients. No complete response (CR) was achieved, while partial response (PR) in 1 patient (5.6%), minor response(MR) in 13 patients(72.2 %), stable disease (SD) in 3 patients(16.7%), and progressive disease (PD) in 1 patient, respectively. Objective response rate (CR+PR), overall response rate (CR+ PR+MR) and disease control rate (CR+PR+MR+SD) were 5.6 %, 88.9 %, and 94.4 %, respectively. Median progression free survival (PFS) was 2.6 months (95% CI:2.49-2.90), 6 month PFS was 36%, and median overall survival (OS) was 6.7 months (95% CI: 3.35-11.1). Myelosuppression including leukopenia and plateletpenia were the most common high grade toxicities (grade 3 and 4). The incidences of grade III and IV leukopenia were 51.4%(36/70) and 25.7 % (18 / 70), and those of grade III and IV plateletpenia were 24.3%(17/70)and 12.9 % (9/70), respectively. The incidences of high grade leukopenias and plateletpenias were not related to prior chemotherapy(p>0.05). **CONCLUSION:** VM-26 and ACNU regimen for MGMT negative glioma patients is acceptable, and may improve overall response rate, disease control rate and 6 month PFS.KEY WORDS:Glioma, Chemotherapy,Teniposide, Nimustine

P199

SAFETY PROFILE OF CARMUSTINE WAFER TREATMENT IN FIRST LINE TREATMENT OF MALIGNANT GLIOMA WITH CARMUSTINE IMPLANTS FOLLOWED BY CONCOMITANT RADIOCHEMOTHERAPY

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Objective
Randomized trials have shown improvement of survival for patients with newly diagnosed malignant gliomas for resection and implantation of BCNU-wafers followed by radiation and also for postoperative concomitant radiochemotherapy with TMZ. This has resulted in protocols combining BCNU-wafer and concomitant radiochemotherapy. We have compiled the treatment experience of 7 neurosurgical centers using implantation of carmustine wafers at primary surgery followed by six weeks of radiation therapy (59-60 Gy) and 75mg/m2/d TMZ in patients with newly diagnosed glioblastoma.

Patients and methods
We have retrospectively analyzed the postoperative clinical course, occurrence and severity of adverse events, progression free interval, and survival in 44 patients who received tumor resection, BCNU-wafer implantation and concomitant radiochemotherapy.

Results
Of 44 patients who received gliadel wafer 25 patients (57%) had died and 3 patients (7 %) had progressed at time of data analysis (mean PFS 7.7 &plusmn 6.3 months). Mean overall survival was 11.3 &plusmn 6.7 months at the time of data closure with 19 patients alive (median follow up 11.6 &plusmn 8.2 months). Adverse events of any kind were reported for 23 patients (52%) and resulted in therapy delays for concomitant radiochemotherapy (18%) or a deferred start of temozolomide monochemotherapy (9%). Surgical complications such as wound healing abnormalities (16%), CSF-leakage (11%), meningitis (7%), intracranial abscess formation (5%), cerebral edema (25%) and hydrocephalus (7%) were observed. Unscheduled readmission was required for 19 patients and surgical intervention was necessary in seven. Medical complications included thrombembolic events and hematotoxicity. Adverse events in 57% occurred during radiochemotherapy and later during TMZ monochemotherapy (39%).

Conclusion
Our data demonstrate that combination of local chemotherapy and concomitant radiochemotherapy is promising, but carries a significant risk of toxicity that currently appears underestimated. A modified follow up is required to account for the frequent occurrence of late adverse events.

P200

THE GROWTH PATTERN OF RECURRENT GLIOBLASTOMA AFTER GLIADEL(TM) WAFER IMPLANTATION IN FIRST RECURRENCIES

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Purpose: Although in recent years several approaches for local therapies in the treatment of glioblastoma multiforme have been tested in clinical trials, carmustine polymeres (Gliadel(TM) wafer) is the only evaluated local therapy to date. Because of the predominance of temozolomide, Gliadel(TM) is frequently used in recurrent gliomas only. However, data about effectiveness and the pattern of re-recurrences are rare. Therefore, we initiated the present MRI-based retrospective study. **Methods:** 37 patients had surgery for first recurrence of glioblastoma, where Gliadel(TM) wafers (n=1-8) were implanted. Early post-op MRI was performed documenting the extent of resection, tumor remnants and wafer placements. Follow-up MRI was performed every two months looking particularly for tumor growth in relation to the wafer placements. Progression-free and overall survivals were recorded. **Results:** 27 patients were available for evaluation, while 10 patients had incomplete data. In 24 (88%) patients an early tumor growth was recorded in the first MRI follow-up, 2 months post-op in areas where the wafers were not implanted. If the wafers had been placed in areas with suspicious tumor according to the early post-op MRI, tumor progression was recorded in the follow-up in 10 (67%) of 15 patients. If the wafers were placed in areas without suspicious tumor remnants (12 patients), regrowth in those areas was recorded only in 4 (33%) patients, meaning 67% of the patients had no early tumor progression when Gliadel(TM) was placed in tumor-free areas. However, tumor pseudo-progression has to be taken into account for all cases. Survival data will be determined. **Conclusions:** The use of Gliadel(TM) wafer in recurrent glioblastoma is most effective in areas with no tumor remnants, underlining the meaning of surgical resection of recurrent tumors, also.

P201

PRE-RADIATION CHEMOTHERAPY WITH ACNU-CDDP IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA: A RETROSPECTIVE ANALYSIS

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OBJECTIVE We evaluated the benefit of pre-radiation chemotherapy with ACNU (nimustine) and CDDP (cisplatin) in patients with newly diagnosed glioblastoma by retrospective analysis. **METHODS** A total of 151 patients were newly confirmed to have glioblastoma between January 2000 and December 2004. All patients underwent surgical resection: 38 (25.2%) patients underwent complete resection, 73 (48.3%) underwent incomplete resection, and 40 (26.5%) underwent biopsy. Pre-radiation chemotherapy using ACNU-CDDP was administered as an initial adjuvant therapy for 87 (57.6%) patients (ACNU-CDDP group), radiation therapy was performed in 31 (20.5%) patients (RT group), and the remaining 33 (21.9%) patients were treated with other regimens or refused to undergo further treatment. **RESULTS** The median survival time was 13 months (95% CI, 11.29-14.71), and the overall survival rate was 54.0% at 1 year and 21.3% at 2 years. The differences in median survival time between the complete resection group and biopsy group and between the ACNU-CDDP group and RT group were significant (15.0 months vs 10 months, p=0.028; 16.0 months vs 12.0 months, p=0.036) in the univariate analyses. Even in the multivariate analysis, pre-radiation chemotherapy using ACNU-CDDP had a significant effect on survival prolongation (HR=0.628, p=0.042). The usage of temozolomide for adjuvant or salvage therapy also had an independent and significantly positive effect on survival (HR=0.511, p=0.006). Grade 3 and 4 hematologic toxicities occurred in 28 (32.1%) patients in the ACNU-CDDP group, but there were no treatment-related deaths. **CONCLUSION** Pre-radiation chemotherapy with ACNU-CDDP as an initial therapy has independently beneficial effects on survival prolongation, with tolerable treatment-related toxicities in patients with newly diagnosed glioblastoma.

P202

A CONCURRENT ASSOCIATION RADIATION THERAPY FOTEMUSTINE FOR NEWLY DIAGNOSED MALIGNANT GLIOMAS, A PHASE II.

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Purpose: Fotemustine, a nitrosourea compound, is used for the treatment of malignant gliomas, especially in France. Recently, EORTC NCI Canada have been showed that a concomitant combination radiation therapy temozolomide (oral cytotoxic drug) improve survival in glioblastomas. We are testing a concurrent combination of fotemustine and radiotherapy for newly malignant gliomas. **Methods:** A prospective phase II study has opened for accrual in September 2004. Patients over 18 years of ages who are able to give informed consent and have histological proven, newly diagnosed supratentorial malignant gliomas are 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 before radiation therapy). Adjuvant chemotherapy, fotemustine, was administered at tumor progression as standard and classic regimen. **Results:** 22 patients have been enrolled in this study, 16 men and 6 women, median age 56 years-old (range 32 to 74), median Karnofsky performance status 70 (range from 60 to 90). Histology included 16 glioblastomas, 3 anaplastic astrocytomas, 2 anaplastic oligodendrogliomas and 1 mixed glioma, Surgery (3 total resections) was performed for 5 patients and stereotactic biopsy for 14 patients. This concurrent association radiation therapy fotemustine has been well tolerated, the toxicity was mild; three hematologic toxicities as grade III-IV has been observed. Median survival from initial diagnosis was 9.9 months, two patients currently remain alive. Median survival was 11 months for surgery and 9 months for stereotactic biopsy. **Conclusions:** Concomitant combination fotemustine radiation therapy is safe and well tolerated. Overall survival of over 10 months for all population compares favorably with other reports.

P203

TOXICITY PROFILE OF VALPROATE AND ETOPOSIDE METRONOMIC CHEMOTHERAPY

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Introduction: Valproate, an antiepileptic drug, also inhibits histone deacetylase. When given with etoposide the drugs synergistically induce apoptosis and cell differentiation in vitro (Das J Neurooncol 85:159-70). Here, we describe the toxicity profile of the valproate and etoposide given as combined oral metronomic dose. **Methods:** A Retrospective chart review was conducted for patients treated with both valproate and etoposide. Dose limiting toxicities were categorized according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). **Results:** Eight patients with relapsed CNS malignancies were included (4 males, 4 females; 6 children, 2 adults). The median of individual maximum blood valproate level was 129 microg/ml (range: 39-221). The most common dose-related side effect was mucosal thickening seen on MRI (6/7). An episode of grade III hand-foot syndrome with redness, pain and swelling of the skin resulted in discontinuation of etoposide. Other dose limiting toxicities were fatigue (n=2), grade III leukocytopenia (n=5) and grade III mucositis (n=2) in children and thrombocytopenia in adults (n=2), resulting in scheduled dose reductions. The median of individual Maximum Tolerated Dose (iMTD) for Valproate was 10 mg/kg/day (range 5-37) when given simultaneously with etoposide (median iMTD 32 mg/m2/day, range 22-54). **Discussion:** The toxicity profile of this combination chemotherapy appears to be age-dependent. Fatigue, mucositis and leukocytopenia were observed as chronic toxicities. All dose related toxicities were reversible. Conclusion: Bone marrow, skin and mucosal toxicities limit the dose of this combination chemotherapy. The inter-person variability in toxicity was large, requiring further studies.

P204

COMBINATION THERAPY WITH CARBOPLATIN AND ETOPOSIDE FOR RECURRENT MALIGNANT GLIOMAS

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Background. The current standard care for newly diagnosed glioblastoma (GBM) includes chemotherapy with temozolomide (TMZ), but no standard regimens have been established for recurrent GBM. We investigated efficacy and toxicity of combined chemotherapy with carboplatin (CBDCA) and etoposide (VP16) (CBET) for recurrent GBM. **Methods.** Forteen patients with recurrent high grade glioma treated with CBET from December 2001 to April 2008 at Kyorin University Hospital (median age of 52 yo, 19-65; median KPS 70) were included. Since March 2003, the intensified dose regimen (CBDCA 360mg/m^2, day 1; VP16 120mg/m^2, day 1-3) was applied to 11 cases (anaplastic astrocytomas 2, GBM 9). **Results.** Among the 11 patients, three received the treatment as the second line, eight as the third line, and ten (91%) had undergone previous TMZ therapy. Six patients received only one cycle, while other five did three or more cycles. Stable disease (SD) was achieved in five patients (45%), but there were no complete or partial responses. The median PFS from the start of CBET was 1.7 months, PFS at 6 months was 14%, the median survival from the start of CBET was 5.6 months. CBET as the second line (p=0.03) and no history of previous TMZ (p=0.02) were associated with longer time to progression in a log-rank analysis. The best response of SD had a statistical impact on overall survival (p=0.02) compared with progressive disease. Grade 3 or 4 neutropenia was observed in 100% of patients, thrombocytopenia in 55%, and anemia in 27%. **Conclusions.** CBET induced a stabilization of disease in a half of recurrent GBMs, but caused a notable hematotoxicity. TMZ-pretreated GBM tended to fail to respond CBET. Dose modification needs to be considered for further application.

P205

PHASE II STUDY OF IFOSFAMIDE, CARBOPLATIN AND ETOPOSIDE FOR PATIENTS WITH GLIOBLASTOMA AT FIRST RELAPSE

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Purpose The prognosis of patients with recurrent glioblastoma is remains unsatisfactory. We conducted a phase II study of ifosfamide, carboplatin and etoposide (ICE) for patients with glioblastoma at first relapse to prolong the useful life. **Patients and methods** This was an open-label, single-center phase II study. Forty-two patients with first glioblastoma at first relapse after surgery followed by standard radiotherapy (60 Gy) with a first-line temozolomide-based or ACNU-based chemotherapy, were enrolled. The primary endpoint was progression-free survival at 6 months (PFS-6), and secondary endpoints were response rate, toxicity, and overall survival. Chemotherapy consisted of ifosfamide (1000 mg / m^2 on day 1, 2 and 3), carboplatin (110 mg / m^2 on day 1), etoposide (100 mg / m^2 on day 1, 2, and 3), every 6 weeks. PFS-6 was 35 % (95%CI, 22 % to 50 %). The median PFS was 17 weeks (95% CI; 10 to 24 weeks). Response rate was 25 % (95% CI, 9 % to 34%). Adverse events were generally mild and consisted mainly of alopecia. **Conclusion** This regimen is well tolerated and has some activity and could be one of the options for patients with recurrent glioblastoma.

P206

PHASE II STUDY OF ANTINEOPLASTONS A10 AND AS2-1 IN PATIENTS WITH BRAINSTEM GLIOMA. PROTOCOL BC-BT-11

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The aim of this study is to evaluate the outcome of the treatment of brainstem gliomas with ANP. A total of 40 patients were enrolled, but twelve were not evaluable due to too short a duration of treatment and lack of follow-up MRIs. Among 28 evaluable patients (ST), there were 23 children and five young adults. Twelve patients were newly diagnosed, and sixteen were previously treated. The additional group of 52 evaluable patients (40 children and 12 young adults) were treated under special exception (SE), and eighteen of these patients were newly diagnosed. In both ST and SE groups, 92% of patients suffered from diffuse intrinsic brainstem gliomas (DBSG). ANP was administered daily through a subclavian venous catheter via a double channel infusion pump. The median duration of treatment was 5.4 (ST) and 5.6 months (SE). The median of average dosages of A10 was 9.0 g/kg/d (ST) and 9.4 g/kg/d (SE), and AS2-1 0.3 g/kg/d in both groups. ANP was well tolerated with serious toxicities occurring in less than 10% of patients in both groups including fatigue, somnolence, hypernatremia, hypokalemia, skin rash, polyuria, anemia, vomiting, elevated transaminases, dyspnea, and subcutaneous extravasation with no chronic toxicities. The responses in both groups were as follows: complete response 18% (ST), 10% (SE), partial response 14% (ST), 4% (SE), stable disease 43% (ST), 54% (SE), progressive disease 25% (ST), 32% (SE). The overall survival (OS) at two years was 36% (ST), 42% (SE), and at 5 years was 25% (ST), 19% (SE). ANP resulted in marked responses and survival rates in patients with uniformly fatal, inoperable brainstem gliomas. These results compare favorably to radiation therapy and chemotherapy (Mandell et al 1999, 7% OS at two years and 0% at five years), but should be confirmed in Phase III trials scheduled to begin in 2009.

P207

TREATMENT OF PRIMARY MALIGNANT GLIOMAS AND MENINGIOMAS WITH RUTA/CALC PHOS

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Purpose: In India, the vast majority of people with brain tumors have no access to conventional therapy. At our community clinic in Kolkata, India, we use the ultra-dilute medicines Ruta 6 and Calc Phos 3X, prepared according to government regulated standard pharmacopeias commonly used in homeopathic practice, to treat all brain neoplasms, including the commonly occurring malignant gliomas and meningiomas. . Previous in vitro studies suggest that Ruta/Calc Phos selectively induces cell death in brain cancer cells but promotes proliferation in normal peripheral blood lymphocytes. The purpose of this study was to document our outcomes using this novel non-surgical approach to treatment of primary malignant gliomas. **Methods:** A retrospective chart review of 90 gliomas, 58 astrocytomas and 144 meningiomas with treatment initiated in 2000 and extending into 2007 was conducted. All available clinical, histological and radiological records for diagnosis and follow-ups were analyzed. Patient reported outcomes (PRO) as well as disease status outcomes when available in the clinical records and reports were tallied. We only report on patients who returned for at least one follow-up visit. **Results:** : Overall survival rates for gliomas were 74%, 53%, 43%, 39% and 32% at 1,2,3,4 and 5 years respectively. For the astrocytomas grades 2-4, survival rates were 81%, 66%, 48%, 38% and 35%. Meningioma survival rates were 99%, 83%, 54%, 39%, and 33%. Of special note is that 21% of the astrocytomas, 22% of gliomas, and 20% of meningiomas went into complete remission without any surgery, chemotherapy or radiation therapy. The majority of patients reported their status as better than before initiating treatment with Ruta/Calc Phos. **Conclusions:** Treatment with Ruta/Calc Phos achieved survival rates comparable to conventional aggressive treatment at a fraction of the cost and with no adverse impact on quality of life. Further prospective trials are needed.

P208

LEPTOMENINGEAL GLIOMATOSIS IN PATIENTS TREATED WITH BEVACIZUMAB

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Anti-angiogenic therapy is utilized in therapy of malignant gliomas with increasing frequency. While dramatic radiographic responses and improved survival can be seen in many patients receiving VEGF inhibitors, rapid progression of the disease has been observed. We describe our experience with two patients who developed leptomeningeal gliomatosis (LG) during therapy with bevacizumab.A twenty-eight year old man was diagnosed with pleomorphic xanthoastrocytoma with anaplastic features, WHO grade III, 22 months prior to presentation with LG. At the time of recurrence tissue diagnosis identified high grade glioma with features of glioblastoma. The patient received therapy with radiation and temozolomide (6 adjuvant cycles). He was then found to have a subependymal nodule in the left frontal lobe and received stereotactic radiosurgery. Bevacizumab and irinotecan were started. After 2 cycles patient worsened clinically and his MRI identified extensive FLAIR changes involving both hemispheres and ventral brainstem with leptomeningeal enhancement. A twenty-eight year old man was diagnosed with ganglioglioma WHO grade I, 35 months prior to presentation with LG. Initial therapy included resection with subsequent radiotherapy. Patient progressed 23 months later and tissue diagnosis confirmed high grade glioma with features of glioblastoma. Temozolomide was introduced for 6 cycles. At the time of second progression patient was treated with bevacizumab and carboplatin. After receiving 1 cycle of therapy he was found to have extensive intracranial disease with leptomeningeal involvement. Leptomeningeal gliomatosis is a rare complication of malignant glioma. It is of great importance to identify which patients are at risk for this complication and as our report indicates age and secondary vs. primary glioblastoma might play an important role. Since it was recently recognized that bevacizumab can alter patterns of recurrence in malignant gliomas, this report is very timely and implicates possible deleterious effects of anti-angiogenic therapies in this malignancy.

P209

RESPONSE TO INTRATHECAL INFUSIONS OF DEPOCYTE IN SECONDARY DIFFUSE LEPTOMENINGEAL GLIOMATOSIS. A CASE REPORT.

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Purpose: Secondary diffuse leptomeningeal gliomatosis, in which a glioma of the brain or spinal cord infiltrates the leptomeninges, is a clinical uncommon metastatic complication of malignant gliomas, for which there is no consensus regarding treatment. In an ante mortem series in which the diagnosis of leptomeningeal gliomatosis was based on neuroradiological results, an incidence of 2% was reported. The appearance of leptomeningeal gliomatsosis is a pre terminal event. **Case Report:** A 44 year-old woman rapidly developed an intracranial pressure and impairment of cognitive function. A huge right temporal tumor was diagnosed, and an incomplete resection was performed. Histology was glioblastoma, a concomitant radiation therapy temozolomide was administered. Her clinical status was sub normal. The first course of adjuvant temozolomide was administered, and in days after, her neurological status suddenly impairment. An alteration of cognitive function status was noted, she was unable to walk, and aphasia was reported. The neurological examination revealed a confusion, aphasia and walk disabilities. The CSF showed an elevated protein content of 2 g/l, and glucose concentration was low. Cytology of CSF showed no malignant cells. Systemic nitrosourea chemotherapy, fotemustine, was administered. Intrathecal sustained release cytarabine, Depocyt, was performed (an induction cycle followed by a consolidation), the patient received 7 intrathecal infusions. Her clinical status dramatically improved, and she was discharged to medical unit. **Conclusions:** Intrathecal infusions of Depocyt, sustained release cytarabine, recommended for treatment of lymphoma neoplastic meningitis, seems to be effective in secondary diffuse leptomeningeal gliomatosis, temporary remission could be obtain.

P210

NEW THERAPEUTIC APPROACH FOR BRAIN TUMORS: INTRANASAL ADMINISTRATION OF RAS INHIBITOR MONOTERPENE PERILLYL ALCOHOL.

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Perillyl alcohol (POH) is a monoterpene with preclinical antitumor activity which proposed mechanism of action involves inhibition of post-translational isoprenylation of small G proteins, including p21-Ras, thereby blocking signal transduction. Deregulated p21-Ras function, as a result of mutation, overexpression or growth factor-induced overactivation, contributes to growth of malignant gliomas. Intranasal administration is a practical and non-invasive approach that allows therapeutic agents which do not cross the blood-brain barrier to enter the Central Nervous System, reducing unwanted systemic side effects. Applying this method we performed a phase I / II study of POH in patients with relapsed malignant gliomas after standard treatment. POH was administrated in concentration (55mg) 4 times daily. The objective of this study was to evaluate the toxicity and progression-free survival after 6 months of treatment. The cohort consisted of a total of 68 patients were investigated, 52 (76.4%) with glioblastoma (GBM), 10 (14.7%) with anaplastic astrocytoma (AA) and 6 (8.8%) with anaplastic oligodendroglioma (AO). Neurological examination and suitable image analysis established disease progression. After 6 months of treatment it was observed the following: Partial Response: 4% (n=2) with GBM and 33% (n=2) with AO; Stable Disease: 44% (n=23) with GBM, 60% (n=6) with AA and 33.3% (n=2) with AO; Progressive Course: 52 % (n= 27) with GBM, 40% (n=4) with AA and 33% (n=2) AO. The progression free survival (sum of partial responses and stable disease) was 48.2% for patients with GBM, 60% for AA patients and 66.6% for AO patients. The present work indicate for the first time, that intranasal administration of the signal transduction inhibitor, perillyl alcohol, is a safe, non invasive, low cost and regression of tumor size in some patients is suggestive of antitumor activity.

P211

CETUXIMAB DOES NOT INFLUENCE SERUM MAGNESIUM HOMEOSTASIS IN PATIENTS WITH GLIOBLASTOMA MULTIFORME TREATED WITH CONCOMITANT RADIOTHERAPY AND TEMOZOLOMIDE

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Purpose: In 2008, 5 years after introducing epidermal growth factor receptor (EGFR) targeting antibodies into cancer therapy, several authors demonstrated that in colorectal cancer patients early serum magnesium (Mg^{2+}) reduction caused by weekly infusions of irinotecan and cetuximab, a monoclonal antibody to EGFR, may serve as a positive predictor of therapy response and outcome. Gradual decrease of serum Mg^{2+} was attributed to insufficient activation of renal epithelial EGF dependent Mg^{2+} channels.

Whether EGFR-inhibition influences Mg^{2+} homeostasis and treatment response in patients with glioblastoma (GB) has not been addressed to date. Following the GERT phase I/II protocol, patients with primary GB are treated with radiation and concomitant temozolomide as well as weekly infusions with cetuximab. We analysed serum Mg^{2+} concentrations in patients and studied possible associations with efficacy and outcome.

Methods: Thirty-nine patients have until now entered the protocol and were included in this analysis. Cetuximab was given at a loading dose of 400 mg/m^2 one week prior to radiochemotherapy, followed by a total of six weekly infusions of 250 mg/m^2. Chemotherapy consisted of concomitant and adjuvant temozolomide according to the EORTC 22981/26981 protocol. Serum Mg^{2+} levels were measured prior to study enclosure and weekly afterwards. Statistical analysis was performed via Students t test.

Results: No significant decrease in serum Mg^{2+} levels was detected, neither during EGFR inhibition nor during the first two hundred days after initial cetuximab exposure. Even though two patients displayed early Mg^{2+} reductions of > 20% within the first 21 days after the loading dose of cetuximab, average Mg^{2+} levels remained stable ($p < 0,05$).

Conclusion: In contrast to reported data on colorectal cancer patients, addition of cumulative doses of 1,900 mg/m^2 cetuximab to standard radiochemotherapy does not cause significant reduction in serum Mg^{2+} levels in patients with glioblastoma.

P212

A RETROSPECTIVE SINGLE INSTITUTIONAL ANALYSIS OF CILENGITIDE (CGT) AND CHEMOTHERAPY FOR HEAVILY PRE-TREATED RECURRENT GLIOBLASTOMA MULTIFORME (GBM).

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Background: CGT, an integrin inhibitor, has been shown to have activity in the treatment of recurrent glioblastoma in combination with chemotherapy in Phase II trials. **Methods:** We conducted a retrospective analysis of all patients treated at our institution for recurrent GBM with CGT containing regimens. **Results:** Between 1/2008 and 12/2008 15 patients with relapsed GBM received CGT 2000mg given by intravenous infusion 2 times per week. The median age was 51. The ECOG performance status was 1-2. Multi-focal GBM was present in 7 patients. All patients had previous postoperative radiotherapy (60Gy in 42 # over 6 weeks) as part of the initial management. 10 patients had multiple craniotomies. 10 patients had primary GBM and 5 were initially grade III with transformation to GBM on subsequent biopsy. 8 patients had prior concurrent chemo-radiotherapy with temozolomide (T). 11 patients had previous bevacizumab (B) in combination with chemotherapy, 6 with good response. All 15 patients had chemotherapy prior to CGT: T (n = 3), T with procarbazine (P) (n = 3), carboplatin (C) and etoposide(E) (n = 3), PT followed by CE (n = 5) or E alone (n = 1).CGT was administered with the following: PT (n = 12), E (n = 3), B with CGT (n = 5). The median number of CGT doses given was 22. Stable disease (SD) was achieved in 10 and progressive disease (PD) in 4 patients. Partial response (PR) was seen in one case. The median duration of response was 10 wks (range 3-21 weeks). Toxicity was minimal and most likely attributable to the concomitant chemotherapy. There was one grade II thrombocytopenia and one grade I neutropenia. There were no infusion reactions, no nausea and vomiting, no acute haemorrhage or thrombotic problems and no wound dehiscence. 4 patients were still on treatment at time of writing this abstract. 1 discontinued because of exhaustion and 10 because of progressive disease. **Conclusions:** In this uncontrolled series CGT was observed to be extremely safe given with chemotherapy and/ or B, with some activity observed. Prospective evaluation of CGT in combination with other treatments is warranted.

P213

CILENGITIDE MODULATES ATTACHMENT AND VIABILITY OF HUMAN GLIOMA CELLS, BUT NOT SENSITIVITY TO IRRADIATION OR TEMOZOLOMIDE IN VITRO

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Cilengitide is a cyclic peptide antagonist of integrins &alpha&nu&beta3 and &alpha&nu&beta5 which is currently evaluated as a novel therapeutic agent for recurrent and newly diagnosed glioblastoma. Its mode of action is thought to be mainly antiangiogenic, but may include direct effects on tumor cells, notably on attachment, migration, invasion and viability. Here we show that cilengitide induces detachment in some glioma cell lines, while the effect on cell viability is modest. Detachment induced by cilengitide could not be predicted by the level of expression of the cilengitide target molecules at the cell surface. Glioma cell death induced by cilengitide was associated with the generation of caspase activity, but caspase activity was dispensable for cell death since ectopic expression of cytokine response modifier (crm)-A or the broad spectrum caspase inhibitor, zVAD-fmk, were not protective. Moreover, forced expression of Bcl-XL or altering the p53 status did not modulate cilengitide-induced cell death. No consistent effects of cilengitide on glioma cell migration or invasiveness were observed in vitro. Neither ectopic expression of MGMT in MGMT-negative cells nor silencing the MGMT gene in MGMT-positive cells altered their response to cilengitide alone or cilengitide in combination with temozolomide. These data suggest that the beneficial clinical effects derived from cilengitide in vivo may arise from altered perfusion which promotes temozolomide delivery to glioma cells.

P214

METABOLIC FACTORS GOVERN THE CYTOTOXIC POTENTIAL OF EGFR INHIBITION IN HUMAN MALIGNANT GLIOMA CELLS

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Inhibition of epidermal growth factor receptor (EGFR) signaling efficiently sensitizes human malignant glioma cells towards apoptosis induced by death ligands, radiotherapy and chemotherapy in vitro. In clinical trials, however, EGFR inhibition has generated disappointing results in glioblastoma patients. We have recently found that EGFR inhibition confers protection from hypoxia-induced cell death by decreasing energy demand. Here, we have characterized in detail the importance of oxygen and glucose availability for the modulation of cell death by EGFR inhibition. The EGFR inhibitors PD153035 and erlotinib profoundly inhibited cell death under starvation conditions (0.1% O2 and 2 mM glucose). With increasing O2 and glucose concentrations, EGFR inhibition progressively induced cytotoxicity. Whereas cell death under starvation conditions was necrotic, cytotoxic conditions induced caspase activation and a sub-G1 peak, indicating apoptosis. EGFR inhibition also resulted in a decrease in mitochondrial membrane potential preceding cell death under cytotoxic conditions, suggesting mitochondrial injury. We further examined whether cell death was mediated by reactive oxygen species (ROS). ROS were not induced by EGFR inhibition, and antioxidants did not inhibit its toxicity. The protective effects of EGFR inhibition towards hypoxia-induced cell death, but not the cytotoxic effects, were mimicked by inhibition of PKB/Akt. Notably, protection from hypoxia was also conferred by EGFR inhibition in HCT116 cells. These data demonstrate that metabolic conditions govern the effects of EGFR inhibitors on glioma and colon carcinoma cell viability and suggest that the microenvironment of solid tumors may be responsible for the low clinical activity of these promising therapeutics.

P215

AN EPIGENETIC GENOME-WIDE SCREEN IDENTIFIES THE SFRP FAMILY OF WNT SIGNALING INHIBITORS AS NOVEL TUMOR SUPPRESSOR GENES IN MEDULLOBLASTOMA

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Medulloblastomas (MB) are the most common pediatric nervous system malignancy. Despite treatment, mortality rates remain 30%. Known mutations account for only a subset of MB cases. We hypothesize that screening for tumor suppressor genes (TSGs) silenced by promoter CpG island methylation may uncover TSGs not previously described in MB. We performed an epigenome-wide screen of 9 MB cell lines treated with decitabine using Affymetrix HG U133 plus 2.0 expression arrays, identifying genes with increased expression following treatment. We identified 3 members of the *SFRP* family of Wnt signaling inhibitors as putative TSGs silenced by promoter methylation in MB. The *SFRP* gene family consists of 5 members: *SFRP1, SFRP2, SFRP3, SFRP4*, and *SFRP5*. Of these, only *SFRP1, SFRP2*, and *SFRP3* were appreciably expressed in normal cerebellum, and demonstrated increased expression in a subset of MB cell lines following treatment. We confirmed methylation of the *SFRP1*, *SFRP2*, and *SFRP3* promoter regions using bisulfite sequencing, and found aberrant methylation in 12/51, 2/51, and 8/51 primary MB tumors by methylation specific PCR, respectively. TSG function was assessed through stable reexpression of *SFRP1*, *SFRP2*, and *SFRP3* in the D283 and ONS76 MB cell lines. Reexpression of each gene reduced MB cell proliferation by MTS assay. *SFRP1*, *SFRP2*, and *SFRP3* reexpression in D283 cells reduced its ability to form colonies in soft agar. Reexpression of these Wnt signaling inhibitors also limited Wnt signaling axis activity, as evidenced by reduced phosphorylated DVL2 levels by western blotting. Aberrant Wnt signaling is known to play a role in the pathogenesis of a subset of MB cases. We have identified for the first time a novel mechanism (loss of normal pathway inhibition) that contributes to upregulated Wnt signaling in this disease.

P216

SILENCING OF NEURONAL DIFFERENTIATION GENES REVEALED BY PROFILING THE MEDULLOBLASTOMA EPIMETHYLGENOME

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In order to identify novel genes and pathways deregulated in medulloblastoma, we carried out a genome-wide screen for genes silenced through methylation of promoter CpG islands. We screened seven medulloblastoma cell lines by treatment with either 5-aza-deoxycytidine (DAC), or Trichostatin A (TSA), followed by hybridization of RNA to expression microarrays. We identified 1102 genes that exhibit increased expression (>2-fold) upon treatment with DAC, but which did not increase in expression after TSA treatment alone (<1.4-fold). We then performed Methylated DNA Immuno-Precipitation (MeDIP) on eight cell lines as well as normal adult and fetal cerebella. Methylation enriched DNA was then compared to methylation impoverished DNA (wash fraction) by hybridization to a genome-wide CpG island promoter array (MeDIP-ChIP). Of the 1102 genes that increased in expression with DAC, 333 were specifically methylated at the proximal CpG island in MB cell lines versus normal adult cerebellum in one or more cell lines ($P<0.01$). To determine if genes targeted by promoter methylation in cell lines were also silenced in human primary tumors, we subsequently integrated gene expression data derived from 112 primary medulloblastomas and 14 normal fetal and adult cerebella. Of the 333 methylated candidate genes identified above, 99 showed greater than two-fold down regulation in >10% of primary tumors as compared to normal controls. Strikingly, 20/99 (20%) genes have known roles in neuronal differentiation and synaptogenesis such as LRRC4, BHLHB5 and CYP26B1, suggesting that avoidance of terminal neuronal differentiation is a critical event in medulloblastoma pathogenesis. Additionally, negative regulators of the Wnt pathway (5/99) and the CXCR4-CXCL12 chemokine axis (5/99) were also frequently targeted. Poorly characterized genes made up a large fraction of silenced, methylated genes (15/99). These data demonstrate the utility of combining DAC screening with MeDIP-ChIP, and suggest that differentiation agents should be considered in the treatment of medulloblastoma.

P217

PEDIATRIC INTRACRANIAL GERM CELL TUMORS OF DIFFERENT PROGNOSIS OUTCOMES AND ETHNIC BACKGROUND INHERIT CHARACTERISTIC EMBRYONIC STEM CELL TRAITS AND MICRORNAOME PROFILES

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Intracranial pediatric germ cell tumors (PGCTs) are rare and heterogeneous neoplasms with different clinical behaviors. About 40 to 50% of intracranial PGCTs are germinomas, which are classified in the good prognostic group with mature teratoma. In contrast, PGCTs of other types, including yolk sac tumor (YST), embryoncal carcinoma (EC), immature teratoma and mixed PGCTs of these subtypes, are recognized as poor prognostic ones which are resistant to drug or irradiation treatment. The underlying mechanisms are not clear yet. We applied genomics approaches to unmask the transcriptome compositions of Asian intracranial PGCTs and then compared them to those of Caucasian cases. Clear microRNAome and mRNA profiles associated with tumor prognosis were identified. Differentially expressed microRNAs were confirmed by real-time PCR. Genes responsible for self-renewing pluripotent and apoptosis phenotypes were abundant in germinoma and mature teratoma, while genes associated with differentiation, tumor invasiveness and epithelial-mesenchymal transition (EMT) in PGCTs of poor prognosis. We also observed complex microRNA and gene networks functioning in different PGCTs, in which Wnt pathway genes such as beta-catenin acted as hubs to maintain the stability and connectivity of the whole genetic network in poor prognostic PGCTs. MicroRNAs inhibiting angiogenesis genes in the same genetic network were also down in poor prognostic PGCTs. We further compared these findings with published Caucasian PGCT data, and found clear segregation between Asian and Caucasian cases, with Asian malignant PGCTs being the closest ones to embryonic stem cells. We conclude that the observed clinical differences between PGCTs of different prognosis types and ethnic background are mirrored by significant differences in global microRNA and mRNA expression, and that unique stem cell traits are associated with clinical outcomes of PGCTs and reflect the origins of different PGCTs. Genes and microRNAs identified hold the potential of being novel therapeutic targets in further differentiation therapy.

P218

THE FREQUENT EXPRESSION OF ARF-BP1 IN PRIMARY INTRACRANIAL GERM CELL TUMORS

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Background and Purpose; The ARF binding protein 1 (ARF-BP1) directly binds and ubiquitinates p53, and this activity is inhibited by ARF. The alterations of p53 gene are rare in intracranial germ cell tumors (GCTs). The ARF gene is frequently deleted in intracranial GCTs. To analyze the role of ARF-BP1, the immunohistochemical study was performed for primary intracranial GCTs. **Materials and Method;** A total of 33 formalin fixed, paraffin embedded intracranial GCT specimens were used and previously diagnosed by H.E., CK, HCG, AFP, PLAP staining. 27 pure GCTs (20 germinomas, 3 yolk sac tumors, 2 choriocarcinomas, and 2 teratomas), and 6 mixed GCTs (including 5 foci of germinomas, 2 embryonal carcinomas, 2 yolk sac tumors, 4 teratomas) were prepared for the immunohistochemical study of ARF-BP1 protein. **Result;** ARF-BP1 was positive in 18 of 20 germinomas, in 2 of 3 yolk sac tumors, in 2 of 2 choriocarcinomas, in 0 of 2 teratomas, and in 6 of 6 mixed GCTs. ARF-BP1 showed relative over-expression in germinoma compared with the other types of GCT. The frequency of nuclear and/or cytoplasmic expression of ARF-BP1 was various in each case. **Conclusion;** The high frequency of the positivity of ARF-BP1 suggests that the ARF-BP1 is a key molecule in the pathogenesis of primary intracranial GCTs.

P219

FREQUENT SINGLE NUCLEOTIDE POLYMORPHISM (SNP) OF BCL10 AND ASSOCIATION WITH POOR PROGNOSIS IN PRIMARY INTRACRANIAL GERM CELL TUMORS

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Intracranial germ cell tumors (ICGTs) are uncommon neoplasms, but they occur more frequently in the Japanese population than the West. Little is known about the mechanisms of ICGTs development and the reasons for its Japanese propensity. Recently, the BCL10 gene, which is a proapoptotic signaling molecule involved in the Apaf-1/caspase-9 cell death pathway, may act as tumor suppressor gene in multiple types of tumors including testicular germ cell tumors. To evaluate whether genetic alterations of the BCL10 gene occur in the genesis of ICGTs, we analyzed the BCL10 gene in 20 ICGTs ; 10 pure germinomas (PG) and 10 nongerminomatous germ cell tumors (NGGCT). While no inactivating mutations were found, five of 10 cases (50%) of PGs and one case of 10 NGGCTs had specific single nucleotide polymorphism (SNP) that carried an amino acid substitution at codon 5 in exon 1 or codon 162 in exon 3. This specific SNP is also known to occur at higher frequency than in healthy individuals. In addition, four of 6 cases (66%) with SNP suffered from CSF dissemination ($p = 0.037$). These data suggested that this specific SNP in the BCL10 gene may be partly responsible for the tumorigenesis of pure germinoma in Japanese individuals, and may associated with aggressive nature of the tumor.

P220

EXPRESSION PROFILES OF MICRORNA IN PEDIATRIC MALIGNANT GLIOMAS

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Purpose: Pediatric malignant gliomas occur rarely as compared to the counterparts of adult patients. The concept of distinct tumorigenesis between pediatric and adult patients leading to the common phenotype of malignant gliomas have gained general acceptance in the decade. In recent years, the studies of microRNA(miRNA) demonstrated that miRNAs could target mRNAs which oncogene and tumor suppressor gene transcribed. Through this way, they will also act the effect of tumor suppression or oncogenesis. **Methods and materials:** In order to elucidate the key contribution of miRNA in glioma tumorigenesis and the related pathways, we carried out expression profiling of miRNA in pediatric malignant gliomas, neural stem cells (NSC) and mesenchymal stem cells (MSCs) by miRNA microarray. **Results:** Our data showed bulk tumor become more stem cell-like during grade 2, 3 & 4 tumors. Many down-regulated miRNAs are also down in stem cells. One of which is miR-296, which can target Nanog and relate to the property of stemness and malignancy. Oncogenic miRNAs, such as miR-122, are also up-regulated in our pediatric glioblastomas samples. **Conclusion:** By targeting the genes and microRNAs we identified, it will hold the potential of discovering novel tumorigenesis mechanism and developing further differentiation therapy for pediatric gliomas.

P221

STRESS LEVELS AMONG PARENTS OF CHILDHOOD BRAIN TUMOR SURVIVORS

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AIM. Few studies of childhood brain tumor survivors have focused on the stress of survivors' parents, which can play an important role in survivor well-being. The purpose of this study was to better understand the range of stress experienced by parents of brain tumor survivors. **METHODS.** 72 parents of childhood brain tumor survivors who were at least one year past treatment were recruited. For survivors, mean age = 10.7 years, mean time since last treatment = 2.6 years, 36.6% had low grade astrocytoma and 15.5% had ependymoma. Parents (79% mothers) mean age=41.2 years, 88.9% were married, 82% had at least college degree. Most parents reported survivors having good (19.1%), very good (33.3%), or excellent (33.3%) quality of life in general. Parents were asked to complete the 15-item Impact of Events Scale (IES), which was designed to evaluate specific life event stress (i.e., their child's brain tumor). In addition to a total subjective stress score, the IES can also produce two subscale scores: intrusion and avoidance. Cut-off scores are available to determine range of stress (subclinical, mild, moderate and severe) for ease of intepretation. **RESULTS.** Only 28.2% of the parents exhibited subclinical stress. 46.1% parents showed mild range of stress, 21.1% moderate and 5.6% severe. There was no significant difference between fathers and mothers on total subjective stress (p=0.09), avoidance (p=0.06) or intrusion (p=0.22). No significant correlation between years since the last treatment and stress, avoidance and intrusion (p&gt0.05 for all three comparisons) were identified. **CONCLUSIONS.** Most parents of childhood brain tumor survivors reported clinically significant levels of stress even considering that they rate their children's quality of life as being good more than one year after treatment. Further research is necessary to determine predictors of paternal stress in this population.

P222

MULTI-TIERED INTERVENTION STUDY FOR PATIENTS, CARERS AND HEALTH PROVIDERS TO ADDRESS CHALLENGING BEHAVIOURS AFTER BRAIN TUMOUR

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Purpose: Untreated cognitive/behavioural impairments (challenging behaviours, CBs) associated with brain tumour (BT) adversely affect functional status and quality of life. A "Behavioural Consultancy" approach is a psychosocial intervention to manage the person with CBs within their naturalistic environment. Compensatory strategies are introduced that can be adopted by the person with BT themselves, or utilised by family members (carers) and staff to structure the environment to minimise the impact of CBs. This study piloted a multi-tiered intervention (individual, carers, staff) to treat CBs after BT utilizing the Behavioural Consultancy model. **Methods:** Participants for the individual and family interventions were recruited from a broader prevalence study of CBs after BT. For the individual case, a single case experimental design was employed to evaluate the efficacy of skill-based training and environmental changes in managing excessive talking in a woman with a benign BT. For the carer intervention, a half-day workshop to train seven carers in the use of compensatory strategies to manage CBs was trialled. Finally, 43 allied health staff from neurosurgical and cancer services attended a one-day training workshop in the Consultancy approach. A pre-post impact evaluation was employed for both workshops, employing a purpose-designed Strategies Use Measure. **Results:** All three interventions demonstrated positive results. The single case (n=1) showed a 71% decrease in time in excessive talking after the Consultancy intervention. Three month follow-up found the subject maintaining a 38% decrease from baseline, emphasising the need for ongoing environmental supports. Participants attending the carer workshop demonstrated significant post-intervention increases in perceived knowledge of Strategy Use ($t = 3.33$, $p<.05$). Similarly, staff attending the full-day workshop recorded significant post-training gains in knowledge of Strategy Use ($t = -10.76$, $p<.001$). **Conclusions:** The potential efficacy of a Consultancy approach can be demonstrated at the level of individual patients, carers and health providers alike.

P223

KNOWLEDGE AND ATTITUDES ON THE DIAGNOSIS, TREATMENT AND PROGNOSIS AMONG PATIENTS WITH BRAIN TUMOR AND THEIR CAREGIVERS IN A TERTIARY HOSPITAL

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Background: Quality of life (QOL) studies show that brain tumors and treatment have physical, cognitive, and emotional effects on patients and caregivers. It is prudent to study first how the subjects understand the disease. This may correct misconceptions, enhance effective treatment and lead to better QOL study results. **Objectives:** This study aims to determine the knowledge and attitudes on diagnosis, treatment and prognosis among the subjects in a tertiary hospital. **Methods:** A 14-item questionnaire was drawn from a previous qualitative study on brain tumors. Descriptive analysis was used. **Results:** During the one month study period, 30 patients and 20 caregivers were tested. Majority were females, unemployed, with mean age of 44.75. Forty-three percent of patients finished secondary education; 40% of caregivers graduated college. Memory has been mainly affected by the illness. Most participants said that brain cancer is different from systemic cancers. Majority assumed that brain tumors can be cured and are not infectious. A few thought it equates to death (26% patients; 45% caregivers). Sixty-six percent of patients and 60% of caregivers said that brain tumors are hereditary. Frequent head trauma was the most commonly cited cause of brain tumors (66% patients; 70% caregivers). Majority (93.3%) did not choose between quality rather than prolongation of life. Family was most influential in treatment choices. Spiritual beliefs greatly helped them cope with their infirmity. Thirty-three percent of patients and 25% of caregivers believed in mercy killing. **Conclusion:** There is lack of knowledge on brain tumors among patients and their caregivers. Most subjects did not understand the concept of quality vs. quantity of life. Hope and spiritual belief are their key strengths. Majority avoided active decision making in treatment options. Therefore, it is essentially important to understand the mindset of brain tumor patients and their caregivers in order to provide comprehensive care.

P224

MANAGEMENT OF PRIMARY MALIGNANT BRAIN TUMORS DURING PREGNANCY

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The incidence of brain tumors is reduced by about 64% in the pregnant population compared to nonpregnant women of similar age. Although rare, they are worthy of consideration in that, of all the diferent cancers seen arising during pregnancy, brain tumors carry perhaps the greatest potential for maternal and fetal death. Moreover, there is almost always a conflict of interest between optimal maternal therapy and fetal well-being that emotionally taxes both patient and physician alike. The maternal interest may require an immediate treatment of the recently diagnosed tumor, however, the optimal therapy may impose great risks to the fetus.This is is an extensive literature review leading to a suggested algorithm for the treatment of brain tumors in pregnancy.An extensive literature review revealed several case reports and a few small retrospective case series. Clearly, randomized studies are not possible in this situation and decisions hence have to be made on a case to case basis. This study is a review dealing with the dilemmas in the diagnosis and treatment of primary malignant brain tumors in pregnancy, the use of diagnostic modalities and its effect on the developing fetus.It provides guidelines for optimum treatment of these tumors, delineating first the present standard of care for the various tumor types as it relates to pregnancy and subsequently the attendant risks of neurosurgery, radiation, chemotherapy and ancillary medications such as corticosteroids and anticonvulsants, in a pregnant patient.

P225

CANCER STEM CELLS FROM GLIOBLASTOMA MULTIFORME PATIENTS

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Glioblastoma multiforme (GBM) in current clinic diagnosis was highly malignancy because of the survival rate of patients was approximately only 15% at two years even those patients have offered to standard-of-care therapy includes surgical resection radiotherapy, and temozolomide, administered both during and after radiotherapy. However, again recurrence revealed that small subset of cells and drugs resistance population sufficient to form a new tumor according to current cancer stem cell hypothesis. In our progress reports, we present data showed that isolated tumor stem cells from GBM patient have seemed to establish a standard protocol including self-renewal in vitro and tumor-initiated in nude mice. In addition, used to identified cancer stem cell marker: CD133 and Nestin was present positive in our tumor spheres. Tumor spheres of differentiation ability of both neurons and astrocytes lineage existed at serum condition. We have also found some properties in tumor spheres including aggregation of tumor spheres, rich in alkaline phosphatase : embryonic stem cell enzymic activity, altered nuclear morphologies at differentiation condition, and to stand seized ability of highly migration and invasion. CD44 surface molecular relating to vessel was high expression at cancer stem cells derived cancer cell at serum condition, but absent at tumor spheres.In summary, we indeed established a method for isolation of cancer stem cells from GBM patients no matter how present data in vitro or in vivo. In further, we hope that these cancer stem cells could help us to understand how the ability of cancer stem cells to against chemotherapy and finally find out several methods for absolute eliminating cancer.

P226

CHARACTERIZATION OF CANCER STEM-LIKE CELLS DERIVED FROM HUMAN GLIOBLASTOMAS

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It has been recognized that cancer stem-like cells play an important role in progression in brain tumors. We aimed to study the characteristics of cancer stem-like cells derived from human brain tumors. Thirteen surgically obtained brain tumor specimens were used that included 9 glioblastomas, 1 medulloblastoma, 1 pleomorphic xanthoastrocytoma (PXA) with anaplastic features, 1 anaplastic astrocytoma and 1 oligodendroglioma. Tumor-derived cells were cultured in serum-free media, containing EGF and bFGF, under the floating culture condition for neural stem cells. The percentage of CD133 positive cells among cancer stem-like cells was measured by flow cytometry. Tumorigenicity of cancer stem-like cells from surgical specimens was evaluated in athymic mice. Cancer stem-like sphere cells or anchored cells cultured under an adherent condition were injected into the right cerebral hemisphere, and the mice were observed for the number of days survived (n=10/group). Stem cell cultures from 2 glioblastomas, PXA with anaplastic features, anaplastic astrocytoma, and oligodendroglioma failed to form spheres, whereas 7 stem cell cultures from glioblastoma specimens and 1 from medulloblastoma developed spheres. Three sphere series from glioblastomas, named TGS01, TGS02 and TGS04, were positive for CD133 at the percentage of 85.0%, 13.7% and 16.3%, and median survival time of mice injected with $5.0x10^4$ cells were 42days, 90days and 66days, respectively. TGS01 cells showed decrease in CD133-positive cells from 85.0% to 28.9% through several passages supplemented with growth factor once a week, and we called them as TGS01-$CD133^{low}$. Median survival time of mice injected with $5.0x10^4$ TGS01-$CD133^{low}$ cells were 56days. Brain tumors of these mice were similar to original tumors pathologically except for less invasiveness. These results indicated that CD133-positive rate might correlated with their propagation ability *in vivo*, even though these rates were unstable under *in vitro* condition.

P227

CHARACTERIZATION OF CANCER STEM CELLS IN HUMAN GLIOBLASTOMAS

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There is a growing body of evidence showing the exsistence of cancer stem cells (CSCs) in malignant brain tumors, and these CSCs are supposed to play pivotal roles in tumor initiation, growth and recurrence. In this study, we isolated putative CSCs from a human glioblastoma cell line U251 and partially characterized the isolated cells. **Methods:** Cells forming spheroid aggregates (sphere cells) and those attaching to plastic dishes (adherent cells) were separately isolated from U251 cells by culturing cells in the presence of growth factor mixture. Proliferation, migration and invasion activities of the original U251 cells, sphere-forming cells and adherent cells were investigated using Alamar blue assay and boyden chamber assay. Protease activity was examined by gelatin zymography. **Results:** When cultured in serum-free medium with the growth factor mixture, some U251 cells formed spheres. Such sphere cells had abilities of self-renewal, forming secondary spheres derived from single cells in spheres and differentiating into neuron-like cells. Sphere cells continued to proliferate for months and formed solid tumors when grafted subcutaneously to nude mice. By contrast, adherent cells did not have these properties. Sphere cells possessed much higher migration and invasion activities than adherent cells. However, there was no correlation between the invasive activity and the enzymatic activity of matrix metalloproteinase. **Conclusion:** U251 cell line contained a sub-population of cells characterized as CSCs. The currently characterized properties of sphere cells may be responsible for the resistance to treatment for glioblastoma.

P228

CHARACTERIZATION OF TUMOR STEM CELLS ISOLATED FORM HUMAN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme tumors contain a sub-population of cancer stem cells that contribute to malignancy and resistance to therapy. Previous studies have characterized these cells based on their similarity to adult neural stem cells and their expression of CD133. In this study, we separated cancer stem cells from non-pluripotent cells comprising the bulk of the tumor based on their physical growth properties using both serum and serum-free cell culture media. We successfully generated cancer stem cells from multiple tumor masses as well as from the surgical flush. Cancer stem cells were able to proliferate as non-adherent spheroids and differentiated along neuronal and glial lineages following culture in serum-supplemented media. Compared to adherent cancer cells and human fetal neural stem cells, tumor spheroids showed increased rates of proliferation, as measured by BrdU incorporation, and greater resistance to differentiation, respectively. Further characterization of these cells were carried out using immunohistochemistry and real-time PCR to reveal molecular markers enriched in cancer stem cells that are absent in both cells comprising the bulk of the tumor as well as in normal neural stem cells. These markers are down-regulated following differentiation and may serve as important diagnostic and prognostic factors. Moreover, the presence of unique markers offers exciting new therapeutic targets.

P229

STAT3 SERINE727 ACTIVATION IN GLIOMA CANCER STEM CELLS

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PURPOSE: Persistent activation of the signal transducer and activator of transcription 3 (STAT3) has been observed in many cancers. Various cytokines and growth factors such as the epidermal growth factor (EGF) are capable of leading to STAT3 phosphorylation at the tyrosine-705 position via recruitment of JAK2. Recent studies showed that neural stem cells are characterized by activation of STAT3 at the serine-727 position by the Akt-mTOR pathway in the absence of tyrosine-705 phosphorylation. Differentiation into terminal lineages was associated with loss of serine activation and gain of tyrosine phosphorylation. Because cancer stem cells (CSC) represent a population of malignant cells with stem-like properties, we investigated the presence and significance of STAT3-S727 activation in glioma-derived CSC. **METHODS:** CSC isolated from acutely resected tumor specimens that are capable of recapitulating the tumor in immunodeficient mice were studied for STAT3 activation. **RESULTS:** Glioma-derived CSC showed STAT3 phosphorylation at both serine and tyrosine sites by immunocytochemistry and immunoblot. STAT3-S727 activation correlated with self-renewal capacity as assessed by sphere forming assay. As seen with neural stem cells, differentiation of CSC with retinoic acid or addition of serum resulted in attenuation of STAT3-S727 phosphorylation, and loss of self-renewal capacity even in the presence of STAT3-Y705 phosphorylation. To determine the identity of the upstream pathway responsible for the activation of STAT3-S727 in CSC, we probed the Akt-mTOR pathway. However, persistent activation of STAT3-S727 was observed even after treatment with mTOR, PI3K, and EGFR inhibitors. **CONCLUSIONS:** STAT3-S727 activation may represent a marker of glioma CSC stemness. This study suggests inhibition of growth pathway may be insufficient in preventing CSC self-renewal. A differentiation-based therapeutic strategy may target the CSC population.

P230

ENHANCEMENT OF STEM-LIKE GLIOMA NEUROSPHERE RADIORESPONSE BY GEFITINIB: BY INCREASED DNA DAMAGE AND REDUCED DNA REPAIR CAPACITY

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Brain tumor stem cells (BTSCs) have been identified as responsible for maintenance and growth of brain tumors, which subsequently contributes to chemo- and radioresistance. In this study, we analyzed the effect of irradiation on glioma neurospheres (that displayed BTSC characteristics) and differentiated glioma cells. We determined whether the tyrosine kinase inhibitor gefitinib could enhance radiosensitivity of glioma neurospheres by increasing DNA damage and reducing DNA repair capacity. Malignant glioma tissues were collected with informed consents from patients in accordance with protocols approved by the institutional review board. Tissues were minced, trypsinized and cultured for growth of neurospheres and differentiated glioma cells. Neurospheres were evaluated for BTSC characteristics, i.e. ability to self-renew, multi-potentiality and tumorigenesis. Dose-dependent effects of irradiation alone, and combined effects of irradiation and gefitinib in neurospheres and differentiated glioma cells were analyzed for CD133+ (neural stem cell marker) cell population, clonogenic formation, γ-H_2AX (double-strand DNA break marker) immunostaining and DNA-PKcs protein expression. Glioma neurospheres displayed characteristics of BTSCs to self-renew; differentiate into neurons, oligodendrocytes and astrocytes; and induce growth of gliomas in NOD-SCID mice. Irradiation significantly increased CD133+ cell population in neurospheres, but not in differentiated glioma cells. Irradiation did not affect clonogenic survival and γ-H_2AX immunostaining of glioma neurospheres, but dose-dependently reduced clonogenic survival and increased γ-H_2AX immunostaining of differentiated glioma cells. Addition of gefitinib significantly inhibited clonogenic survival and increased γ-H_2AX immunostaining of irradiated-neurospheres, without affecting irradiated-differentiated glioma cells. Irradiation inhibited DNA-PKcs expression in neurospheres, with further inhibition by combined gefitinib and irradiation. In differentiated glioma cells, irradiation enhanced DNA-PKcs expression without further effect by combined gefitinib and irradiation. In summary, stem-like glioma neurospheres are resistant to irradiation-induced DNA damage, with greater clonogenic survival following irradiation than differentiated glioma cells. Gefitinib enhances radiosensitivity of stem-like glioma neurospheres by increasing DNA damage and reducing DNA repair capacity.

P231

INHIBITION OF TUMOR GROWTH AND ANGIOGENESIS BY TARGETING CXCL12/CXCR4 AXIS IN STEM CELL-LIKE GLIOMA CELLS

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Stem cell-like glioma cells (SCLGCs), or glioma stem cells (GSCs), produce high levels of vascular endothelial growth factor (VEGF) with yet to be defined mechanisms. We hypothesized that the chemokine CXCL12 and its receptor CXCR4 might participate in SCLGC-mediated tumor angiogenesis. We therefore isolated CD133+ cells from a human glioblastoma cell line U87 and primary glioblastoma tissues with the properties of self-renewal, multi-potency and tumorigenicity. These CD133+ SCLGCs expressed more CXCR4 than their CD133- counterparts and were capable of forming tumorspheres in vitro. Activation of CXCR4 in SCLGCs with the ligand CXCL12 induced calcium mobilization and increased VEGF production through the PI3K/AKT pathway. Knock-down of CXCR4 by RNA interference or treatment with a CXCR4 antagonist AMD3100 reduced the capacity of SCLGCs to form colonies in soft agar and inhibited the growth and angiogenesis of tumors grown in SCID mice. We further observed co-expression of CD133 and CXCR4 in tumor cells of surgically removed human primary malignant gliomas and such cells were located adjacent to microvessels. These results suggest that CXCL12/CXCR4 axis may play an important role in mediating the tumorigenesis and neovascularization initiated by SCLGCs. Thus, CXCL12/CXCR4 axis constitutes a promising target for the development of novel anti-glioma therapeutic agents.

P232

CULTIVATION AND IDENTIFICATION OF THE CELLS FROM CENTRAL NERVOUS SYSTEM HEMANGIOBLASTOMAS AND ITS STEM CELLS ORIGIN

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Objetive: To cultivate and identify the tumor cells from central nervous system hemangioblastomas(CNS HB) for illustrating its histological origin and pathogenesis. **Methods:** 13 CNS HBs were primary and passaging cultured using enzyme combined digestion. The cells were identified by morphological observation, immunofluorescence, ultramicrostructural observation, EPO and VEGF concentration measurement from culture fluid supernatant and CNS HB tissues immunohistochemical stain. **Results:** 11 CNS HBs were successfully cultivated. The cells monolayer appeared after 3 weeks. The results of immunofluorescence showed Vimentin+, VEGF+, CD133+, Nestin+, EPO+, CD34-, SMA-, GFAP-. EPO and VEGF concentration measurement showed the cells had the ability of exocrine EPO and VEGF. CNS HB tissues immunohistochemical stain suggested the cells cultured actually were the stromal cells of CNS HBs. **Conclusion:** A novel method was successfully created for attainment of the cells from CNS HB, and firstly demonstrated it may derived from the stem cells of mesoderm mesenchymal tissues.

P233

SCREEN AND IDENTIFY THE CRITICAL PROTEINS OF CENTRAL NERVOUS SYSTEM HEMANGIOBLASTOMA TUMOR CELLS USING ON-LINE CAPILLARY 2D-HPLC COMBINED WITH LTQ-ORBITRAP MS

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Object: We carried out a pilot study in which we analyzed the proteomes of central nervous system hemangioblastoma (CNS HB) tumor cells by using on-line capillary 2D-HPLC combined with LTQ-Orbitrap ESI MS so as to screen and identify the critical proteins related to CNS HB occurring. **Methods:** CNS HB cells were cultured and identified by enzyme digestion from CNS HB tissues collected during operations. Normal human nerve cells were obtained as control. Total proteins of cells were extracted and enzymolysed. And then LC packing Ultimate 2D-HPLC systemcombined with LTQ-Orbitrap hybrid mass spectrometer were used to segregate and idenify the proteins of CNS HB tumor cells and normal human nerve cells. And shot gun quantitating technique was used to compare the protein expressional abundance of CNS HB cells with normal human nerve cells so as to screen the critical proteins. **Results:** 674 and 531 proteins were segregated and identified in this study. Of the total proteins identified, 360 proteins were found exclusively in CNS HB tumor cells while 217 proteins were found only in normal human nerve cells. And 55 proteins including 30 up-regulated and 25 down-regulated with distinguished difference in proteins expressional abundance were found, too. The identified proteins according to their functions could be divided into several groups. Some critical proteins related with CNS HB occurring such as 14-3-3, vimentin, carbonic anhydrase were screened. **Conclusions:** The whole proteins of CNS HB tumor cells with distinguished difference from normal nerve cells suggests CNS HB tumor cells may not come from normal never cells but have a origin of mesenchymal stem cells of brain. The process of CNS HB occurring is an intricate, multi-step processmediated by a variety of important proteins.

P234

NOTCH1 INDUCES MORE EXPRESSION OF DLL1 IN U251MG GLIOMA CELL LINE

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Notch signaling pathway takes part in coordinated regulation of cell growth, survival and differentiation. Notch receptors cleave into Notch intracellular domain (NICD) and Notch extracellular domain (NECD) when Notch ligand binds membrane receptors. NICD dissociates from NECD, translocates into the nucleus and binds to members of the CSL transcription factor family. And then the CSL family member CBF-1/RBP-JK becomes transcriptional activator and activates large family of &beta &beta helix loop helix (bHLH) transcription factors known as HES genes. The expression of Notch ligand as the first beginning of Notch signaling pathway is crucial for its activation. Previous findings have shown that Notch1 and Delta-like1 (DLL1) over express in many glioma cell lines and primary human gliomas. Down-regulation of DLL1 by RNA interference inhibits proliferation and induces apoptosis in multiple glioma cell lines. On the other hand, previous studies had found that the expression of Notch1 inhibited delta ligand of neighbors in the development of nervous system of Drosophila and vertebrates. However in glioma, it is still unknown whether the secretion of Notch ligand is effected by Notch signaling pathway. In this regard, we examined the expression of DLL1 after up-regulating Notch1 by transfecting Notch1 expression plasmid and blocking Notch1 receptors by Notch1 antibody. We showed that Notch1 expression plasmid induced more expression of DLL1 in U251MG glioma cell lines. Adversely the way of blocking Notch1 receptors down-regulated the expression of DLL1 and induced apoptosis and growth inhibition in U251MG cells, which had no statistical difference from the effect of knocking down DLL1 by siRNA. But jointing knocking down DLL1 and blocking Notch1 receptors induced much more apoptosis and growth inhibition than sum of single factor. These results suggest that Notch1 could induce more expression of DLL1 and there may be a potential positive feedback loop between Notch1 and DLL1 in U251MG glioma cells. And DLL1 and Notch1 may become joint therapeutic targets.Keywords: Notch signaling; Glioma; Notch1; DLL1; Feedback loop; U251MG

P235

HEDGEHOG PATHWAY AND GLIOMAGENESIS

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Gliomas are one of the most malignant cancers for human. Malignant gliomas contain small fractions of cells which have both tumorigenicity and neural stem cell properties, such as self-renew, multipotent, so called Cancer Stem-like Cells(CSCs). CSCs have features of radioresistance and chemoresistance, so they could be a crucial target for brain tumor therapy. We have established two glioma stem-like cell(GSC) lines from fresh human glioma tissues. GSCs formed floating spheres in serum-free-medium containing bFGF, EGF and human LIF. They were multipotent, expressed a number of stem cell markers(by RT-PCR, immunocytochemistry, immunohistochemistry) and formed tumors when transplanted in nude mouse brains. We focused on three signaling pathways those activated by Notch, Hedgehog(Hh), Wnt/Frz, all of which are involved in brain tumorigenesis. Cyclopamine, which is a Hh pathway inhibitor, blocked cell proliferation of GSCs, we considered that Hh pathway was essential for proliferation of GSCs. We confirmed that Gli2 bind to the enhancer region of Sox2 promoter and that dominant negative form of Gli2(dnGli2) suppressed Sox2 mRNA/protein level in NSCs *in vitro* and *in vivo*. When we transfected the dnGli2 expressing vector to GSCs, dnGli2 also suppressed Sox2 expressions and cell proliferation in GSCs. Inducible dnGli2 expressing GSCs prolonged mean survival times of transplanted nude mice, but broke the tumorigenicity *in vivo*. As NSCs might be the origin of brain tumors, applying the principles of stem cell biology make it possible to conquer malignant gliomas.

P236

THE FUNCTIONAL ANALYSIS OF A NOVEL KDR (VEGFR II)-PDGFR-ALPHA FUSION GENE IN GLIOBLASTOMA: FROM DISCOVERY TO CLINICAL APPLICATION

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Chromosomal rearrangements leading to translocation and fusion gene are the most frequent form of cancer genes discovered in hematological disorders and child sarcomas. Because gene rearrangements are harder to detect in solid tumors, they are probably underestimated. Recent advances in array-based analysis have identified frequent gene fusions in prostate and lung cancer. Gene rearrangement in the form of intragenic deletion is the primary mechanism of oncogenic mutation of EGFR and PDGFRα in gliomas, while the incidence of gene fusion is unknown. We applied an array-based comparative genomic hybridization (aCGH) strategy and sequencing to identify gene rearrangements within tyrosine kinases and identified the first case of fusion gene with oncogenic potential from a glioblastoma patient sample. This fusion contains the 5, segment of the KDR gene and the 3, segment of the PDGFRα gene by a small interstitial inversion on chromosome 4q. When this KP (KDR-PDGFRα) fusion gene is introduced into NIH3T3 cells by retroviral infection, fusion gene product has constitutively elevated tyrosine kinase activity *in vitro* originating from homodimerization in ligand-independent manner. NIH3T3 cells expressing KP fusion have a transformed-phenotype *in vitro* and are tumor-forming in nude mice. The transformed phenotype of these cells is reversed by PDGFR blockade. Subsequently, to identify novel phenotype reversing drugs with potential use in the clinic, we performed high throughput (HTP) drug screening of the NIH3T3 cells expressing KP fusion and found several promising candidates. HTP drug screening also indicated that Akt pathway is necessary for the transformed phenotype. These results suggest that KP fusion gene behaves as a potent oncogene in a subset of gliomas and can be used as a tool for drug development.

P237

ISOLATION AND CHARACTERIZATION OF AN N-LINKED OLIGOSACCHARIDE THAT IS INCREASED IN GLIOBLASTOMA TISSUE AND CELL LINE.

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We have isolated and characterized N-linked oligosaccharides that are significantly increased in glioblastoma tissue and cell lines. The structures of N-linked oligosaccharides present in 3 human normal brain tissues, 15 patients with glioblastoma and 3 glioma cell lines were analyzed by partially automated technique for the isolation and fluorescent labeling of N-linked sugar chains from glycoproteins. Characterization of the sugar chains was achieved with the use of a combination of HPLC columns and a highly sensitive fluorescence detector at femtomole levels. By collecting peaks which accounted for 0.1 % or more, sixteen different oligosaccharide structures were characterized from glioblastoma tissue and cell lines. The 16 oligosaccharide structures accounted for 48.9 % of the total N-linked oligosaccharides present in glioblastoma tissue. The major components of total oligosaccharides were similar to those of normal brain tissue. The amount of a biantennary bigalactosylated structure with one core fucosylation (A2G2F) was present in increased levels in glioblastoma tissue (mean= 2.90 %) and glioma cell lines (mean= 5.60 %), while being less than 0.1% in normal brain tissues. Expression of highly branched tetra-antennary N-glycans that are usually detected in lungs or hepatocellular cancer was not observed. Tissue glioma cells and cultured cells also displayed strong LCA lectin binding, which binds to sugar chains with core fucose (including A2G2F), while normal brain tissue did not. Moreover, LCA lectin inhibited proliferation of glioma cells, and this inhibition resulted from induction of apoptosis. A2G2F on glioma specimens may provide a novel marker and target for the diagnosis and treatment of glioblastoma, respectively.

P238

INHIBITION OF GLIOMA PROLIFERATION BY MODIFIED P53 PROTEIN FUSED WITH PROTEIN TRANSDUCTION DOMAINS

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Protein transduction domains (PTDs), short basic peptide sequences, in such as human immunodeficiency virus type 1 (HIV-1)-encoded trans-activator TAT domain and artificial cell membrane permeable poly-Arginine (11R) domain are capable of mediating proteins across the plasma membrane into nearly all eukaryotic cells.P53 protein is one of the altered proteins in cancer cells, and is mutated or deleted in more than half of human tumors including malignant glioma. Therefore, tumor suppressor protein therapy with wild-type p53 protein has been proposed as a possible therapy for malignancies lacking active p53. However, the half-life of 11R-p53 is less than 24 hr. Therefore, repeated transduction of 11R-p53 is needed for the inhibition of cell proliferation.In this study, we show that the COOH-terminal region of p53 modulates the susceptibility of p53 to Mdm2-mediated degradation. Moreover, p53 mutant in which lysine residues 370, 372, 373, 381, 382 and 386 in the COOH-terminal were replaced by arginine residues was resistant to ubiquitin-proteosome-mediated degradation, and that the transcriptional activity was higher than that of wild-type p53. We investigate the effect of protein transduction therapy of the mutated p53, which the multiple lysine residues were substituted by arginine on the stabilization of the protein and the inhibition of the growth of malignant glioma cells. The p53 mutant proteins fused with 11R were effectively delivered in glioma cells and localized in nucleus. Moreover, the delivered proteins were resistant to Mdm2-mediated ubiquitination and the expressions of 11R-p53 mutant were more stable than those of wild-type 11R-p53. Finally, the mutant 11R-p53s displayed a higher transcriptional activity and a more powerful inhibition of the proliferation of glioma cells compared with wild-type 11R-p53.

P239

EXPRESSION OF THE LYSOSOMAL-ASSOCIATED MEMBRANE PROTEIN-1 (LAMP-1) IN ASTROCYTOMAS

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Targeting lysosomes is a novel approach in cancer therapy providing a possible way of killing the otherwise apoptosis-resistant cancer cells. Recent research has thus shown that lysosome targeting compounds induce cell death in a cervix cancer cell line. Tumor stem cells in glioblastomas have recently been suggested to possess innate resistance mechanisms against radiation and chemotherapy possibly explaining the high level of therapeutic resistance of these tumors. Since the presence and distribution of lysosomes in tumor cells and especially in tumor stem cells in astrocytomas is unknown, the aim of this study was to investigate the immunohistochemical expression of LAMP-1, a membrane bound protein in lysosomes, in formalin fixed paraffin embedded tumor tissue from 23 diffuse astrocytomas, 17 anaplastic astrocytomas and 72 glioblastomas. The LAMP-1 expression was scored and compared with both tumor grade and patient survival. Moreover double immunofluorescence stainings with LAMP-1 and the stem cell marker CD133 as well as the macrophage marker CD68 were performed. The results showed that LAMP-1 was expressed in the vast majority of tumors being present in the cytoplasm of single tumor cells, cell clusters and in blood vessel endothelial cells. The LAMP-1 expression in glioblastomas was significantly higher than in diffuse and anaplastic astrocytomas ($p<0.001$). No association between the expression of LAMP-1 and patient overall survival was found. Double immunofluorescence staining with LAMP-1 and CD133 showed some degree of coexpression both in niches and single cells but coexpression of LAMP-1 and CD68 was also found. In conclusion, this study shows that lysosomes are widely distributed in astrocytomas, especially in glioblastomas. With the localization of LAMP-1 in CD133 positive putative tumor stem cells, these results suggest that targeting lysosomes may be a promising strategy of improving astrocytoma treatment.

P240

EVALUATION OF ELK-1 ACTIVATION BY PHOSPHATIDYLINOSITOL 3-KINASE/AKT PATHWAY IN GLIOBLASTOMA

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Purpose: Glioblastoma multiforme is the most malignant tumor among the glial tumors. A survival transcription factor, Elk-1 has been shown to be in the downstream of MAPK and PKC pathways and these pathways exerts some of its proliferative effect via Elk-1 activation in GBM. However, a possible interaction with Elk-1 and another major survival pathway PI3K has not been studied in GBM. **Methods:** Western blotting for phosphorylation of MAPK and PI3K pathways and Elk-1 in the downstream, Egr-1 PCR and survival, proliferation and apoptosis assays were performed to evaluate the Elk-1 role upon stimulation by EGF and TPA. **Results:** Elk-1 transcriptional activation was demonstrated after TPA or EGF stimulation of MAPK, PI3K and PKC pathways in rat C6 and human U138 glioma cells as in vitro. Elk-1 transcriptional activity was parallel to the phosphorylation of its serine 383 residue in response to EGF or TPA stimulation. This activation was blocked mainly by UO 126, a MEK inhibitor. Inhibiting PI3K pathway with LY 294002 did not affect Elk-1 transcriptional activation but the phosphorylation of Elk-1 was blocked partially by LY 294002. EGF stimulated preferentially the PI3K pathway, while TPA did so for MAPK pathway. PI3K had no direct effect in activation of Elk-1, rather interacted with MAPK to phosphorylate Elk-1. Elk-1 activation did have a physiological corresponse in regard to viability, but not in proliferation and apoptosis. **Conlusion:** Our results suggest that Elk-1 is activated after phosphorylation at serine 383 residue mainly by MAPK pathway and PI3K pathway has an indirect role in Elk-1 activation via MAPK pathway. Elk-1 may be a survival factor in C6 rat and U138 human glioma cells.

P241

ANTINEOPLASTONS INHIBIT MCM COMPLEX IN GLIOBLASTOMA CELLS

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Phenylacetylglutaminate (PG) is an active ingredient of antineoplastons A10 and AS2-1 (ANP) and is also a metabolic byproduct of phenylbutyrate (PB). Antineoplastons have recently been granted FDA orphan drug designation for the treatment of gliomas. In the antineoplaston AS2-1, PG is used in combination with phenylacetate (PN), a histone deacetylase inhibitor. The aim of this study is to further understand the mechanism of action of PG on brain tumor cells in vitro. We performed a screen to detect changes in gene expression in response to PG and PN in U87 glioblastoma cells using the Affymetrix Human Genome plus 2.0 oligonucleotide arrays. Pathway analysis was performed using tools such as DAVID, Onto-express, Genespring, and GeneMAPP to identify pathways that show fold enrichment of genes based on the expression data. Antineoplastons appear to inhibit cancer cell proliferation by targeting multiple pathways in the cells. A total human gene array study in glioblastoma cells showed that major metabolic pathways such as glycolysis and TCA are downregulated. Many pro-apoptotic genes such as CASP3, CASP4, several TNFRs, TRAF3 are up-regulated. The cell cycle is disrupted and major checkpoint proteins are suppressed leading to apoptosis of glioblastoma cells. The Minichromosome Maintenance Complex (MCM) proteins are highly expressed in malignant human cells and pre-cancerous cells undergoing malignant transformation. On the other hand they are not expressed in differentiated cells that have withdrawn from the cell cycle. Therefore, these proteins are ideal diagnostic markers for cancer and promising targets for anti-cancer drug development. All six genes of the MCM are markedly suppressed by PG and PN in our gene array study. We are currently investigating the role of corresponding proteins in apoptosis of glioblastoma cells. In conclusion ANP inhibit MCM complex in glioblastoma cells which may play important role in control of tumor growth.

P242

ENHANCED EXPRESSION OF NADPH OXIDASE NOX4 IN HUMAN GLIOMAS AND ITS ROLES IN CELL PROLIFERATION AND SURVIVAL

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[Purpose] Reactive oxygen species (ROS) has been attracting attention as a mediator of various cell signaling pathways. Nox-family NADPH oxidases have been proven to be a major source of ROS production in various cell types and have crucial roles in various physiological and phathological processes. In this study, we have evaluated expression of Nox-family genes in various neuroepithelial tumors, and also analyzed its functions in cultured glioma cells. **[Methods and Results]** We found that Nox4, a homolog of gp91phox /Nox2 (a phagocytic NADPH oxidase catalytic subunit) is prominently expressed in various neuroepithelial tumors by reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical studies. We quantified Nox4 mRNA expression by real time PCR in specimens from 58 patients with astrocytic tumors, and found that the expression levels of Nox4 mRNA in glioblastomas (WHO grade IV) were significantly higher than other astrocytomas (WHO grade II and III). We also found that specific knockdown of Nox4 expression by RNA interference resulted in cell growth inhibition and enhanced apoptosis induction by cisplatin in cultured glioma cell lines, KNS42 and 81. **[Conclusions]** Based on these observations, enhanced expression of Nox4 appeared to be involved in cell growth and survival in glioma cells.

P243

EXPRESSION PATTERN OF EGFRS, INTEGRINS, AND RELATED MOLECULES IN HUMAN GLIOBLASTOMA VERSUS INTRACEREBRAL LUNG ADENOCARCINOMA METASTASIS

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Glioblastoma is the most common and most malignant primary tumor of the central nervous system. The therapy of these tumors is often ineffective mainly because of their invasive behavior. Contrarily, metastases of the highly dedifferentiated anaplastic lung adenocarcinoma are well-circumscribed intracerebral lesions with a very moderate infiltration activity to the adjacent brain tissue. Epidermal growth factor receptors (EGFRs), integrins and their most important ligands: collagens, fibronectin and laminins play a pivotal role in the invasion process. To understand the background of the different invasion activity of glioblastoma and adenocarcinoma metastasis, the expression of these molecules were determined and compared to each other and to peritumoral brain. Neurosurgical tissue samples were investigated. The mRNS expression of 29 molecules was measured by QRT-PCR and immunohistochemical (IHC) investigation was also performed in the case of 9 molecule. EGFR amplification by fluorescence in situ hybridization (FISH) and EGFRvIII mutation by IHC were also determined. Significant difference was detected in the mRNA level 10 molecules in comparison of glioblastoma to metastasis. The EGFR was amplificated both in the glioblastoma and metastasis. EGFRvIII mutation was detected predominantly in glioblastoma. ErbB2, 3, 4, laminin &alpha2, &alpha4, &beta1, integrin &alpha3, &alpha7, &alpha9 and &beta1 seem to play some role in the different invasion activity of glioblastoma and intracerebral adenocarcinoma metastasis. These molecules might serve as possible targets for anti-invasive therapy.

P244

INHIBITION OF LRIG3 GENE EXPRESSION VIA RNA INTERFERENCE MODULATES THE PROLIFERATION, CELL CYCLE, CELL APOPTOSIS, ADHESION AND INVASION OF GLIOBLASTOMA CELL

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Background LRIG3 (leucine-rich repeats and immunoglobulin-like domains, LRIG) gene is both under-and over-expressed in human cancers and its role on tumor growth is not fully clarified. This study was undertaken to explore the effects of inhibition of the expression of LRIG3 gene on proliferation, apoptosis, adhesion and invasion of the human glioblastoma cell line (GL15) and explore possible mechanisms by using small interfering RNA (siRNA) targeting the LRIG3 gene.

Methods The plasmids pGenesil2-LRIG3-shRNA1 and pGenesil2-LRIG3-shRNA2 which containing U6 promoter and LRIG3-specific short hairpin RNA (shRNA) and the plasmid pGenesil2-negative-shRNA containing unspecific shRNA were transfected into GL15 cells. Stable cell clones were selected by G418. The changes in LRIG3, EGFR and P-EGFR expression levels were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot. Cell proliferation was detected by MTT assay. The expression of PCNA and Ki-67 in GL15 cells were examined by SABC immunohistochemistry. The apoptosis rate and cell cycle were analyzed by flow cytometry. The changes of the GL15 cells adhesive and invasive ability were measured by cell adhesion assay and Transwell chamber.

Results Compared with those in control cells, the mRNA levels of LRIG3 transcripts were reduced by 52.4% and 63.8% in shRNA1- and shRNA2-transfected cells, respectively; its protein levels were reduced by 50.9% and 67.4%, respectively. The EGFR and P-EGFR protein level in pGenesil2-LRIG3-shRNA (siRNA) transfected cells were significantly higher than that in negative shRNA (neg) transfected cells p&lt0.05. MTT showed that the LRIG3-specific siRNA cell had higher proliferation rate than neg cell p&lt0.05. The positive rate of PCNA was significantly higher in shRNA1- and shRNA2-transfected cells than in control cells [(72.13&plusmn5.64)% and (81.93&plusmn5.23)% vs. (35.40 &plusmn5.69)%, P &lt 0.01]. The positive rate of Ki-67 was also significantly higher in shRNA1- and shRNA2-transfected cells than in control cells [(82.27&plusmn5.50)% and (88.67&plusmn3.52)% vs. (49.73&plusmn5.73)%, P &lt 0.01]. PCNA expression was positively correlated to Ki-67 expression (r =0.932, P&lt0.001). Cell cycle analysis showed that silencing LRIG3 increased the percentage of G2 / M phase cells and improved the proliferation index significantly (p&lt0.01). Silencing LRIG3 can inhibit the apoptosis of GL15 cells p&lt0.05. Treatment of GL15 cells with pGenesil2-LRIG3-shRNA can enhance adhesive and invasive ability p&lt0.05.

Conclusions Our results demonstrated that RNAi against LRIG3 could effectively down regulate LRIG3 gene expression, and then effects cell proliferation, apoptosis, adhesion and invasion on the human glioblastoma. LRIG3 might be involved in the regulation of EGFR signaling, and serve as a tumor suppressor gene in the pathogenesis of glioma.

P245

PROMYELOCYTIC LEUKEMIA PROTEIN INDUCES APOPTOSIS BY THE REPRESSION OF NFκB ACTIVATION IN GLIOBLASTOMA CELLS.

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Purpose: Promyelocytic leukemia (PML) protein plays an essential role in the induction of apoptosis; its expression is reduced in various cancers. As the functional roles of PML in glioblastoma multiforme (GBM) have not been clarified, we assessed the expression of PML protein in GBM tissues and explored the mechanisms of PML-regulated cell-death in GBM cells.**Experimental Design:** We examined the PML mRNA level and the expression of PML protein in surgical GBM specimens. PML-regulated apoptotic mechanisms in GBM cells transfected with plasmids expressing the PML gene were examined.**Results:** The protein expression of PML was significantly lower in GBM- than non-neoplastic tissues; approximately 10% of GBM tissues were PML-null. The PML mRNA levels were similar in both tissue types. The overexpression of PML activated caspase-8 and induced apoptosis in GBM cells. In these cells, PML decreased the expression of transactivated forms of NFκB/p65 and c-FLIP gene expression was suppressed. Therefore, PML-induced apoptosis resulted from the suppression of the transcriptional activity of NFκB/p65. PML overexpression decreased phosphorylated IκBα and nuclear NFκB/p65 and increased the expression of the suppressor of cytokine signaling (SOCS)-1. A proteasome inhibitor blocked the reduction of activated p65 by PML.**Conclusions:** The reduction of PML is associated with the pathogenesis of GBM. PML induces caspase-8-dependent apoptosis via the repression of NFκB activation by which PML facilitates the proteasomal degradation of activated p65 and the sequestration of p65 with IκBα in the cytoplasm.This novel mechanism of PML-regulated apoptosis may represent a therapeutic target for GBM.

P246

THE CELLULAR ONCOGENE ETS-1 COUNTERACTS P53 TUMOUR SUPPRESSOR ACTIVITY BY AFFECTING THE LEVEL OF P53 PROTEIN.

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Study purpose
The proto-oncogenic factor ets-1 is often found overexpressed in malignant brain tumours and has been implicated in promoting glioma invasion. We have previously shown that ets-1 transcriptional activity is negatively regulated by tumour suppressor p53 via a direct physical interaction between the two proteins. In this study, we addressed the possibility of a reciprocal relationship between ets-1 and p53 and investigated the impact of ets-1 on transcriptional activity of p53.

Methods
Transcriptional activity of endogenous or ectopically expressed recombinant p53 and ets-1 proteins was evaluated by semi-quantitative RT-PCR or western blot analyses. Protein interaction was assessed by co-immunoprecipitation of endogenous proteins or by performing pull-down assays with recombinant proteins expressed in insect cells. DNA binding of p53 was assessed in vitro by gel shif assay (EMSA) or in vivo by chromatin immunoprecipitation (ChIP).

Results

We have identified a mutually antagonistic relationship between tumour suppressor p53 and the cellular oncogene ets-1. ets-1 expression and transcriptional activity are inhibited in the presense of high levels of wtp53. However, the p53-mediated inhibition of the ets-1 expression is overcome through the increase in ets-1 protein levels. Furthermore, the enhanced expression of ets-1 leads to a reduction in the p53 protein levels and consequently, diminishes transcriptional response mediated by p53. Our investigations revealed a previously unknown interaction between ets-1 and MDM2, a major regulator of p53 stability. Our results strongly suggest that the ability of ets-1 to modulate p53 levels is mediated by the interaction of ets-1 and MDM2.

Conclusion
Increased expression of ets-1 affects p53 at the protein level through an mdm2-dependent mechanism that may operate in glioma cells with wild type p53 to diminish its tumor suppressor function.

P247

COOPERATIVE CYTOTOXICITY BY HDAC INHIBITORS AND RTKIS: INDUCTION OF CX43 IN GBM CELLS

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Glioblastoma multiforme (GBM) display a remarkable biological heterogeneity and redundancy of tumour cells, most likely explaining the poor effects of the present available treatment options. This necessitates development of new therapeutic strategies. We have previously addressed this issue demonstrating induction of the gap junction protein and tumour suppressor connexin 43 (Cx43) by the histone deacetylase inhibitor 4-phenylbutyrate (4-PB). Here we further evaluated combination treatments with 4-PB and the receptor tyrosine kinase inhibitors gefitinib (EGFR) and vandetanib (EGFR,VEGFR-2, RET). The present study demonstartes that gefitinib and vandetanib increase expression levels and relocalizes Cx43, most likely also including a 20-25 kDa protein entity with similarity to the previously identified C-terminal of Cx43. Cooperative cytotoxic effects were also evident when cells were subjected to combination treatment with 4-PB and gefitnib or vandetanib. It is suggested that this novel approach could be a possible strategy to overcome the dismal efficacy in the treatment of GBM.

P248

A MDR1 (ABCB1) GENE SINGLE NUCLEOTIDE POLYMORPHISM PREDICTS OUTCOME OF TEMOZOLOMIDE TREATMENT IN GLIOBLASTOMA PATIENTS

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Purpose: Some patients with glioblastoma multiform do not respond to temozolomide even though they have aberrant promoter methylation of the DNA repair enzyme MGMT. This suggests that additional factors hamper temozolomide cytotoxicity. We aimed to confirm first that temozolomide is a target for the multidrug-resistance transporter MDR1/ABCB1, and second to investigate whether genetic variants of the MDR1 gene are associated with the survival of glioblastoma patients treated with temozolomide.**Methods:** Temozolomide mediated cytotoxicity was determined by the colorimetric methyl-thiazol-tetrazolium (MTT) assay in MDR-expressing and-non-expressing cell lines. Genotypes of three single nucleotide polymorphisms of the MDR1 gene (C1236T, G2677T, and C3435T), MDR1 mRNA expression levels and the MGMT promoter methylation status were analyzed in 112 glioblastoma patients who had been treated either by surgery plus radiotherapy alone or by additional temozolomide chemotherapy. **Results:** In vitro analysis revealed that temozolomide mediated cytotoxicity is dependent on MDR1 expression. Multivariate analysis of MDR1 genotypes showed that the C/C variant of the exon12 C1236T SNP is predictive for survival of patients treated with temozolomide. This effect was independent of the MGMT methylation status. Patients with the C/C genotype had a 2-year overall survival of 37% compared to 8% and 10% for patients with C/T and T/T genotypes, respectively (p=0.02). No influence was seen in the group of patients with radiotherapy only. **Conclusion:** The genotype of the MDR1 exon12 C1236T SNP is a novel independent predictive factor for outcome of temozolomide treatment in glioblastoma patients

P249

DIVERSITY OF DNA DAMAGE RESPONSE OF ASTROCYTES AND GLIOBLASTOMA CELL LINES WITH VARIOUS P53 STATUS TO TREATMENT WITH ETOPOSIDE AND TEMOZOLOMIDE

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Phosphorylation of histone H2AX is a sensitive marker of DNA damage, particularly of DNA double strand breaks. Using multiparameter cytometry we explored effects of etoposide and temozolomide (TMZ) on three glioblastoma cell lines with different p53 status (A172, T98G, YKG-1) and on normal human astrocytes (NHA) correlating the drug-induced phosphorylated H2AX (γH2AX) with cell cycle phase and induction of apoptosis. Etoposide induced γH2AX in all phases of the cell cycle in all three glioblastoma lines and led to an arrest of T98G and YKG-1 cells in S and G_2/M. NHA cells were arrested in G1 with no evidence of γH2AX induction. A172 responded by rise in γH2AX throughout all phases of the cycle, arrest at the late S- to G_2/M- phase, and appearance of senescence features: induction of p53, p21, p16, and β-galactosidase, accompanied by morphological changes typical of senescence. T98G cells showed the presence of γH2AX in S phase with no evidence of cell cycle arrest. A modest degree of arrest in G1 was seen in YKG-1 cells with no rise in γH2AX. While frequency of apoptotic cells in all four TMZ-treated cell cultures was relatively low it is conceivable that the cells with extensive DNA damage were reproductively dead. The data show that neither the status of p53 (wild-type vs mutated, or inhibited by pifithrin-α) nor the expression of O^6-methylguanine-DNA methyltransferase significantly affected the cell response to TMZ. Because of diversity in response to TMZ between individual glioblastoma lines our data suggest that with better understanding of the mechanisms, the treatment may have to be customized to individual patients.

P250

THE DECREASED EXPRESSION OF GANP IS ASSOCIATED WITH CHROMOSOMAL INSTABILITY IN MALIGNANT GLIOMAS

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Purpose: Malignant gliomas are highly proliferative and invasive, resulting in poor prognosis, which are associated with aneuploidy and chromosomal instability (CIN). GANP is a germinal center-associated nuclear protein containing a region homologous to *Saccharomyces* Sac3, which is involved in mRNA export and the regulation of DNA recombination. GANP expression was decreased in the advanced cases of human breast cancers. This observation was confirmed by experimental mouse model of *ganp*-heterodeficiency, which caused mammary tumors with hyperploid cells at a high incidence. We studied how GANP expression changes and associates with CIN in malignant gliomas. **Materials and Methods:** The *ganp* mRNA was assessed by real-time PCR in 101 cases of adult malignant glioma including anaplastic astrocytoma (AA) and glioblastoma (GBM) by comparing with various clinicopathologic factors. We examined the cell cycle change of five human malignant glioma cell lines by *ganp* RNA interference (RNAi)-transfection. The generation of CIN was examined after *ganp* RNAi-treatment of glioma cell lines with various *p53* mutations. **Results:** GBM showed a significantly lower level of *ganp* mRNA than those of AA. We classified malignant gliomas into $ganp^{Low}$ and $ganp^{High}$ groups. The $ganp^{Low}$ group displayed more malignant character with loss of heterozygosity on chromosome 10 (LOH10) and *EGFR* gene amplification, and showed the significant poor prognosis. *Ganp* RNAi caused hyperploidy of glioma cell lines by flow cytometric analysis, and caused CIN with multiplication of chromosomes and the increase of *EGFR* gene in the fluorescence *in situ* hybridization (FISH). **Conclusions:** Malignant gliomas of the highly malignant character showed a significant decrease of GANP expression and caused hyperploidy and CIN.

P251

ELK4 NEUTRALISATION SENSITISES HIGH GRADE GLIOMA TO APOPTOSIS THROUGH DOWN REGULATION OF THE ANTI-APOPTOTIC PROTEIN MCL-1

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Purpose: Bcl-2 family anti-apoptotic proteins are elevated in many human malignancies and are attractive therapeutic targets. The purpose of this study was to measure anti-apoptotic Bcl-2 family expression in high grade glioma to investigate potential targets for molecular therapy. **Methods:** Gene expression was investigated by QPCR and Western blot in 37 primary high grade glioma surgical specimens and 12 GBM cell lines. Targeted reduction of gene expression was performed in GBM cell lines using transfected siRNAs or plasmid-based shRNAs. Increased gene expression was achieved using plasmid-based cDNAs. Spontaneous, ABT-737- or cisplatin-induced apoptosis was measured by FACS analysis of annexin V-stained cells. Gene promoter function was analysed by luciferase reporter assay. **Results:** We found Mcl-1 to be the highest expressed anti-apoptotic Bcl-2 family member in the majority of malignant gliomas. This was functionally important as neutralisation of Mcl-1 induced apoptosis and increased sensitivity to the BH3 mimetic ABT-737 or cisplatin treatment in GBM cells. Sequencing of the Mcl-1 promoter identified a novel functional single nucleotide polymorphism (SNP) in a previously uncharacterised consensus ETS transcription factor binding site. We identified the ETS domain transcription factor ELK4 as a key regulator of Mcl-1 in glioma, since ELK4 down regulation was shown to reduce Mcl-1 expression. Importantly the presence of the SNP, which ablated ELK4 binding, whilst uncommon in gliomas, was associated with low Mcl-1 levels and greater susceptibility to apoptosis following chemotherapy treatment. Furthermore, down regulation of ELK4 by siRNA, resulting in loss of Mcl-1 expression, increased sensitivity to ABT-737 and cisplatin treatment. Conversely, ELK4 overexpression increased Mcl-1 levels and this was shown to be protective against higher concentrations of the chemotherapy agent cisplatin. **Conclusions:** These findings demonstrate ELK4 to be a critical regulator of Mcl-1 expression in GBM and highlight both ELK4 and Mcl-1 as molecular therapeutic targets in high grade glioma.

P252

XCT AS A POTENTIAL MOLECULAR TARGET IN GLIOBLASTOMA MULTIFORME

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xCT is a light chain of the cystine/glutamate antiporter system xc-, and its expression is correlated with the system xc- activity. Cystine taken up by the cell via system xc- is rapidly reduced to cysteine, which is incorporated into glutathione. At the same time, released glutamate promote killing of surrounding neurons. Recent studies have suggested the important role of glutamate transporters and exchangers in glioblastoma multiforme (GBM). We have investigated the expression of xCT in 38 patients with newly diagnosed GBM. Expression of glial glutamate transporter GLT-1(EAAT2) was also investigated. All patients were treated by temozolomide plus radiation therapy. xCT expression was determined by immunohistochemical staining with an antibody against xCT (PA1-16893, Affinity BioReagents, CO). Immunoreactivity was graded and determined as weak or strong. xCT protein expression level was also investigated in 18 patients using semiquantitative western blotting. In the normal brain area, weak staining for xCT was observed in the leptomeningeal cells. No significant immunostaining in neurons or astrocytes was seen in the uninvolved normal brain. The result of immunohistochemical staining was tightly correlated with the result obtained by western blotting. Kaplan-Meier analysis of progression-free survival showed a statistically significant association between strong xCT exprssion and poor outcome (log-rank, $p<0.05$). Median progression-free survival was 11.1 months (weak xCT expression: n=10) versus 5.7 months (strong xCT expression: n=28). Cox regression analyses demonstrated that xCT expression was one of significant predictors, independent of other variables. GLT-1(EAAT2) expression was invariably very weak in GBM. These findings suggest that xCT could be one of the molecular targets in therapy for GBM. Sulphasalazine is known to inhibit the activity of system xc-. We have initiated a phase I/II trial of radiotherapy with concomitant temozolomide plus sulfasalazine for newly diagnosed GBM.

P253

A NOVEL MMAC/PTEN GENE MUTATION IN A GBM PATIENT

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The MMAC/PTEN tumor suppressor gene has an essential biological role in the formation of Glioblastoma multiforme (GBM). It is known that there are variations in genetic alterations in tumors that develop in patients with different ethnic backgrounds so, we aimed to evaluate the presence of MMAC/PTEN mutations and protein expressions in Turkish patient with GBM. We investigated mutations of the MMAC/PTEN gene using single strand conformational polymorphism (SSCP) method followed by DNA sequencing. Additionally the level of MMAC/PTEN protein expression in tumor was assessed by immunohistochemistry (IHC). As a result of our investigation, a novel MMAC/PTEN sequence variant was identified: it was G deletion at codon 159. This frameshift mutation was caused premature stop codon at downstream 7 codons. Moreover, it was observed that MMAC/PTEN protein expression was highly reduced in Turkish patient with GBM. **Keywords:** GBM; MMAC/PTEN; IHC; novel mutation; Turkish population

P254

ATTRACTION OF HEMATOPOIETIC PROGENITOR CELLS BY EXPERIMENTAL GLIOMAS IN VIVO: TOWARDS A CELL-BASED THERAPY FOR MALIGNANT GLIOMAS?

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Adult progenitor and stem cells are novel candidates for a cell-based gene delivery to cancers. Hematopoieitc progenitor and stem cells (HPC) are an easily accessible cell population. Thus, we investigated their potential as autologous cellular vehicles in the treatment of malignant gliomas. Experimental gliomas attracted HPC in vivo by a transforming growth factor (TGF)-beta-regulated CXC chemokine ligand (CXCL) 12-dependent pathway. Irradiation and non-lethal hypoxia promoted attraction of HPC by a hypoxia-inducible factor (HIF-)1-alpha-dependent induction of CXCL12. Next, we studied the role of adhesive interactions for the glioma tropism of HPC. Exposure of human cerebral endothelial cells (SV-HCEC) to supernatants of glioma cells induced CD62E expression. Transendothelial migration assays showed enhanced HPC migration after CD62E induction which was impaired by neutralizing CD62E antibodies. Tissue microarrays containing human glioblastoma and normal brain samples showed CD62E expression on glioblastoma vessels. Glioma supernatant-mediated induction of CD62E on endothelial cells required TGF-beta signaling in glioma cells as well as vascular endothelial growth factor receptor 2 (VEGF-R2) signaling in endothelial cells. Genetic modification of HPC by lentiviral transduction ex vivo did not interfere with the glioma tropism of HPC in vitro or in vivo. Importantly, lentivirally transduced HPC were not tumorigenic.These data suggest that HPC might be promising vectors in the treatment of experimental gliomas. Given the essential roles of TGF-beta and VEGF-A, a potential HPC-based therapy might not work in combination with TGF-beta-antagonistic strategies or VEGF-A neutralizing approaches. Instead, HPC-based therapy might be compatible with irradiation and chemotherapy.

P255

OLIGODENDROGLIOMAS WITH LOH 1P/19Q: IDENTIFYING GENES ASSOCIATED WITH THERAPEUTIC SENSITIVITY

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Background: Loss of heterozygosity of chromosomes 1p and 19q occurs in approximately 70% of oligodendrogliomas and is associated with therapeutic sensitivity and better overall survival. The precise genetic contributors to the improved outcome for patients with LOH of 1p and 19q are unknown.

Aim: To use microarray profiling to compare gene expression in oligodendrogliomas with LOH of 1p and 19q to those without, in order to identify genes that are implicated in the improvement in outcome for patients with LOH, then investigate the role of these genes in chemosensitivity, tumorigenesis and survival in the biology of gliomas.

Methods: Exon microarrays were used to identify differential expression patterns of genes located on the 1p and 19q chromosomes. Genes located throughout the rest of the genome were also screened in order the ascertain whether these chromosomal aberrations may mediate therapeutic responsiveness or simply act as genetic markers for chemosensitive tumours due to other genetic alterations. qPCR and immunohistochemistry was performed to validate the differential expression of these genes in a larger sample set.

Results: PLAG1, IGF2, IQGAP1 and CHI3L1 genes were all found to be have low expression in oligodendrogliomas with LOH of 1p and 19q compared to those without LOH. Interestingly, these genes have also been reported to be aberrant in glioblastomas. Thus, underexpression of these genes in oligodendrogliomas with LOH of 1p and 19q may contribute to a more favourable prognosis seen in these patients.

Conclusions: The genes identified by this study are potentially associated with the improved response to therapy and survival seen in oligodendrogliomas with LOH of 1p and 19q. Further investigation into the function of these genes could ultimately lead to improved efficacy of therapy in the majority of gliomas that retain the 1p and 19q chromosomes.

P256

ASSOCIATION OF 7Q34 COPY NUMBER GAINS AND KIAA1549-BRAF GENE FUSIONS WITH JUVENILE PILOCYTIC ASTROCYTOMA

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PURPOSE: The purpose of this study was to determine whether specific genomic and transcriptional alterations are associated with specific pediatric astrocytoma malignancy grades . **METHODS:** DNAs and RNAs were extracted from pediatric astrocytomas. DNA copy number aberrations (CNAs) were determined using a customized molecular inversion probe (MIP) panel. Gene expression and fusion gene detection were determined by RT-PCR. **RESULTS:** MIP profiling was conducted on 10 previously diagnosed grade II tumors and 22 WHO-III and WHO-IV tumors. Among the grade II tumors, 7q34 duplications, involving the BRAF oncogene, were observed in 3/10 cases. Review of these 10 cases revealed two of three with BRAF duplications as having histopathologic features associated with juvenile pilocytic astrocytoma (JPA), with classification of the third BRAF duplication case being uncertain due to insufficient archival material. Subsequently we acquired 6 and 5 additional, histopathologically verified JPAs (WHO grade I) and grade II tumors, respectively, and identified BRAF gene duplications in all grade I cases, but in none of the grade II diffuse-type astrocytomas. RNAs from all grade I-II tumors were examined by RT-PCR for determination of KIAA1549-BRAF fusion transcripts. The results showed that all tumors with BRAF gene duplications had detectable fusion transcripts (9/9), whereas none of the 12 grade II tumors showed evidence of this fusion product. Analysis of the expression profiles from Grade I and II tumors revealed that tumors with KIAA1549-BRAF fusion transcripts expressed elevated PDGFRA, and this observation was supported by quantitative RT-PCR analysis which revealed significantly increased PDGFRA expression in tumors with single copy gain of BRAF ($p = 0.02$). **CONCLUSIONS:** Our results indicate a complete concordance between the detection of BRAF gene duplications and KIAA15449-BRAF fusion transcripts in pediatric low grade malignancy astrocytomas, and that detection of either alteration is strongly correlated with, and perhaps indicative of, WHO grade I JPA.

P257

IDH-1 MUTATIONS ARE FREQUENT IN LOW GRADE GLIOMA AND DO NOT CORRELATE WITH OUTCOME TO TEMOZOLOMIDE CHEMOTHERAPY

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Introduction. Mutations in the IDH-1 gene have recently been described to occur infrequently in glioblastoma, and have been reported to occur more frequently in low grade astrocytoma, oligodendroglial tumors and secondary glioblastoma. We analysed a series of 70 low grade astrocytoma cases treated with temozolomide for the presence of IDH-1 mutations. **Methods.** A consecutive series of patients with at central pathology review histologically confirmed progressive low grade astrocytoma after radiation therapy treated with temozolomide chemotherapy were analysed for IDH-1 mutations. Response was assessed using Macdonalds criteria. The presence of IDH-1 alterations in the mutational hotspot codon R132 was assessed by sequencing analysis of DNA isolated from formalin fixed paraffin embedded material. TP53 mutation status was assessed by sequence analysis of exons 4-9. A series of 30 pheochromocytoma was also analysed for the presence of IDH-1 mutations. **Results.** From 48 patients material was available for molecular analysis, from 27 patients material from two consecutive operations was available. IDH-1 mutations were present in 85% of samples. No IDH-1 mutations were found in the pheochromocytoma. In 6 of the paired samples results of the two samples were different (different mutation (n = 3), appearance or disappearance of a mutation. In 72% of samples a TP53 mutations was found. Six tumors showed no TP53 mutations but a IDH-1 mutations, in 4 a TP53 mutation was found but no IDH-1 mutation. Nineteen of the 43 (44%) evaluable patients responded to temozolomide. No clear difference in outcome to temozolomide treatment was observed. **Conclusion.** IDH-1 mutations are frequent in low grade glioma. No correlation was found between IDH-1 mutations and TP53 mutations. No significant difference in outcome to temozolomide treatment was observed. IDH-1 mutations may be an early event in the gliomatogenesis. More data will be presented on oligodendroglioma.

P258

PPAR-γ ACTIVATORS: OFF-TARGET AGAINST TGF-β MEDIATED GLIOMA CELL MIGRATION AND BRAIN INVASION

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Gliomas are the most common primary tumors of the central nervous system, with glioblastomas as the most malignant entity. Rapid proliferation and diffuse brain invasion of these tumors are likely to determine the unfavourable prognosis. Considering its pro-migratory properties, the transforming growth factor β (TGF-β) signaling pathway has become a major therapeutic target. Analyses of resected glioma tissues revealed an intriguing correlation between tumor grade and the expression of TGF-β 1-3. Using an organotypic glioma invasion model, we identified the peroxisome proliferator-activated receptor γ (PPAR-γ) agonist troglitazone (TRO), which has been developed for the treatment of type II diabetes mellitus, as a potent inhibitor of TGF-β mediated glioma migration and brain invasion. The anti-migratory property of TRO occurred at clinically achievable levels resulting in dramatically reduced levels TGF-β released by glioma cells. Thus, TRO may represent a promising drug for adjuvant glioma therapy. Here, we show that the PPAR-γ inactive TRO-derivative delta2-TRO antagonized TGF-β signaling and glioma cell migration in a variety of glioma cell lines as well as ex vivo glioma progression, giving clear evidence that the anti-glioma properties of TRO occur in a PPAR-γ independent manner. Based on the promising in vitro and ex vivo anti-glioma activities of TRO and delta2-TRO, respectively, in vivo studies have been initiated. Moreover, the identification of the yet unknown target protein(s) of delta2-TRO may provide new therapeutic strategies for gliomas and other highly migratory tumor entities.

P259

TIGHT JUNCTION PROTEIN OCCLUDIN IN HUMAN BRAIN TUMORS: RADIOLOGICAL CORRELATION AND PROGNOSTIC IMPLICATION ON THE SURVIVAL

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Cerebral edema develops in the brain tumors by loosening of the endothelial tight junction. The development of peritumoral brain edema (PTBE) is influenced by many factors including tight junction proteins, such as occludin. We studied the correlation with PTBE volume and survival outcome related to occludin expression. Sixty fresh-frozen specimens from patients with brain tumor were obtained during surgery and confirmed pathologically. Occludin expression was investigated by Western blot analysis. PTBE volume was measured by using preoperative magnetic resonance image (MRI) and survival time in each patient was also estimated retrospectively. Occludin was detected in 41 (68.3%) brain tumors and the other 19 (31.7%) was not. Although its expression was variable according to pathology of brain tumors ($p > 0.05$), high grade glioma (1/4 = 25.0%) exhibited lower expression rate of occludin than low grade one (7/10 = 70.0%). The difference of PTBE volume between occludin-positive and negative brain tumors was significant ($p = 0.002$). The mean survival time was prolonged in occludin-positive tumors comparable to negative one ($p = 0.001$). This study suggests that occludin expression is well correlated to the radiological finding of PTBE development in brain tumors and might be a prognostic indicator for survival of patients.

P260

EXPRESSION OF THE TIMP-1 INTERACTING CELL SURFACE PROTEIN CD63 IN ASTROCYTOMAS

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Recent research has suggested that the cell surface protein CD63 is a new TIMP-1 (tissue inhibitor of metalloproteinases-1) interacting protein or even a TIMP-1 receptor. Since TIMP-1 has been shown to be a negative prognostic marker in different cancers including breast cancer and colorectal cancer, CD63 might also represent a new important biomarker. In a study, using human breast epithelial cells it was demonstrated that TIMP-1 interacts with CD63 and this interaction was shown to be necessary for the TIMP-1 anti-apoptotic pathway. We have recently shown that the TIMP-1 immunohistochemical expression increased with tumor grade in astrocytomas and furthermore, a high TIMP-1 protein expression in glioblastomas was associated with a shorter overall patient survival suggesting TIMP-1 and possibly also CD63 as new biomarkers in glioblastomas. In the present study, we therefore investigated the immunohistochemical expression of CD63 in 112 formalin fixed paraffin embedded astrocytomas. The CD63 immunostainings were scored according to the percentage and staining intensity of CD63 positive tumor cells and blood vessels for comparison with tumor grade and overall patient survival. In addition, CD63 scores were compared to TIMP-1 scores obtained from the same tumors in a previous study. The CD63 protein was detected in both tumor cells and blood vessels in the vast majority of the astrocytomas, with the highest intensity found in tumor cells. The total staining score increased from the mean total score 5.6 in diffuse and anaplastic astrocytomas to the mean total score 7.5 in glioblastomas. There was no association between the CD63 protein expression and the overall patient survival and no correlation was found between TIMP-1 and CD63 immunohistochemical scores. In conclusion, this study showed that the CD63 immunohistochemical expression in astrocytomas increased with tumor grade but was without association with overall survival in these tumors.

P261

MOLECULAR VARIATIONS IN TUMOR-HOST INTERACTIONS BETWEEN INVASIVE AND ANGIOGENIC PHENOTYPE IN HIGH GRADE GLIOMAS

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In solid tumor growth, a crucial turning point is the transition from the avascular to the vascular phase, leading to a more aggressive tumor growth. Antiangiogenesis therapy is a powerful approach aimed at blocking/reducing tumor growth. The identification of novel angiogenesis specific proteins is crucial for the development of new antiangiogenic therapies, and such proteins are potential new biomarkers in cancer. A xenograft animal model of human glioblastoma multiforme (GBM) has been developed in our lab. The model includes 4 generations of rats with the GBM tumor phenotype varying from very invasive, nonangiogenic in the first to less invasive, fully angiogenic in the last generation rat. The objective of our study was to explain the molecular background of the phenotypic change, the angiogenic switch, at the protein level and to identify potential biomarker and target candidates we applied quantitative proteomics based on iTRAQ 2DLC MALDI TOFTOF and bioinformatics on a membrane enriched fractions of the tumors. In four generations of tumors, we identified 1038 nonredundant, host or tumor specific proteins (C.I. >= 95%). Species specific separation of the proteins allowed tumor host interaction studies at the proteome level and may reveal novel biomarkers involved in the angiogenic switch. Bioinformatic analyses over four tumor phenotypes revealed distinct groups of proteins with specific expression profiles that may be involved in the angiogenic switch and tumor angiogenesis. It appears from these profiles that many of the regulated proteins are involved in alternative tumor specific metabolic pathways for energy production. The expression of particular proteins representing these profiles was validated by non-proteomic methods (e.g. GBM tissue arrays) as well as by functional assays using a novel GFP expressing NOD/Scid mouse model, recently developed in our laboratory, which will allow us to confirm the tumor/host interactions involved in the angiogenic switch.

P262

MULTI-MODAL IMAGING OF ACUTE AND CHRONIC UP-REGULATION OF HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1) IN TUMORS

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Objective: Detection and assessment of tumor hypoxia, blood flow dependent hypoxia (acute) and oxygen diffusion dependent hypoxia (chronic), are important to understand tumor biology. This study addresses whether in vivo multi-modal imaging systems can be used for assessment of acute and chronic up-regulation of hypoxia-inducible factor-1 (HIF-1) in tumors. **Methods:** A multimodality reporter gene system for radionuclide, fluorescence, and bioluminescence (BLI) imaging was developed and placed under the control hypoxia responsive elements (HRE/TGL), and transduced to C6 and RG2 cells. HRE/TGL-RG2 and -C6 were implanted subcutaneously in mice. Constitutively expressing TGL and wild-type tumor cells were also implanted in the same animals as positive and negative controls, respectively. Acute tumor hypoxia was induced by injection of Hydralazine (HYZ). To investigate the feasibility of BLI to image acute up-regulation of HIF-1, sequential BLI was performed with or without HYZ treatment. Thereafter, autoradiographic imaging was performed following [18F]-FDG, [3H]-Xanthine and [14C]-FIAU administration to investigate the quantitative and spatial relationship between glucose metabolism, tumor blood flow and HIF-1 expression, respectively. **Results:** HYZ treatment significantly increased BLI photon emission intensity in the HRE/TGL tumors, but not in positive and negative control tumors. Radiotracer evaluations revealed that HYZ reduced tumor blood flow from 0.36 to 0.10 (ml/min/g), suggesting that HYZ can induce acute tumor hypoxia by decreasing blood flow. Autoradiographic images showed a good spatial relationship between FDG and FIAU uptake in viable portions of the tumors, indicating enhanced glucose metabolism in the same tumor regions with up-regulation of HIF-1 expression. **Conclusion:** The multimodality HRE/TGL reporter system can be used to visualize hypoxic conditions by fluorescence, BLI and radiotracer imaging. BLI appears to be very sensitive to changes induced by acute hypotension, and to chronic hypoxic conditions in large tumors. HIF-1 expression in tumors, as assessed by FIAU uptake, occurred in a blood flow-dependent manner.

P263

RELATIONSHIP BETWEEN ABC TRANSPORTER PROTEINS AND VASCULOGENESIS IN GLIOBLASTOMAS

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(BACKGROUND) Glioblastoma patients have a median survival of approximately 15 months following surgical resection, radiotherapy and temozolomide. However, as with other regimens, it does not lead to cure, because the blood brain barrier (BBB) is one of the problems in chemotherapy for the central nervous system (CNS). BBB forms a very effective barrier to the free diffusion of many polar solutes into the brain. Many metabolites that are polar have their brain entry facilitated by specific inwardly-directed transport mechanisms. These molecules are substrated for the ABC (ATP-binding cassette) transporter proteins which are present in BBB, and the activity of these transporters very efficiently removes the drug from CNS. By the way, glioblastomas are among the highest vascularized tumors. Neovascularization encompasses both angiogenesis and vasculogenesis. In the process of vasculogenesis, de novo formation of blood vessels from bone marrow-derived endothelial progenitor cells (EPCs) takes place. So, we hypothesized that glioblastoma patients have increased levels of EPCs involved in vasculogenesis because the increased EPCs and amplified ABC transporter proteins play important roles in cancer chemotherapy problems. **(METHODS)** We investigated the ABC transporter proteins (MDR1, MRP1, MRP2 and ABCG2) and surface markers of primitive EPCs (CD133 and VEGFR) in 25 glioblastoma specimens using RT-PCR assay and immunohistochemical staining. **(RESULTS)** We observed overexpression of CD133 and VEGFR with ABCG2 amplification in the microvessel endothelium. Glioblastomas with MDR1, ABCG2, CD133 and VEGFR amplification have a median progression free survival (PFS) of approximately 4.6 months, but glioblastomas with no amplification of CD 133 and VEGFR have a median PFS of 8 months. **(CONCLUSIONS)** Glioblastomas with MDR1, ABCG2, CD133 and VEGFR amplification tend to have a shorter median PFS. The increased EPCs and amplified ABC transporter proteins probably resist cancer chemotherapy with consequent poor outcomes in glioblastomas.

P264

HISTONE DEACETYLASE INHIBITOR, VALPROIC ACID INHIBITS GLIOMA ANGIOGENESIS IN VITRO AND IN VIVO IN THE BRAIN

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Valproic acid (VPA) is one of the histone deacetylase inhibitors and now under the clinical trial for anticancer drug including glioma. We investigate the antiangiogenic action of VPA in glioma cells and endothelial cells in vitro and glioma in vivo in the brain. In vitro, the anti-proliferative effect of VPA on human glioblastoma U87MG cells, rat glioma C6 cells and endothelial cells was assessed by cell proliferation assay. We investigated the effects of VPA on the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 (HIF-1) by glioma cells under normoxic and hypoxic condition. The effect of VPA to inhibit for tube formation in human umbilical vein endothelial cell was assessed by angiogenesis kit. In vivo, a brain tumor model of malignant glioma with Wister rat was used. After administration of VPA and CPT-11 to rat, angiogenic status of tumor tissues were assessed by vessel densities and the expression of VEGF and HIF-1. In vitro, VPA inhibited the proliferation of human endothelial cells preferably compared to glioma cells. VPA reduced VEGF secretion into conditioned media and VEGF mRNA expression of glioma cells under normoxic and hypoxic condition. VPA was also found to inhibit tube formation. In vivo, treatment with either VPA and CPT-11 reduced VEGF mRNA expression and CD31 expression as vessel densities in vivo in the brain tumor. In conclusion, VPA inhibit glioma angiogenesis by mechanisms involving a decrease in VEGF expression and inhibition of tube formation. VPA would be useful as an adjuvant medicine for malignant gliomas through its anti-angiogenic action.

P265

THE P14ARF TUMOR SUPPRESSOR INHIBITS GLIOMA-INDUCED ANGIOGENESIS BY A NOVEL MDM2/SP1/TIMP3 SIGNALING AXIS

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The Ink4a/Arf locus on chromosome 9p21 is frequently inactivated in the progression of malignant astrocytoma suggesting its role in tumor suppression. This locus encodes two tumor suppressor proteins, the p16INK4A cell cycle inhibitor and the alternative reading frame product, p14ARF, which can activate p53 by blocking mdm2 function. The importance of ARF loss in glioma formation is further underscored by the fact that it leads to glioma formation in knockout mice and the astrocytoma-melanoma syndrome in humans. The mechanisms on how p14ARF loss facilitates the development of malignant gliomas is poorly understood, especially in p53 mutated tumors. It is noteworthy that this genetic alteration occurs with the transition from WHO grade III to IV, and coincides with the associated pathological feature of dramatically increased neovascularization. **Here, we present a novel p53-independent tumor suppressor function for ARF as an inhibitor of glioma-induced angiogenesis.** We demonstrate that ARF restoration in glioma cells induces the secretion of an inhibitor of angiogenesis and identified this inhibitor as tissue inhibitor of metalloproteinase-3 (TIMP3), a negative regulator of proteolytic activities required for vascular remodeling. We show that ARF-induced expression of TIMP3 inhibits endothelial cell migration and vessel formation *in vitro* and *in vivo* in response to angiogenic stimuli produced by cancer cells. We further investigated the mechanism connecting ARF to TIMP3, and found that ARF induces the transcription of the *TIMP3* gene by activating the binding of transcription factor SP1 to the promoter. ARF does this indirectly, by relieving Sp1 from an inhibition by MDM2, allowing it to activate *Timp3* expression. The discovery of this novel angiogenesis regulatory axis provides new insights into p53-independent-ARF tumor suppressive mechanisms that have implications for the development of novel therapies directed at malignant gliomas and other diseases characterized by vascular pathology.

P266

DICER-REGULATED MICRORNAS 222 AND 339 PROMOTE RESISTANCE OF CANCER CELLS TO CYTOTOXIC T-LYMPHOCYTES BY DOWN-REGULATING ICAM-1 EXPRESSION

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The RNase III endonuclease Dicer plays a key role in generation of microRNAs (miRs) in cells. We hypothesized that Dicer regulates cancer cell susceptibility to immune-surveillance through miR processing. Indeed, Dicer disruption up-regulated intercellular cell adhesion molecule (ICAM)-1, and enhanced the susceptibility of tumor cells to antigen-specific lysis by cytotoxic T-lymphocytes (CTLs), while expression of other immuno-regulatory proteins examined was not affected. Blockade of ICAM-1 inhibited the specific lysis of CTLs against Dicer-disrupted cells, indicating a pivotal role of ICAM-1 in the interaction between tumor cells and CTL. Both miR-222 and -339, are down-regulated in Dicer-disrupted cells and directly interacted with the 3' untranslated region (UTR) of ICAM-1 mRNA. Modulation of Dicer or these miRs inversely correlated with ICAM-1 protein expression and susceptibility of U87 glioma cells to CTL-mediated cytolysis, while ICAM-1 mRNA levels remained stable. Immunohistochemical and quantitative RT-PCR analyses of 30 primary glioblastoma tissues demonstrated that expression of Dicer or miR-222 was inversely associated with ICAM-1 expression. Taken together, Dicer is responsible for the generation of the mature miR-222 and -339, which suppress ICAM-1 expression on tumor cells, thereby down-regulating the susceptibility of tumor cells to CTL-mediated cytolysis. This study suggests development of novel miRNA-targeted therapy to promote cytolysis of tumor cells.

P267

HYPOXIA-INDUCED STAT3 ACTIVITY CONTRIBUTES THE HIF-1, VEGF, MMP2 AND TWIST EXPRESSION IN HUMAN GLIOBLASTOMA, WHICH INDUCE ANGIOGENESIS AND CELL MIGRATION.

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P268

NOVEL MONOCLONAL ANTIBODY 13R2.C3 AGAINST IINTERLEUKIN 13 RECEPTOR ALPHA-2 REVEALS A SPECIFIC AND MORE WIDE-SPREAD EXPRESSION OF THE RECEPTOR IN VARIOUS BRAIN TUMORS

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Purpose. Interleukin 13 receptor alpha 2 (IL-13Rα2), an IL-13 plasma membrane receptor, is over-expressed in a vast majority of patients with glioblastoma multiforme (GBM) and also in dogs with malignant gliomas. We have thus decided to generate monoclonal antibody against the human receptor that would cross-react with the canine receptor. **Methods.** A 15-amino acid peptide spanning from residue 86 to 101 and located within D1 region of the extracellular portion of human IL-13Rα2 was synthesized as immunogen for the study. The peptide has 100% homology between human and canine receptors. A standard protocol was used for generation and purification of monoclonal antibody. Selected clone, 13R2.C3, was used for Western blot analysis of human and canine tumor lysates.Results. 13R2.C3 antibody revealed a single protein band of expected molecular size for IL-13Rα2 in Western blots of cell and tissue lysates. IL-13Rα2 protein was readily detected in human GBM with 9/14 showing high and 4/14 moderate over-expression. This is higher than seen in studies using a polyclonal antibody (CCR 14:199-208, 2008). All (6/6) canine GBMs tumor were positive for IL13Rα2 with 50% exhibiting high over-expression. Human and canine astrocytomas and oligodendrogliomas were all positive for IL-13Rα2 to various degrees. Interestingly, human meningiomas exhibited strong positivity (5/6). In addition, IL-13Rα2 was highly expressed in canine choroid plexus papillomas. Normal human and canine brain samples were negative for IL-13Rα2 with 13R2.C3 antibody. **Conclusions.** We have obtained a monoclonal antibody against IL-13Rα2 cross-reacting with human and canine receptors. 13R2.C3 antibody detects immunoreactive IL-13Rα2 in up to 90% of human GBM. In addition, other brain tumors, such as high grade oligodendrogliomas, meningiomas and canine choroid plexus papillomas, appear to express the receptor at high levels and thus may be appropriate candidates for IL-13Rα2-targeted therapies.

P269

SYNERGISTIC EFFECT OF HUMAN IFN-BETA AND TMZ ON MALIGNANT GLIOMA CELL LINES

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IFN-β is known to display pleiotrophic biological activities including antitumor effects. On the other hand, TMZ has demonstrated efficacy and become a key agent in patients with malignant gliomas; however, its survival benefit remains unsatisfactory. More recent studies have indicated that there might be favorable therapeutic interactions between IFN-β and TMZ, although the therapeutic advantages of such a combination have not yet been fully explored. The aim of the present study was to elucidate whether an antitumor effect could be potentiated by a combination of human IFN-β and TMZ.The antitumor effect of and cell sensitivity to IFN-β and TMZ, and the synergistic potential of IFN-β and TMZ in combination, were evaluated in malignant glioma cell lines. The correlations among the MGMT methylation status, quantitative level of MGMT mRNA, MGMT protein expression, and antitumor effect of these agents were also evaluated, since one of the most prominent resistance mechanisms to TMZ involves MGMT.Cell growth inhibitory effects of IFN-β and TMZ on all tumor cell lines were observed in a dose-dependent manner, and the human malignant glioma derived cell lines differed in their sensitivity to TMZ. MGMT status, including promoter hypermethylation, quantitative mRNA expression, and protein expression, was strongly correlated with TMZ sensitivity. A synergistic cell growth inhibitory effect and down-regulated MGMT mRNA levels were significantly observed when a clinically achievable CNS dose of IFN-β was combined with TMZ, as compared to treatment with IFN-β or TMZ alone in TMZ resistant T98G cells.These results suggest that the clinical therapeutic efficacy of TMZ might be improved by combination with IFN-β in malignant gliomas unmethylated at the MGMT gene. The data provide an experimental basis for future strategies in TMZ chemotherapy, although further studies are needed to determine the detailed role of combined IFN-β and TMZ chemotherapy in increasing tumor sensitivity.

P270

THE ROLE OF CHEMOKINE SDF-1/CXCR4/CXCR7 FOR GLIOBLASTOMA ANGIOGENESIS AND GLIOBLASTOMA DERIVED ENDOTHELIAL CELLS

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Glioma cells and tumor endothelial cells (ECs) in hypoxic area obtain prominent angiogenesis and invasiveness, resulting in resistance to radiation and chemotherapy. We investigate the role of SDF-1α/CXCR4 and CXCR7 in glioma angiogenesis and invasiveness both for tumor cells and tumor derived ECs under normoxic and hypoxic condition in order to suggest new treatment strategy for gliomas.Immunohistochemical detection of SDF-1, CXCR4, CXCR7 were obtained in 54 astrocytic tumors. SDF-1 was strongly positive in high grade tumor cells and endothelial cells and CXCR7 was constantly positive in high grade endothelial cells. Four ECs from 13 glioblastoma patients were isolated and cultured with endothelial growth medium (GBMEC-1, 3, 7, 8). SDF-1α/CXCR4/CXCR7 mRNA expression in vitro were different between normal (HUVEC) and glioma derived ECs (GBMEC). GBMEC showed strong expression of VEGF, up-regulation of SDF-1α; expression with hypoxia, no expression of CXCR4, and constant expression of CXCR7. GBMECs that secreted a large amount of SDF-1 into the conditioned medium migrated well and their tube formation was inhibited by AMD3100. Also glioma cell expressed SDF-1 under hypoxic condition and their invasiveness was inhibited by AMD3100. In conclusion, SDF-1α/CXCR4/CXCR7 were key target molecules in glioma angiogenesis and invasiveness. The characteristics of glioma derived ECs were completely different from those of normal ECs. GBMECs secrete SDF-1 and VEGF, resulting enhancement of autocrine angiogenesis through VEGFR and CXCR7 as well as paracrine glioma invasiveness. In order to inhibit glioma angiogenesis and invasiveness, glioma derived ECs should be targeted, for example by antagonizing SDF-1α or CXCR7 expression.

P271

SOLUBLE CD70 CONFERS AN IMMUNE-MEDIATED SURVIVAL ADVANTAGE IN EXPERIMENTAL GLIOBLASTOMA

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Purpose of the study: Expression of CD70 on the surface of glioma cells was shown to induce a potent anti-tumor immune response in a murine glioma model in vivo. There is evidence that also the soluble form of CD70 is active in vivo. A soluble co-stimulatory ligand is attractive as it might have a wider distribution into the tissue surrounding the tumor in vivo. **Methods:** The murine glioma cell line SMA-560 was engineered to produce a soluble from of CD70 (sCD70). The expression of sCD70 was confirmed by immunoblot.. Markers of immune cells were assessed by flow cytometry. The proliferation of immune cells was investigated in a ^{3}H-Thymidine assay. The secretion of IL-2 and IFN-γ was assessed by ELISA. SMA-560 cells were implanted stereotactically in the right striatum of syngeneic VM/Dk mice. The infiltration of immune cells in gliomas was assessed by immunohistochemistry. CD8+ or NK cells were depleted by the injection of appropriate antibodies intraperitoneally. **Results:** In vitro expression of sCD70 enhances proliferation of syngeneic splenocytes and their secretion of IL-2 and IFN-γ. In syngeneic VM/Dk mice bearing intracranial gliomas, sCD70 enhances the survival rate. The infiltration of CD8+ cells is enhanced compared to controls. Depletion of CD8+ cells abrogates the survival advantage. **Conclusion:** These data suggest that sCD70 is a potent stimulator of anti-glioma immune response with cytotoxic CD8+ T-cells playing a major role.

P272

ABERRANT HYPERMETHYLATION OF CPG ISLANDS PROFILE OF CELL CYCLE REGULATORY GENES IN MALIGNANT ASTROCYTOMAS

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Purpose: Aberrant hypermethylation of CpG islands in the promoter region plays a casual role in the inactivation of various key genes involved cell cycle regulatory cascade, which could result in loss of cell cycle control. The aim of the present study was to investigate the role of promoter methylation of genes with a proven role of the cell cycle regulation in malignant astrocytomas. **Experimental Design:** We profiled the CpG island methylation status of *RB1, p14ARF, p15INK4b, p16INK4a, p21Waf1/Cip1, p27Kip1,* and *p73* genes by methylation-specific polymerase chain reaction assay in a homogeneous cohort of patients with malignant astrocytomas, and assessed their relationships with clinical behavior. **Results:** Promoter hypermethylation of the *RB1, p14ARF, p15INK4b, p16INK4a, p21Waf1/Cip1, p27Kip1,* and *p73* genes was detected in 4 (4%), 3 (7%), 2 (5%), 6 (5%), 6 (8%), 7 (7%), and 11 samples (22%) of the 53 newly diagnosed malignant astrocytomas, respectively. A total of 50% of the cases carried methylation of at least one gene, and only 20% of the cases displayed concordant hypermethylation of two gene. None of the tumors disclosed three or more methylated loci. The presence of methylation of these genes or a group of genes was not associated with any distinct clinicopathological characteristics including tumor grade, proliferation activity, responsiveness to adjuvant therapy, and patient survival. p73 protein accumulation examined by immunohistochemical staining was found in 6 (23%) of the 40 samples examined, with no significant association with the methylation status of *p73* and any of the clinicopathological parameters tested. **Conclusions:** Aberrant hypermethylation of the key cell cycle regulatory genes occurs at a relatively high frequency in malignant astrocytomas, independent of each other methylation and clinicopathological parameters. This epigenetic change may be an important early event during the pathogenesis of malignant astrocytomas.

P273

AN IN VITRO STUDY OF SELECTED SYNTHETIC CHALCONE ANALOGUES ACTIVITY AGAINST BEN-MEN-1 MENINGIOMA CELL LINE

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Introduction: Ben-Men-1 is a human benign meningioma cell line established from meningothelial meningioma cells immortalised by retroviral transduction of hTERT. The cells display monosomy 22 typical for WHO gr.I tumours. Despite their benign nature, 20-25% of these neoplasms recur within 10 years after initial therapy. This fact inspired authors to study potential anti-meningioma effects of selected synthetic analogues of chalcones, intermediates in the biosynthesis of flavonoids with relatively well-characterised anticancer properties. **Material and Methods:** Cytotoxic effects of Q-510 (E-2-(4-methoxybenzylidene)-1-benzosuberone), Q-705 (E-2-(2,4-dimethoxybenzylidene)-1-tetralone and Q-766 (E-2-(4-hydroxybenzylidene)-1-benzosuberone) on Ben-Men-1 cells were assessed using the colorimetric MTT assay. Apoptotic activities of the tested agents were analysed by flow cytometry using Annexin V/PI staining and DNA cell cycle analysis. **Results:** Of the tested chalcone analogues, Q-510 displayed a significant cytotoxic effect on Ben-Men-1 cells in a concentration-dependent manner with an EC50 of 2.682 ± 1.999 microM. At 1 microM, Q-510 induced apoptosis in Ben-Men-1 cells increasing significantly the percentage of both apoptotic (AnnexinV+/PI-) and necrotic (AnnexinV+/PI+) cells from 1.0 ± 0.4% to 7.6 ± 2.6% and from 2.5 ± 0.9% to 4.3 ± 0.5% in untreated vs. treated cells, respectively. A similar increase was also observed in the percentage of sub-G0/G1 cells: from 2.6 ± 3.4% in untreated cells to 21.3 &plusmn 9.7% in cells treated with 1 microM Q-510 for 72 hours. For Q-705 and Q-766, no cytotoxic or apoptotic activities against meningioma cells were observed. **Conclusion:** Q-510, a synthetic chalcone analogue, possesses a significant anti-meningioma activity at micromolar concentrations. Further studies are necessary to elucidate its mechanism of action as well as its potential utility in the treatment of recurring benign or anaplastic/malignant meningiomas.Acknowledgement: This work was supported by the Slovak Research and Development Agency under the contract No. APVT-20-032504, APVV-20-052005 and VEGA grant 1/3361/06.

P274

NEW CCM1 GENE MUTATIONS IN CHINESE SPORADIC INTRACRANIAL CAVERNOUS ANGIOMA PATIENTS

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Objectives: To detect and analyze the CCM1 gene (7q11.2-q22) mutations in Chinese sporadic intracranial Cavernous Angioma (CA) patients which may be the cause of CA occurring. **Methods:** The sporadic CA group included nineteen unrelated patients of Han people with CA confirmed by postoperation pathology and treated at Department of Neurosurgery, HuaShan Hospital, FuDan University during 2005-2007, and the study was approved by the local ethics committee. Thirty healthy people were enrolled as the control group. Genomic DNA was extracted from the peripheral blood. Exon 8, 9, 11, 12, 13, 15, 16, 17 and 18 of CCM1 and part of intervening sequences near both sides of these exons were amplified by PCR. The PCR products were sequenced directly and then compared with reference sequences from GeneBank. **Results:** Four exclusive mutation sites of CCM1 gene were detected from 11 Chinese sporadic CA patients. Total mutational rate of CCM1 was 31.6/100. Among the four exclusive mutations, there were one missense mutation: exon12, 1172C to T(S391F), one insertion mutation: exon8, 704insT(K246stop), one intervening sequence mutation: IVS12-4C to T, and one synonymous mutation: 1875C to T(F625F). No any mutations were detected in the control group. **Conclusion:** There are CCM1 gene mutations in Chinese sporadic CA, which lead to functional loss or changes of the encoding KRIT1 protein and are related with the sporadic CA occurring.

P275

THE FUNCTIONAL ROLE OF EIF4E IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background and objects
Primary central nervous system malignant lymphoma (PCNSL) is defined as a diffuse lymphoma presenting in the brain or spinal cord without systemic lymphoma. Although the incidence of PCNSL has increased among both immunocompetent and immunodeficient patients, the pathogenesis and histogenetic origin of PCNSL is poorly understood. The mRNA cap-binding protein, eukaryotic initiation factor 4E (eIF4E), plays an important role in mRNA translation and its activity is implicated in cell growth and proliferation. Increased eIF4E expression has been associated with tumor formation and progression in human malignancies including leukemias, lymphomas and several types of cancer. Moreover, eIF4E is known to be activated by MAP kinase-interacting kinase-1 (Mnk-1), which attracts the attention as a molecule related to cell proliferation. Therefore, we investigated the participation of eIF4E in PCNSL.

Methods
The expression of eIF4E in the specimens from PCNSL was examined by immunohistochemical analysis using anti-eIF4E and anti-phospho-eIF4E Ab. In order to examine the physiological role of eIF4E in PCNSL cells, we used a cell line derived from human brain malignant lymphoma (HKBML).

Results
(1) We observed the overexpression of eIF4E and phosphorylated eIF4E in the specimens from the PCNSL patients. (2) Western blotting analysis showed that eIF4E was activated in HKBML, (3) the activation of eIF4E wasn't inhibited by the inhibitors of MAPK (PD98059) and p38MAPK (SB203580). However, Mnk-1 inhibitor (CGP57380) could inhibit the activation of eIF4E. (4) Furthermore, CGP57380 inhibited the cell proliferation and induced a G1 arrest and apotosis in HKBML cells.

Conclusions
These data suggest that Mnk-1/eIF4E pathway plays an important role in pathogenesis of PCNSL. The inhibition of the signal pathway may provide a basis for developing a novel therapeutic approach in PCNSL.

P276

IS IT TOTAL REMOVAL OF GLIOBLASTOMA POSSIBLE? - TUMOR CELL INFILTRATION FROM AUTOPSY BRAIN

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Even if glioblastoma can do total extirpation by an operation macroscopically, it recurs by all means. **[Materials and methods]** We reviewed an infiltrate of tumor cells from autopsy brain in a tumor peripheral zone. We compared it with an image from autopsy brain of 6 examples and reviewed cell infiltration by mapping of tumor cells by form and FCM. Autopsy brain did a horizontal plane in 10mm slice in parallel with OM line and compared it with an image (CT, MRI). The other side made a graft to the homonymous brain surface mainly on a tumor, too, and the autopsy brain dyed HE and VEGF. We compare a peripheral zone from a tumor edge contrasted from an image by contrast media with autopsy brain, and it is number of the cellular infiltrate (A: 100-60%, B: 60-20%, C: We measured less than 20%) and, from autopsy brain (3 examples) at the time of autopsy, obtained tissue according to a part of a tumor and analyzed DNA contents by FCM. **[Results]** Autopsy brain was easy to grasp the tumor more macroscopically than fixed autopsy brain. (1)In MRI, an infiltrate of tumor cells was present in high signal with brain edema (2)It extended over 3-13mm from tumor border, and the tumor cells of an A domain accepted an infiltrate. (3)The tumor had few infiltrates so that in T2 WI hyperintense area around a tumor was small. (4)The DNA index, the proliferating index related to morphologic cell infiltration from tumor border by FCM in a peripheral zone. **[Conclusion]** In an operation of glioblastoma, it was thought that we were insufficient in macroscopical total extirpation of tumor contrast enhanced area in MRI.

P277

CLINICAL SIGNIFICANCE OF VASCULOGENIC MIMICRY IN HUMAN GLIOMAS

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Introduction: Vasculogenic mimicry (VM) is a newly recongnized phenomina in many malignancies. However, we do not know its clinical significance in gliomas. This study was designed to investigate VM in gliomas and compare clinical data of the patients. **Methods:** One hundred and nine glioma specimens were collected for immunohistochemical staining of CD34 and PAS staining (dual staining) to verify existing of VM. The clinical data of those glioma patients were collected and analyzed with existence of VM. **Results:** The VM has been considered for those stained positively for PAS, but negatively for endothelial marker-CD34. There were 13 out of the 109 specimens found VM phenotype (11.9%). Most of the VM were found in higher grade gliomas: 8 of 27 grade IV(29.6%), 3 of 41 grade III (7.3%), and 2 of 36 grade II(5.5%). While there was no VM found in grade I gliomas. The higher grade gliomas had higher incidence of VM than that of lower grade gliomas (P=0.012). There was no any association between existence of VM and the sex, age and preoperative epilepsy of the patients. However, the patients, who's tumor with VM, survived short than that of without VM (P=0.031). **Conclusions:** The VM exists in gliomas, and the higher grade gliomas exhibit more VM than the lower grade gliomas. The glioma patients with VM may have poorer prognosis.Key words: Glioma, Vasculogenic mimicry

P278

VALIDATION OF PROGNOSTIC AND THERAPEUTIC WORTH OF MOLECULAR MARKERS BY ROUTINE TOOLS IN DIAGNOSTIC AND CLINICAL NEUROPATHOLOGY: A STUDY OF 193 GLIOBLASTOMAS.

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The application of molecular biology technologies to cerebral tumours have permitted the identification of genetic and molecular alterations specific for some oncotypes, allowing indications on tumour biological behaviour and to suggest new therapeutic strategies. This issue has changed the clinical management of the patients and has required the implementation of some biomarkers to routine laboratory. In order to give early information about biomarkers, aim of this study was to verify the transferability of molecular biology data to routine diagnosis performing an immunohistochemical study on surgical specimens from 193 glioblastoma patients: 64 female, 129 male with median age 58 years. The results were related to the clinical data obtained from the Tumour Register and, in some cases, compared to the molecular biology tests. The specimens were fixed in Carnoy alcohol-based fixative, processed for histology and immunhistochemistry for EGFR, P53, MGMT, PTEN and YKL-40. Immunohistochemical results showed a different expression pattern: PTEN was positive in 58% of cases, EGFR in 32%, p53 in 37% and YKL-40 in 60%. A correlation of EGFR and YKL-40 expression to shorter time to tumour progression was found. MGMT was considered as positive when more than 20% of neoplastic cells were immunostained: 65% of glioblastomas showed positivity. A correlation to molecular data by methylation-specific PCR was assessed on 100 cases with 90% of correspondence. The immunohistochemical profile of diagnostic, prognostic and predictive biomarkers in glioblastoma patients could be, with adequate technical handling and knowledge, a useful, easy and fast tool for pathologist in routine clinical practice, supporting oncologists to make treatment decisions.

P279

OLIGODENDROGLIAL TUMOR CLASSIFICATION: PHENOTYPE VS GENETIC SIGNATURE

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PURPOSE: Oligodendroglial Tumors (OT) are constituted by Oligodendrogliomas or pure tumors (OD), and Oligoastrocytomas or mixed tumors (OA) and their respective anaplastic grades. Oligodendrogliomas are one of the most chemosensitive solid tumors and loss of chromosome (LOH) 1p is tightly associated with response to chemotherapy.We have previously developed a molecular classification for oligodendroglial tumors and the purpose of this study was to compare it to a histology-based classification. **METHODS:** Microarray analysis was used to study a set of 19 OD and 10 OA. Supervised learning approaches were used to build a two-class prediction model based on the histological class. We performed an evaluation of 3 algorithms (DLDA, 1-NN, and PAM) and 8 different prediction models were built in each one (2, 5, 10, 20, 35, 50, 75, 100 features). The training error of this prediction models were determined using CV-10, and LOO. Finally we selected the best number of genes that result in the smallest cross-validation error. **RESULTS:** No Gene-Ontology based functional enrichment was found in 94 more significant and differentially expressed genes among defined histological classes. We identified 72 features frequently used by predictors. To assess the usefulness of both classifiers in terms of prognosis, we next performed a supervised analysis of genes involved in chemoresistance; groups defined by molecular classification showed differences in the expression of this set of genes which could not be detected considering histological classes. A similar result was obtained when the expression of several genes linked to proliferation and stemness was inspected. **CONCLUSION:** More functionally significant and differentially expressed genes were detected among molecular status than defined histological classes. Gene expression profile was decisively conditioned by 1p/19q allelic deletions. Molecular predictors seem to be more efficient in prognosis and could be complementary to pathological diagnosis.

P280

HISTOLOGIC ANAPLASIA IN SPORADIC AND NF1-ASSOCIATED PILOCYTIC ASTROCYTOMAS PREDICTS UNFAVORABLE BEHAVIOR

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The clinical significance of histologic anaplasia in pilocytic astrocytoma (PA), a rare event, is not fully established. We reviewed 37 histologically anaplastic PA among approximately 2,200 PA cases from the Mayo Clinic file (1.7%), including 22 males and 15 females, with a median age of 36 years (range 5-75). All tumors had either previously documented (n=10) (27%) or coexistent PA precursors (n=17)(46%), or exhibited typical pilocytic features in an otherwise anaplastic astrocytoma (n=10)(27%). Most were located in the cerebellum (54%). Clinical features of NF1 were present in 22%, and a history of radiation for a PA precursor in 11%. Histologically, the anaplastic components were classified as pilocytic-like (38%), small cell (35%), fibrillary (14%) or epithelioid in appearance (14%). Median overall and progression-free survival for the whole group was 24 and 14 months, respectively. Overall and progression-free survival was shorter when there was a history of radiation (p=0.0069, 0.0283), increasing levels of mitotic activity (p=0.0344, 0.0188), and necrosis (p=0.0226, 0.0242). When compared to historical cohorts of St. Anne-Mayo graded infiltrating astrocytomas, overall survival rates of anaplastic PA was similar to that of infiltrative astrocytomas grade 2 and 3, depending on the absence/presence of necrosis respectively. In summary, anaplastic PA exhibits a spectrum of morphologies and is associated with decreased survival compared to typical PA.

P281

APPLICATION OF A CYTOGENETC PROGRESSION SCORE (GPS), TUMOR LOCATION AND GENETIC PROGRESSION IN MENINGIOMAS: HACETTEPE EXPERIENCE

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Purpose of the study: Meningioma is a common intracranial tumor. Monosomy 22 or loss of 22q are the most frequently and early detected abnormality, especially in WHO Grade I tumors. Partial or complete loss of chromosome1 or 1p has been suggested as a significant event in the initiation and progression of meningiomas. Recently, a sophisticated cytogenetic progression model for meningiomas and the relationship between tumor location and genetic progression has been proposed. According to genetic progression score (GPS), in the Group O, no monosomy 22 was detected. Monosomy 22 was observed in the Group I and lost of chromosome 1p was also found in the Group 2 as well as monosomy 22. We investigated the karyotypes associted with multiple and atypical/malignant meningiomas. **Methods:** Tissue cultures from meningioma biopsies and chromosome preparations with Giemsa banding were carried out according to standard procedure. **Results:** None of the patients had NF2. One of two grade II chondroid meningioma was GPS Group 2 (monosomy 22 and loss of 1p). Other WHO Grade II had 46 XX (GPS Group 0). A multiple meningioma patient had monosomy 22 (GPS group I) although the lesion was graded as WHO Grade I. Two giant meningiomas (parafalcine and tentorial) with atypical radiological features showed multiplex trisomy (GPS group 0) and monosomy 22 (GPS group I), respectively. **Conclusion:** The GPS as a quantitative measure allow us for a more precise assesment of the prognosis of menigiomas than classical cytogenetic markers.

P282

TARGETING THE TUMOR STROMA A NOVEL THERAPEUTIC STRATEGY BASED ON SEPARATE ANALYSIS OF THE MALIGNANT AND STROMAL CELL COMPARTMENTS IN BRAIN TUMORS

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The recruitment of host vasculature and the infiltrative behaviour of gliomas underscore the significance of tumor-stroma interactions in brain tumor pathogenesis. The aim of this project is to identify cancer-related changes in the stroma during brain tumor progression that can be targeted therapeutically. However, targeting tumor-activated stromal cells require further insight into the mechanisms that regulate the tumor-stroma interplay. Since, any tumor biopsy contains a mixture of cancer cells and stromal cells, we are unable to determine whether a given gene expression profile or protein signature is derived from stromal or cancer cells. For the same reason, we are also unable to specify the directions of cross-talk between compartments; whether an influence is excerted upon the tumor by the surrounding stroma, or vice versa. In this project, we have generated a green fluorescent protein (GFP)-expressing on the nude rat by crossing nude rat with a transgenic GFP-expressing line. We implant human glioma biopsies in green-fluorescent (GFP) immunodeficient rats. The resulting xenograft tumors are dissociated into a cell suspension and FACS-sorted into GFP-positive stromal cells and GFP-negative tumor cells. We also obtained cell suspensions of stromal cells from normal brain. Human specific nuclei antibody staining has confirmed that sufficient purity of the sorted cells. Using this tool, we intend to delineate the gene expression profiles and protein signatures unique to the tumor-activated stromal cells. This information will subsequently be used to tailor drug regimens that target tumor-activated stroma and tumor-stroma interactions.

P283

THERAPEUTIC EFFICACY OF A POLYMERIC MICELLAR DOXORUBICIN INFUSED BY CONVECTION-ENHANCED DELIVERY AGAINST INTRACRANIAL 9L BRAIN TUMOR MODELS

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Convection-enhanced delivery (CED) with various drug carrier systems has recently emerged as a novel chemotherapeutic method to overcome the problems of current chemotherapies against brain tumors. Polymeric micelle systems have exhibited dramatically higher in vivo antitumor activity in systemic administration. This study investigated the effectiveness of CED with polymeric micellar doxorubicin (micellar DOX) in a 9L syngeneic rat model. Distribution, toxicity, and efficacy of free, liposomal, and micellar DOX infused by CED were evaluated. Micellar DOX achieved much wider distribution in brain tumor tissue and surrounding normal brain tissue compared to free DOX. Tissue toxicity increased at higher doses, but rats treated with micellar DOX showed no abnormal neurological symptoms at any dose tested (0.1-1.0 mg/mL). Micellar DOX infused by CED resulted in prolonged median survival (36 days) compared to free DOX (19 days; P=0.0173) and liposomal DOX (16 days; P=0.0007) at the same dose (0.2 mg/mL). This study indicates the potential of CED with the polymeric micelle drug carrier system for the treatment of brain tumors.

P284

LABEL-FREE DIFFERENTIATION OF INDIVIDUAL HUMAN GLIOMA CELLS BY OPTICAL FTIR SPECTROSCOPIC IMAGING

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The discrimination of cell types at different stages of development is a crucial task in cell biology. Available techniques, based on an irreversible treatment of the cells, do not allow a sensitive label free characterization under in situ conditions. The purpose of this study is to evaluate the ability of infrared spectroscopic imaging (IRSpec) to differentiate individual cells between different glioma cell populations. **Methods :** Monolayers of cultivated U343, T1115 and T508 human glioma cells were characterized using IRSpec. A classification algorithm based on linear discriminant analysis was developed to distinguish different cells without labeling. The classification is based upon specific spectral patterning. **Results :** An accuracy of 91% and 84% was obtained for U343 and T1115 cells. T508 cells exhibit some misclassifications resulting in a lower accuracy rate of 73%. However, even different mixtures of these cell types were recognized and correctly classified. **Conclusions :** As demonstrated, the potential of IRSpec to assess the overall molecular composition of cells in a non-destructive manner opens the possibility to characterize cells on a molecular level without labels or an irreversible treatment. Many different aspects with regards to treatment response and differentiation are now open for nondestructive analysis.

P285

APPLICATION OF MULTIPLE-STAGE TANDEM IMAGING MASS SPECTROMETRY TO THE EVALUATION OF LIPIDS IN A RAT BRAIN C6 GLIOMA MODEL

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Background: Direct molecular analysis of biological tissue using mass spectrometry (MS) is a subject of much interest in the field of metabolomics. Furthermore, MS on 2D samples, also known as is imaging mass spectrometry (IMS) allows visualization of molecular distributions on a tissue surface. This technology has been developed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometers (MALDI-TOF MS). Lipids can perform both structural and functional roles within the body and are known to be important mediators of cell signaling, acting as second messengers in cellular events. Many previous experiments using IMS to image samples have focused on measuring the distribution of elements and small molecules in normal rat brain sections.

Purpose: This study focuses on mapping the lipid distribution in rat brain tissue sections of a C6 glioma model, for comparison with the normal cerebral area.

Methods: $1x10^5$ C6 glioma cells were infused into the brains of Sprague-Dawley rats. The rats were killed on day 21, and their brains were removed and sliced at a 7μm thickness, then placed directly onto plate inserts. MS and IMS analyses were acquired using MALDI-TOF MS (QSTAR XL, Applied Biosystems) in positive ion mode.

Results: Relative intensities of the ion signal of *m/z* 798.5 [phosphatidylcholine (PC) 16:0/18:1 + K]$^+$ were reduced in the tumor area as compared with the normal cerebral area. However, relative intensities of the ion signal of *m/z* 741.4 [sphingomyelin (SM) d18:1/16:0 + K]$^+$ were increased in the tumor area. The decreased PC ion signal in the tumor area suggests the collapse of normal tissue while the increased SM ion signal is regarded as having a tumor cell origin.

Conclusions: Direct molecular analysis using IMS in the rat brain C6 glioma model was performed. The results raise possibility of analyzing brain tumor tissues using this novel approach combining IMS with other imaging methods.

P286

SPECTROSCOPIC IMAGING OF CEREBRAL MICROMETASTASES IN A MURINE MODEL

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Optical spectroscopic imaging has the potential to be introduced into the surgical microscope. Both Raman and Fourier-transformed infrared spectroscopy (RSpec and IRSpec) detect the complete biochemical information. The purpose of this study was to evaluate a murine model of brain metastases with regards to morphological mapping and detection of metastases. **Methods** Hematogenous brain metastases of a malignant melanoma cell line were induced in one brain hemisphere while the other hemisphere remained tumor free. Dried, thin sections for IRSpec, hematoxylin and eosin-stained thin sections for histopathological assessment, and pristine, 2-mm thick sections for RSpec were prepared. IRSpec images were recorded using a multi-channel detector. Raman maps were collected serially using a spectrometer coupled to a fiber-optic probe. The images/maps were segmented by cluster analysis. **Results** The clusters coincided well with the morphology of mouse brains in stained tissue sections. More details in less time were resolved with IRSPec (25 μm resolution) than RSpec (60 µm). The spectral contributions of melanin in tumor cells were resonance enhanced in RSpec which enabled their sensitive detection in Raman maps. **Conclusions** FTIR and Raman spectrsocopic imaging have sensitive mapping capabilities but reveal different specificities to detect metastatic melanoma cells in murine brain.

P287

SIGNIFICANCE OF NMR BASED METABOLOMICS (METABOLOME) FOR THE PREDICTION OF MALIGNANT TYPE MENINGIOMA USING THE ANALYSIS OF WATER AND LIPID-SOLUBLE METABOLITES

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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 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 ^{1}H-NMR spectra. And 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 two 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 2/3 domain. Lipid-soluble metabolites: Malignant type meningiomas were distributed near location in the grade 3 domain. However, grade 2 domain was isolated.[Conclusion] This study suggests that NMR based metabolomics are very useful for prediction of malignant type meningiomas which were histologically benign.

P288

ROLE OF PROTEOMIC STUDY OF CSF AS A DIAGNOSTIC TOOL IN THE PATIENTS WITH MENINGIOMAS

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Object: Meningiomas are common intracranial tumors and have been considered as benign because of their slow growth rates and the feasibility of surgical cure and meningiomas represent the second most common central nervous system neoplasms in adults and account for 26% of all primary brain tumors. Even Most meningiomas can be graded into WHO grade 1 some of them are classified atypical(grade 2), and anaplastic meningiomas(grade 3). Cerebrospinal fluid contains a rich proteins source of biomarker and has a potential to be used as diagnostic and prognostic indicators for brain tumors. We tried to evaluate possibility of new diagnostic tools from the CSF in patients with meningioma. **Methods:** Proteome profiling patterns were compared in 10 human CSF samples: 5 meningiomas, 5 non-brain tumors CSF. We could completely differentiate specific proteins in CSF associated with human meningioma using the 2-D difference gel electrophoresis analysis and thereafter identified specific proteins by mass spectrometry for ESI-Q-TOF. These proteins were verified with western blotting and immunohistochemistry method. **Results:** There were 10 proteins more than 2-folds differentially expressed between meningioma and non-brain tumor including abundant proteins. 7 proteins (Apo E, Apo J, AAT) were increased in meningoma whereas 3 proteins (PTGDs, TTR, beta-2-microglobulin) were decreased. Through the western blotting method and immunohistochemistry we identified similar pattern with 2-dimensional electrophoresis. **Conclusions:** The goal of our study was to identify for the first time, from the CSF of patients with meningioma by 2-DE method and resulting pattern was confirmed by using western blotting and IHC. These proteins showed the possibility for candidate biomarker for meningoma. These results may provide useful information with respect to preclinical research of diagnostic tools for brain tumor.

P289

IL-6 IN MENINGIOMA; EXPRESSION IS CORRELATED WITH PERITUMORAL EDEMA AND VEGF

Cancelled

P290

ANTITUMOR EFFECT OF HUMANIZED ANTI-IL-6 RECEPTOR ANTIBODY (TOCILIZUMAB) ON GLIOMA CELL PROLIFERATION

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Objective: Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates diverse physiological functions, including cell proliferation and survival. Recent studies showed that IL-6 expression was often elevated in response to several types of cancers and tumors, including glioma. Although IL-6 is known to play an important role in glioma, the involvement of IL-6 signaling has been quite controversial. The aim of this study was to evaluate the involvement of IL-6 signaling in glioma and the inhibitory effect of IL-6 signaling on glioma tumor proliferation. **Methods:** The expression of IL-6 receptor (IL-6R) was evaluated in glioma tissues by immunohistochemistry and also the involvement of IL-6 signaling in glioblastoma U87MG cell proliferation was determined. In addition, to examine the inhibitory effect of IL-6 signaling on glioma cell proliferation, we investigated the effects of IL-6- and IL-6R-specific siRNA and AG490, a specific inhibitor of JAK2 phosphorylation in U87MG cells. Furthermore, we investigated the effects of tocilizumab, a clinically developed humanized anti-human IL-6 receptor antibody in U87MG cells. **Results:** Increased immunoreactivity for IL-6R was predominantly found in the cytoplasm of endothelial cells in all glioblastoma samples. Inhibition of IL-6 signaling by IL-6 and IL-6R -specific siRNA and AG490 suppressed glioma cell proliferation, respectively. Furthermore, tocilizumab exerted the anti-proliferative effect on glioblastoma cell line U87MG via IL-6R-dependent JAK-STAT3 pathway. **Conclusions:** IL-6 signaling pathway plays an important role in glioma cell proliferation, and that tocilizumab exerts anti-tumor effect in U87MG glioma cells. These results may bring a new insight into molecular pathogenesis of glioma and may lead to a new therapeutic intervention.

P291

THE PLASMA CONCENTRATION OF ET-1 IN THE BRAIN TUMORS

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ET-1is produced by a large variety of cells from a wide rang of mammalian species, and has been identified in plasma. The difference between plasma concentrations of ET-1 in prostate cancer patients, as compared to those without prostate cancer has been reported. To our knowledge, the plasma concentrations of ET-1 in different types of brain tumor have not been reported. If the different types of brain tumor express significantly various concentration of plasma ET-1, endothelin receptor antagonists can be administered to inhibit proliferation of tumor cells. Therefore in this study, we test the difference of serum concentrations of ET-1 in brain tumor.The plasma concentrations of ET-1 in different brain tumors were as following: control: 8.0 ±1.953 pg/ml, meningioma: 8.8 ± 2.521 pg/ml, brain metastasis: 8.6 ± 3.098 pg/ml, glioblastoma multiforme: 5.0 ± 2.133 pg/ml, and pituitary adenoma: 8.4 ± 1.980 pg/ml. There were no significant differences among the subtypes of meningioma. Among the brain metastasis group, the plasma concentration of ET-1 of the lung carcinoma with brain metastasis showed high level concentration (9.1 ± 3.650 pg/ml). There were no significant differences between the subgroup of metastasis except the colon carcinoma. The concentration of ET-1 in the meningioma and brain metastasis group is significantly higher than that of glioblastoma multiforme group($p<0.001$). The cause of different plasma concentration of ET-1 may be the different blood supply system. Most meningiomas are feeded from extracranial blood system and brain metastasis is seeded from systemic blood supply. The glioblastoma multiforme is feeded via local brain blood supply.

P292

PLASMA IG E LEVELS CORRELATE WITH DIAGNOSIS AND RESPONSE TO THERAPY IN GLIOMA PATIENTS

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Objective: To determine the plasma IgE levels in different grades of glioma patients and its relationship with therapy response. **Methods:** Enzyme-linked immunosorbance assay (ELISA) was employed to determine the plasma IgE levels of 25 normal subjects and 155 glioma patients (including 52 grade II glioma patients, 31 grade III glioma patients and 72 GBM patients).We also compared the plasma IgE levels of 30 GBM patients who are receiving chemotherapy after operation without signs of recurrence and 16 GBM patients who have a recurred disease after operation. **Results:** Plasma IgE level in normal subjects and glioma patients were 429.3+/-162.9ng/ml and 320.9+/-193.2ng/ml respectively. Plasma IgE level was significantly lower in glioma patients($t=2.60, p=0.01$). Plasma IgE level in the low-grade glioma patients (269.6+/-203.2ng/ml) was significantly lower than that in the high-grade glioma patients(grade III and GBM,346.8+/-183.6ng/ml)($t=2.39, p=0.018$). No significant differences were observed from the plasma IgE levels within the high-grade glioma patients($p=0.74$). Plasma IgE increased($t=4.92, p<0.001$) after successful removal of the tumor(572.7+/-249.0ng/ml).But did not decrease after recurrence($p=0.903$). **Conclusions:** Plasma IgE levels of the glioma patients especially the low-grade glioma patients were significantly lower than the normal subjucets. Plasma IgE levels increased after successful tumor removal in the high-grade glioma patients.

P293

DELETION OF PTEN IN CLIVAL CHORDOMAS

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PURPOSE: Chordoma is a bone cancer restricted to the axial skeleton and believed to arise from the remnants of the notochord. Clinical disease recurrence is characterized by invasion into surrounding structures by incompletely removed cancer cells. Accepted treatment regimen consists of surgery followed by radiation therapy. A phase II clinical trial revealed that use of imatinib may be warranted in chordomas with activated platelet-derived growth factor receptor (PDGFR). Because this clinical experience and our in vitro observations demonstrated inconsistent response to PDGFR inhibition, we investigated the downstream signaling pathway in chordoma cells. **METHODS:** We performed detailed molecular pathologic analyses on 15 chordoma specimens from 12 patients, and established primary cultures from acutely resected chordoma tissue for in vitro experiments. DNA isolated from manually microdissected tumor tissue was used to perform polymerase chain reaction-based loss of heterozygosity (LOH) analysis for the phosphatase and tensin homolog (PTEN) gene (10q23). Primary cultures were established by mechanical dissociation of chordoma tissue and propagated in both serum-free and serum-containing conditions at 5% and 20% oxygen tensions. **RESULTS:** LOH at the PTEN locus was observed in 2 specimens (17%). This was confirmed at the protein level by Western blot analysis. Cell culture studies showed increased proliferative rate of PTEN deficient tumors compared to PTEN wild-type chordoma specimens. In addition, chordoma cells derived from PTEN deficient tumors were less responsive to PDGFR tyrosine kinase inhibition. **CONCLUSIONS:** To our knowledge, this is the first report of PTEN deletion in chordomas. Because patients with PTEN deleted chordomas may be refractory to PDGFR inhibition, screening tumor tissue may be of value prior to initiating therapy.

P294

PROMOTER METHYLATION PROFILES OF SIX TUMOR-RELATED GENES IN VARIOUS BRAIN TUMORS

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BACKGROUND: Hypermethylation of tumor-related genes represents a primary mechanism in the inactivation of this gene. In this study, we investigated the methylation status of the promoter of six genes in human brain tumors. **METHODS:** Genomic DNA was isolated from formalin-fixed paraffin sections of 279 brain tumors, including 58 oligodendroglial tumors, 56 ependymomas, 114 astrocytomas, 14 central neurocytomas and 37 chordomas, and then bisulfite-converted, and analyzed by methylation-specific polymerase chain reaction. **RESULTS:** Among the brain tumor samples, 81.4% (227/279) revealed hypermethylation in at least one gene analyzed. Especially in oligendroglial tumors, all but one sample (98.3%) displayed anomalies in at least one gene. RASSF1A was hypermethylated in more than half of the tumors in each classification except in chordoma. P16 was the least frequently hypermethylated gene in this study, showing no methylation in central neurocytomas and chordomas. MGMT was more commonly methylated in gliomas, including 55.1% in oligodendroglioma, 66.1% in ependymoma, and 31.6% in astrocytomas, in contrast to the 7.1% in central neurocytoma and 0% in chordoma. **CONCLUSION:** The major subtypes of brain tumors display differential aberrant promoter methylation profiles. Such information may have substantial diagnostic and prognostic value.

P295

SHORT COURSE OF RADIOTHERAPY AND CONCOMITANT TEMOZOLOMIDE IN PATIENTS AFFECTED WITH GLIOBLASTOMA WITH V-VI PROGNOSTIC CLASSES. A PILOT STUDY

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Purpose: To evaluate the outcome and toxicity profile of glioblastoma patients treated with hypofractionated radiotherapy (HPF) (Roa 2004) and concomitant Temozolomide (TMZ) (Stupp 2005) in unfavorable V and VI prognostic classes (Mirimanoff 2006). **Materials and Methods:** We are monitoring clinical outcome, survival, and acute and long term toxicities of patients treated with HPF scheme (40 Gy in 15 fractions) concomitantly with TMZ (75 mg/m2 for 2 patients and 85 mg/m2 for 5 patients) (21 days in total), followed by adjuvant TMZ (150-200 mg/m2). MST is calculated from surgical procedure. Dexamethasone and neurological status are being registered. **Results:** Eight patients have been recruited till 12/2008, 7 of them are able to be validated (4 men and 3 women; median age of 69 years). Classification according to the RTOG/EORTC recursive partitioning analysis was as follows: class V for 3 patients and class VI for 4 patients. Surgery consisted of partial resection (n=2) or only biopsy (n=5). Median survival time was 24 weeks (4.5-63) for the whole group, but some differences were found by 3 class V patients (4.5, 54, 63 w) and 4 class VI patients (8, 13, 24, 58 w). In 3 patients MGMT gene promoter was methylated. Main acute toxicity was asthenia and thrombocytopenia, with one case of pneumonia. Two patients died during treatment because progression. No long term neurological complications have been found. No steroid dependence was observed in 2 patients. After progression, 3 patients were entered in schedule of CPT-11 and bevacizumab, with MRI and SPECT monitoring. **Conclusions:** Hypofractionated Radiotherapy concomitant with Temozolomide can be used for selected poor prognostic GBM patients to reduce the overall treatment time, without apparent increased toxicity.

P296

ASSESSMENT OF 11C-METHIONINE PET CHANGES FOR MONITORING THERAPEUTIC RESPONSE FOLLOWING SIMULTANEOUS INTEGRATED BOOST TECHNIQUE FOR GLIOBLASTOMA MULTIFORME

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Purpose We developed simultaneous integrated boost technique (SIB) planned by 11C-methionine (MET)-PET, and quantified the effect of MET-PET in monitoring the response to radiation for SIB with chemotherapy. **Methods** We performed SIB in 17 patients with GBM with subtotal resection. GTV-1 was defined as the area of intensive MET uptake. GTV-2 was defined as the area of mild MET uptake. PTV-1 encompassed GTV-1 plus 5 mm margin, and PTV-2 encompassed GTV-2 plus 2 mm margin. SIB was performed in 8 fractions, planning the dose for GTV-1 at 68 Gy, PTV-1 at 56 Gy and PTV-2 at 40 Gy with concurrent temozolomide at a dose of 75mg/m2. Each patient underwent a series of MET-PET exams: baseline prior to SIB, 3 months following SIB and 6 months following SIB. The change of MET uptake value in the PTV-1 and PTV-2 (PTVs) after SIB was investigated. **Results** MET-PET exams were performed in all 17 patients 3 months after SIB and in 10 patients 6 months after SIB. At 3 months following SIB, MET uptake value in the PTV-1 was reduced in all 17 patients (average 32.3%). The uptake value in the PTV-2 was reduced in 14 of 17 patients (average 22.8%), and increased in three patients (average 14.6%). At 6 months following SIB, the uptake value in the PTV-1 was reduced in all 10 patients (average 35.4%). The uptake value in the PTV-2 was also reduced in all 10 patients (average 25.3%). In three of those 10 patients, new abnormal MET uptake at the outside of PTVs was seen. **Conclusion** Preliminary results demonstrated that the uptake value of MET decreases after SIB at the PTV-2, as well as at the PTV-1 in the majority of patients. In some cases, however, it is difficult to prevent tumor recurrence at the outside of PTVs.

P297

RECURRENCE PATTERN AFTER [(18)F]FLUOROETHYLTYROSINE- POSITRON EMISSION TOMOGRAPHY-GUIDED RADIOTHERAPY FOR HIGH-GRADE GLIOMA.

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P298

RHABDOMYOSARCOMA AS A LATE COMPLICATION OF STEREOTACTIC RADIOTHERAPY IN THE PATIENT WITH NEUROFIBROMATOSIS TYPE 2

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Neurofibromatosis type 2 (NF2) is a rare autosomal dominant disorder characterized by the development of benign tumors of the peripheral and central nervous system (CNS) including schwannomas, meningiomas, and ependymomas. The gene responsible for the development of NF2 (schwannomin or merlin) acts as a tumor suppressor gene. Disease expression is quite variable resulting in a spectrum of different phenotypes. While there is an association between NF1 and malignant neurofibroma, the development of malignant tumors is not considered a feature of NF2.The hallmark of NF2 is development of bilateral vestibular schwannomas with a consequent hearing loss. Patients often have a large burden of intracranial tumors, particularly meningiomas, and require frequent interventions. Surgery has been a standard therapy, but stereotactic radiotherapy (SRT) has been increasingly used for the management of these tumors. It offers some enticing advantages over surgery particularly in regards to short-term risk. Because of the high frequency of multiple intracranial tumors in NF2 patients, SRT is an attractive option in this patient group. Common long-term complications of radiosurgery include cranial neuropathies, and less commonly vasculopathy, while the risk for malignancy after SRT is not well delineated. While there are no known instances of spontaneous malignant degeneration of tumors in NF2 patients, there are a few reports of malignant schwannoma, meningioma and ependymoma after the SRT. We present the first documented case of rhabdomyosarcoma following SRT for a NF2-associated vestibular schwannoma. Patients with NF2 have a tumor suppressor gene defect and may be more vulnerable for development of secondary malignancy after treatment involving radiation, when compared to patients with isolated tumors. Decision about selection of therapy in NF2 patients, particularly ones in young age, must include consideration of long term complications, particularly radiation-induced malignancies.

P299

RADIATION-INDUCED CEREBRAL CAVERNOUS HEMANGIOMAS IN ADULTS.

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Background: Cerebral cavernous hemangiomas are a documented delayed complication of cranial irradiation in pediatric primary brain tumors. They are considered rare in adults and only sporadic cases have been described. **Objective:** To evaluate the frequency of radiation-induced cerebral cavernous hemangiomas (RICH) in routine surveillance imaging of adult long-term survivors of brain tumors therapy. **Methods:** Neuro-oncology patients with RICH detected on routine surveillance MRI within a period of 12 months in our service were retrospectively analyzed . Early CT studies were retrospectively reviewed as well as recent MRI, with particular attention to T2-weighted gradient echo (T2GE) and susceptibility-weighted (SWI) sequences. **Results:** We identified 15 patients (6 men and 9 women) with RICH diagnosed after a mean period of 8 years (range: 5-13) following external beam cranial irradiation delivered as treatment for high grade glioma (5), medulloblastoma (5) ependymoma (2), and either germinoma, pituitary adenoma and meningioma (1 each). The mean age of patients at the time of this analysis is 43 years (range:24-66). The mean cumulative radiation dose was 48Gy (range:40-60) with mean daily fractions of 1.8Gy (range:1.6-2). Characteristically, multiple (3 or more) RICH are detected within the radiation field either supra- or infratentorially. It appears as multifocal areas of round hypointensity on T2GE and SWI images. The lesions are asymptomatic in 10 patients, associated with seizures in 2, recurrent headaches in 2, and acute intracerebral hemorrhage in 1. No specific treatment was given to RICH . **Conclusions:** RICH may be more common than is currently appreciated in adult long-term survivors of brain tumor therapy . The clinical and imaging features are similar to RICH in children. Based on these findings, a prospective analysis of RICH seems warranted in order to evaluate its incidence, prevalence, associated morbidity and risk of bleeding.

P300

HEMATOTOXICITY OF CRANIOSPINAL IRRADIATION IN ADULT PATIENTS

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Background: Craniospinal irradiation (CSI) is part of the standard care in patients with malignant embryonal brain tumours as medulloblastomas, intracranial primitive neuroectodermal tumours (PNETs) and ependymoblastomas. As the radiation field covers large parts of the haematopoietic bone marrow bone marrow depletion is a common side effect of CSI. **Methods:** The incidence, grade and duration of bone marrow depletion in adults treated at our institution with CSI for an embryonal brain tumour during the last 10 years was investigated retrospectively. **Results:** Thirty patients, 12 women and 18 men, aged 23 to 64 years were treated with CSI. Radiation therapy consisted of 1.8 Gy/5 fractions per week to a dose of 36 Gy to the skull and the spinal axis followed by a boost of 18.8 Gy in 10 fractions to the posterior fossa (up to a total of 54.8 Gy). Twenty-two (73%) patients developed aplasia during or shortly after the CSI, with a median duration time of 136 days (range 32 to 480 days). Most common hemato-toxicity (HT) of WHO Grade III or IV was thrombocytopenia (15/30; 50%) and leukocytopenia (19/30; 63.3%), which caused limitations to eventual subsequent chemotherapy (CHT). Granulocyte-colony stimulated factor was used for more than 2 weeks in 23 (76.6%) patients. Further, ten (33.3%) patients presented HT of WHO Grade I/II, for a median of 67 days. Anaemia requiring red blood cell transfusion was observed in seven (27%) patients. The myelosuppression was associated with opportunistic infection, like fungal infection, herpes encephalitis or pneumonia. One patient died of septicaemia. In ten cases, HT combined with infections resulted in interruption or stopping adjuvant CHT. There was no gender or age specific difference in appearance of HT. **Conclusions:** The risk of significant bone marrow depletion is high in adult patients treated with CSI for embryonal brain tumours.

P301

INSPECTION ABOUT RECURRENCE AND PROGRESSING DEMENTIA AFTER WBRT TO METASTATIC BRAIN TUMOR - PROBLEM PRESENTATION AGAINST WBRT -

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(Background and Objective)
Streotactic radiosurgery has come to be used frequently as the initial therapy to metastatic brain tumors, but still whole brain radiation therapy (WBRT) is the standard therapy after surgery. This report deals with cases of emergence of new metastases after WBRT and cases of deterioration of QOL after WBRT, and aims to address issues about the timing and the role of WBRT.

(Cases discussed)
26 cases have been treated in our hospital for metastatic brain tumors during April 2006 and June 2008, and 17 cases of them were treated with surgery and WBRT as the initial therapy. Within these cases, there were 3 cases of recurrence as new intracranial metastatic lesions (all of them were breast cancer), and one case of severe deterioration of QOL as a result of severe brain atrophy and dementia. Representative cases are as follows; (Case 1) 58 year-old female patient with breast cancer was diagnosed with single metastasis in the occipital lobe one year after the treatment of the original cancer. Operation was performed, followed by 40 Gy (2 Gy times 20 fractions) of WBRT. 12 months later multiple small metastases appeared, and cyberknife treatment was brought to those lesion. Since WBRT was administered before cyberknife treatment, full dose could not be used. The patient died of uncontrollable intracranial tumors. (Case 2) 79 year-old male patient with colon cancer was diagnosed with single brain metastasis in the superficial motor cortex 3 months after the treatment of the original cancer. Operation followed by 30 Gy (3 Gy times 10 fractions) of WBRT was performed. 14 months later brain atrophy progressed and dementia advanced. Deterioration of QOL led the patient to the state of cripple.

(Conclusion)
We propose, in cases that long course is expected and emergence of new metastatic lesion has high possibility like breast cancer cases, and in cases of old age, WBRT after surgery should not be performed as a routine protocol. WBRT should be limited to cases of multiple brain lesions which are intractable with operation or stereotactic radiosurgery.

P302

NOVALIS SHAPED BEAM RADIOSURGERY FOR CRANIAL AND SPINAL TUMORS: 8 YEARS OF EXPERIENCE IN KOREA

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Objective: Our experience indicates that Novalis Shaped Beam Radiosurgery offers some distinct clinical advantages in the treatment of a diverse range of cranial and spinal tumors. Over the past eight years we have focused on the development of optimal treatment protocols for local tumor control with minimal collateral damage. In this paper we review 1,041 clinical experiences of cranial and spinal stereotactic radiosurgery(RS) and analyze the results. **Methods:** By April 2008, we had completed 1041 cases of cranial and spinal RS. Of the cranial RS (795 cases), 59% were for malignant tumors and 34% were for benign tumors while of the 245 cases of spinal RS most were for malignant tumors; 70% metastatic tumors with the balance made up of benign tumors and AVM. We analyzed the treatment protocols used and the clinical outcomes according to the different dose-fraction regimes used, the patients' condition and the various stages of tumor development. **Results:** Where we used single session cranial RS we were generally able to achieve excellent levels of local tumor control for most types of benign and metastatic tumors. For more challenging cases involving limitations due to size, type of tumor, dose restrictions and histology we were able to achieve positive outcomes using fractionation and intensity modulation. Many of these tumors were large, glial or located close to critical structures such as the optic nerve or brainstem. **Conclusion:** Hypofractionated schedules for treating sellar & parasellar tumors, multiple and large tumors have been applied with great success. Spinal Radiosurgery is now established as a clinically viable treatment option for a wide range of challenging lesions that previously required invasive surgical procedures. Further study is in progress to expand the range and efficacy of our treatment protocols.

P303

ULTRAFRACTIONATED RADIATION THERAPY (3 DOSES PER DAY) FOR INOPERABLE GLIOBLASTOMAS. A NEW AND PROMISING REGIMEN.

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Background: Ultrafractionation radiation therapy consists in irradiating cells or tumors several times daily, delivering low doses at which hyperradiosensitivity occur. We recently reported the high efficiency of ultrafractionation radiotherapy in glioma cell lines and xenografts and are now conducting a phase II clinical trial to determine the effect of an ultrafractionation regimen for glioblastoma patients. **Methods:** A prospective, multicenter, phase II study has opened for accrual in September 2003. Patients over 18 years of ages who are able to give informed consent and have histologically proven, newly diagnosed and unresectable, supratentorial glioblastoma (WHO grade IV) are eligible. Three doses of 0.75Gy spaced by at least four hours are delivered daily, five days a week for six consecutive weeks for a total of 67.5Gy. Conformal irradiation includes the tumor bulk including a margin of 2.5 cm. Tolerance and toxicity is the primary endpoints; survival and progression-free survival are secondary endpoints. **Results:** To date 31 patients have been enrolled in this study, 16 men and 15 women, median age 58 (range 37 to 76), median Karnofsky performance status (80 range from 60 to 100). The median time between histological diagnosis and the start of treatment is 6 weeks. The ultrafractionated radiation therapy has been well tolerated; no acute grade 3 and/or 4 CNS toxicity has been observed. Minor responses at the end of irradiation were seen in 5 patients. Median survival from initial diagnosis was 10 months, eight patients remain alive. **Conclusions:** Ultrafractionated radiation therapy is safe and well tolerated. No acute CNS toxicitiy has been observed. Overall survival of over 10 months for patients without prior debulking surgery compares favorably with other reports. Updated definitive results will be presented.

P304

STEREOTACTIC RADIOSURGERY (SRS) IN RECURRENT HIGH-GRADE GLIOMA

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Despite the failure of the RTOG 9305 randomized trial to demonstrate the efficacy of SRS in newly diagnosed glioblastoma, this treatment was reported in several institutional series to be modestly effective, particularly in the setting of limited focally recurrent disease. The objective of the current presentation is to retrospectively analyze our experience with 17 patients treated by SRS at the Tel Aviv Medical Center from 2004-2008. There were 9 patients with primary glioblastoma, 2 with secondary glioblastoma, and 6 with anaplastic glioma. The median age was 52 (36-75); there were 8 males and 9 females. The indication for use of SRS was rapidly progressing neurological deficit in 3 patients, and a new, asymptomatic, limited enhancing focus on T1-contrast MRI in 14 patients. Two patients with anaplastic oligodendroglioma received SRS twice. Three of 19 treatments resulted in immediate progression whereas in 16 other treatments (84%) stable disease or partial responses were achieved. Median time to progression in responding patients was 5 months (1-51). Longstanding responses (> 12 months) were observed in 5 patients (3 with GBM and 2 with anaplastic gliomas). In one GBM patient with rapidly progressing hemiparesis due to a new enhancing lesion in the internal capsule region, SRS resulted in significant improvement of limb weakness within 2-3 weeks with clinical stabilization for two months. One patient with anaplastic astrocytoma developed clinically significant radiation necrosis 3 months after SRS and required a palliative operation, but since then has remained asymptomatic and without radiological evidence of active disease for more than 51 months. In the remainder of patients the treatment did not result in worsening of clinical symptoms and was not associated with significant side effects. In conclusion, SRS may be considered an effective salvage procedure in selected patients with recurrent high-grade gliomas, providing long term responses in some cases.

P305

TREATED WITH CYBERKNIFE STEREOTACTIC RADIOSURGERY: TWO CYBERKNIFE CENTERS EXPERIENCE

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Background: The stereotactic radiosurgery such as Gamma-knife or linear accelerator radiosurgery has been showed to reduce the re-hemorrhage annual risk in patients with cavernous malformations. Cyberknife is a new frameless system and the role of Cyberknife stereotactic radiosurgery in the management of cavernous malformations has not been discussed. The authors present the 3-year results of cavernous malformation treated by Cyberknife stereotactic radiosurgery in two medical centers.

Material and Methods: From September 2005 to September 2008, 14 patients with 17 cavernous malformations were enrolled in this study. There were 6 men and 8 women with mean age of 40 years. Thirteen patients (93%) had only one hemorrhagic episode in the pre-radiosurgery period. All patients were treated by Cyberknife stereotactic radiosurgery and followed at least more than one year. Magnetic resonance imaging was performed before and after treatment.

Results: The mean volume was 0.45 ml, whereas the mean maximal dose was 2203 cGy. The median marginal dose was 1600 cGy. The mean prescribed isodose was 79%. The mean follow-up time was 21.5 months. Only two patients re-bled after Cyberknife stereotactic radiosurgery. The annual re-hemorrhagic rate accounts for approximately 7.9%.

Conclusions: Cyberknife stereotactic radiosurgery seems to be effective in reducing annual rehemorrhagic risk in patients with cavernous malformations in the 3 years follow-up. The long-term outcome should be investigated in the future.

P306

PERITUMORAL EDEMA AFTER CYBERKNIFE RADIOSURGERY FOR MENINGIOMA: REPORT OF TWO CASES AND REVIEW OF LITERATURE.

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In the past decades, stereotactic radiosurgery including gamma-knife, linear accelerator or cyberknife becomes an effective tool for patients with intracranial meningiomas, despite of primary or adjuvant therap. It can offer a noninvasive minimal access, achieve a good tumor control rates and maintain the vital neurological functions. However, peritumoral edema induced by radiosurgery during the treatment of intracranial meningioma has been reported in the literature and the mechanism still remains unclear. Parasagittal meningioma has been described as a predisposing factor in developing peritumoral edema after radiosurgery. In our presented cases, one parasagittal meningioma and one convexity meningioma developed peritumoral edema at 6 and 7 months after Cyberknife stereotactic radiosurgery, respectively. The duration was compatible with the findings of Kondziolka et al. (the peak time to edema was 6 to 8 months after treatment). The prescribed dosage has been suggested as a predisposing factor for peritumoral edema after radiosurgery. However, in our other supratentorial meningioma cases, these tumors were all treated with a prescribed dose of 1800 cGy. The peritumoral edema seems to be less likely related with the prescribed dosage. Otherwise, interestingly, although our second case was a convexity meningioma, these two meningiomas were all located around the major draining sinus. Our results support the hypothesis of peritumoral edema as a radiation side effect related to venous cause including damaging bridging veins draining into the adjacent venous sinuses or thrombosis of circulation of sinus. It seems possible meningioma around the major draining sinus may developed this complication after radiosurgery. Our cases provide an important formation that the patient with meningioma around the drainage sinus treated by stereotactic radiosurgery should be followed closely. However, this phenomenon is rarely seen in other kind of brain tumors such as acoustic schwannoma or metastatic lesions. This also should be investigated in further studies.

P307

PRELIMINARY RESULTS OF INITIALLY TRATED PATIENTS-FEASIBILITY OF APPLYING THE SYNERGY-S SYSTEM FOR STEREOTACTIC IRRADIATION SPINAL TUMORS

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Background: Radiation treatment of spinal and paraspinal tumors has been limited by the tolerance of the spinal cord. As such, therapeutic options are restricted to surgically accessible lesions or the use of sub-optimal dosing of external beam irradiation. During the last decade, several systems have been developed to implement extra-cranial stereotactic image guided treatment delivering focused high dose radiation. **Methods:** Synergy-S (Elekta Corp, Crowley, UK) represents a linear accelerator based system that enables the delivery of sophisticated stereotactic-based treatment with high-resolution multi-leaf collimation and volumetric image guidance. The system is predicated on rigid, reproducible, non-invasive body fixation. We sought to determine the applicability of this system to the irradiation of spinal tumors within a busy university-based practice. **Results:** Between November 2007 and June 2008 10 patients were treated for spinal tumors. Six patients were female, median age was 50.2 years (39-79 years) and median KPS 85% (50-100). We treated metastases (renal cell carcinoma, malignant melanoma, leiomyosarcoma and prostate cancer) and primary spinal tumors (malignant meningioma, chondrosarcoma). The spinal levels included cervical (C1-2, C6-7), thoracic (T5), lumbar (L1-2, L2, L5) and sacral (S1). Four cases were treated with radiosurgery (median 1550 cGy) and 3 were treated via fractionation (Median: 450 cGy x 5). All patients tolerated therapy without acute complications. Local control has been achieved in all cases, albeit at short median follow-up time of 9.4 weeks (7.2 -27.1 weeks). **Conclusions:** The feasibility of delivering single and multiple fraction stereotactic spinal irradiation has been demonstrated. No unique difficulties were encountered as a function of the level of disease. Although a head-to-head comparison was not performed, this approach is considerably less time-consuming than other image-guided systems (e.g., Cyberknife). Formal biologically-driven protocols are now in submission to the Institutional Review Board to standardize this promising technology.

P308

DOSE-VOLUME ANALYSIS OF WHOLE VENTRICULAR IRRADIATION BY PROTON BEAM COMPARING WITH INTENSITY MODULATED RADIOTHERAPY AND STANDARD PHOTON THERAPY

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Purpose: To assess the advantage of proton beam therapy (PBT) for whole ventricular irradiation (WVI), treatment plans with proton beam therapy, intensity modulated radiotherapy (IMRT) and standard photon radiotherapy (SPR) were compared. **Methods:** Computed tomography data set of a patient diagnosed germ cell tumor was utilized for the treatment planning. Clinical target volume (CTV) were defined as the volume consisted of lateral ventricles, third ventricle and forth ventricle with 1 cm margins. Treatment plans with 24 GyE for CTV were performed with each treatment technique. Each treatment plan included non-coplanner beams to reduce the dose to organ at risk. Inverse optimization was applied for treatment plan for IMRT. On the treatment plans efficient dose deliveries in CTV were evaluated by the coverage and the homogeneity of doses to the target. The mean dose of organ at risk including supratentorial and infratentorial brain, eyes and cochlea were measured on the treatment plans. **Results:** Three treatment techniques achieved sufficient coverage to CTV. However, the inhomogeneity coefficient values for IMRT were higher than those of PBT and SPR. Mean dose of the supratentorial and infratentorial brain with PBT was lower than those of IMRT and SPR. The doses to eyes and cochlea were decreased in PBT comparing IMRT and SPR. **Conclusion:** Excellent conformality to target and homogenous dose deliver in target by proton beam treatment enable to decrease doses to critical organs. Whole ventricular irradiation by proton beam expected to reduce the late toxicity after treatment of germinoma.

P309

IN VIVO BIODISTRIBUTION OF BORON AFTER INTRAVENOUS 4-DIHYDROXYBORYLPHENYLALANINE-FRUCTOSE (BPA-F) INFUSION IN MENINGIOMA AND SCHWANNOMA: A FEASIBILITY STUDY FOR BORON NEUTRON CAPTURE THERAPY(BNCT)

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BNCT is an experimental neutron activated pharmacotherapy. The radiation energy is for the most part so high that radioresistance does not play a significant role. BNCT is feasible for superficial tumors with high boron uptake. It is thus attrac-tive to develop for inoperable meningiomas and Schwannomas in the elderly and in NF2 patients if sufficiently high and selective boron concentrations are achieved. As no data of boron uptake in vivo in the aforementioned tumors is available, we performed a feasibility study as part of the Finnish BNCT development project.Four meningioma and four Schwannoma patients participated in the study. 100mg of BPA-F /kg of body weight of the patient was infused during 60 min prior to the operation. The tumor was then removed and sampled. The time to tumor removal varied, the earliest being 80 min after the start of infusion. Some of the Schwannomas were removed piecemeal, the last bits taken up to 300 min from the start of the infusion. Boron was measured from tumor and from whole blood and plasma by a previously pub-lished method utilizing atomic emission spectrometer. The mean tumor to whole blood boron concentration ratios was 3.6:1 for meningiomas and 2.4:1 for Schwannomas and respective tumor to plasma concentration ratios were 3.1:1 and 2.1:1. The range of these ratios varied from 2:1 to 6:1.The data shows promising concentration ratios for boron uptake. Although the unavoidable manipulation of the samples in operation and other local factors may alter the concentrations somewhat we believe the results are reliable, as the boron measurement methods have been tested previously. Our results motivate to continue developing BNCT for these difficult to treat tumors.

P310

CLINICAL RESULTS OF MODIFIED BORON NEUTRON CAPTURE THERAPY FOR MALIGNANT GLIOMA

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Boron neutron capture therapy (BNCT) is based upon the nuclear capture and fission reactions that occur when non-radioactive ^{10}B is irradiated with low energy neutrons to produce high energy α particles ($^{10}B[n,\alpha]^{7}Li$). In order for BNCT to be successful a sufficient amount of ^{10}B and neutrons must be delivered to the tumor. We utilized epithermal neutron for deep penetration and simultaneous use of two different boron compounds, sodium borocaptate (BSH) and boronophenylalanine (BPA), with different accumulation mechanism. BNCT group including 21 newly histological confirmed glioblastoma patients treated with surgical removal followed by BNCT in Osaka Medical College during 2002 to 2006. Ten patients were treated with BNCT only, and in the other 11 patients, 20 to 30 Gy fractionated external beam X-ray irradiation was combined after BNCT. No chemotherapy was applied until tumor progression was observed. Treatments were well tolerated. Any kinds of acute systemic or local severe toxicity were not demonstrated. Median overall survival of the patients treated by BNCT was 15.6 months with 2-years survival of 25%. Stratification by RPA criteria showed 6, 6, 8 and 1 patients in class III to VI, respectively. Median survival time for the BNCT group compared to the RTOG database was as follows: 20.6 months vs. 17.9 months for class III; 16.9 months vs. 11.1 months for class IV; 13.2 months vs. 8.9 months for class V. BNCT showed a survival benefit in all of the RPA classes of the RTOG database not only for the good prognosis group.

P311

BRAIN TUMORS BRACHYTHERAPY BY USING RIGID IR192 CATHETER

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AIM: To design a methodology of the insertion of the catheters into the tumor cavity. We used rigid Ir192 catheter with VariSource system for high dose rate (HDR) brachytherapy in the different types brain tumours. **Methods and Materials:** Over the year 12 patients were treated: six patients with recurrent glioblastoma multiforme (GBM), 2 patients with inoperative anaplastic astrocitoma (AA), 3 patients with metastatic tumours (MTS) and 1 patient with recurrent meningioma. Minimum three Ir192 catheters were placed in the resection cavity at the time of surgical resection. We used an original method to insert and fix the catheters in the brain. For HDR brachitherapy we used VariSource workstation. Ir192 wire was delivered into previously calculated and designed shape of tumour by VariSource system. Patients received radiation therapy: a mean dose of 24Gy and a single local dose of 6Gy. Catheters were kept from 4 to 7 days depending the dose. All patients had confirmed pathology of the tumour. The median Karnofsky performance status (KPS) was 80. **Results:** All patients were followed-up over the year. At the time of analysis 3 (50%) patients with GBM, both with AA and one with MTS showed no radiological signs of tumour progression. Acute side effects were wound infection and liquorrhea in three cases. Two patients got mild-nausea and/or headache. 2 incidents of symptomaticradiation necrosis with surrounding edema were observed. **Conclusions:** We designed original safe system to use rigid IR192 wire for brachytherapy that allowed to create a precise shape of radiation field and precisecalculated dose of radiation in functional sites of the brain. Treatment was generally well tolerated.

P312

IMAGING FEATURES IN PARENCHYMAL LYMPHOMATOUS MASSES IN BRAIN: PRIMARY CNS LYMPHOMA OR SYSTEMIC INVOLVEMENT OF THE BRAIN?

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Purpose: We sought to determine whether there is any imaging features to enable differentiation of primary (group 1) and secondary (Group 2) central nervous system (CNS) lymphoma. **Material and methods:** Computed tomography, conventional MR imaging including Gadolinium enhanced series and diffusion-weighted MR imaging (DWI) findings of 19 patients, of which 12 were primary and 7 were secondary CNS lymphoma were re-evaluated. Pathological subtype of the tumours were diffuse large B cell in all primary and systemic tumours except 2 (1 lymphoblastic, 1 Burkitt type). **Results:** An infiltrating lymphoma of butterfly pattern involvement were observed in three patients who had a history of extracranial lymphomatous mass. None of the patients of group 1 had this type of involvement pattern.The lymphomatous lesions had increased signal on DWI in 13 of 19 patients (8 in group 1, 5 in group 2) and decreased ADC values in all. There was no correlation between CT density values of the lesions and ADC values. In five patients (4 in Group 1) a very infiltrative pattern was observed. In a background of profound vasogenic edema, scattered T2-hypointense nodules and stripes oriented parallel to medullary veins suggesting perivascular infiltration were present. These T2-hypointense nodules and stripes enhanced markedly on postcontrast T1-weighted series. In all four patients with infratentorial involvement either brain stem or cerebellum were of primary CNS lymphoma group. The basal ganglia were involved in five patients in group 1 and none in group 2. **Conclusion:** Involvement of infratentorial structures and the basal ganglia, hemorrhagic lesions appreciated as T1 hyperintensity and T2-hypointense nodules and stripes probably showing perivenular infiltration and Gd enhancement along the perivascular veins have been shown in primary CNS lymphomas. Bifrontal transcallosal lesions are suggestive of secondary involvement of CNS by lymphoma.

P313

LONG-TERM SURVIVAL IN PRIMARY CNS LYMPHOMA TREATED BY HIGH-DOSE METHOTREXATE MONOCHEMOTHERAPY: ROLE OF STAT6 ACTIVATION AS PROGNOSTIC DETERMINANT

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We report a single-center experience of sixteen immunocompetent patients diagnosed with primary central nervous system lymphoma and treated with monochemotherapy with high-dose methotrexate (MTX) and deferred radiotherapy. MTX was given at a dose of 8.0 g/m2 for induction and at a dose of 3.5-8.0 g/m2 for maintenance. There were eight complete responses (CR), one partial response, one stable disease, and six patients whose tumors progressed in spite of the chemotherapy. At the final follow-up, five of 5 CRs were alive and well without radiotherapy, with a median follow-up of 26 months. Overall survival in 8 non-CRs treated with the subsequent radiotherapy was 36 months. In the immunohistochemical study, STAT6 was positively expressed in eight out of 13 cases. They included all non-CRs and 2 CRs. This observation suggests that STAT6 expression can be used as a prognostic determinant to the MTX chemotherapy.

P314

TREATMENT FOR PRIMARY CNS LYMPHOMA WITH HIGH-DOSE MTX AND SALVAGE THERAPY WITH ESHAP

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Primary CNS lymphoma (PCNSL) is usually treated with high-dose methotrexate (MTX) which is mostly effective for PCNSL. However, when use of high-dose MTX is repeated, MTX-resistant PCNSL gradually increases. In addition, high-dose MTX therapy is not able to be applied for patients of poor renal function, and early recurrence after high-dose MTX therapy is often encountered. Therefore, although the salvage therapy instead of high-dose MTX therapy is required, it has not been established. Here, we show treatment outcome of high-dose MTX and salvage therapy with ESHAP (cisplatin, etoposide, methypredonisolone and high-dose cytarabine). In 2002 to 2008, we have treated 30 patients bearing PCNSL (mean age, 63.5 years; female, 8, male, 22) . Among them, 7 cases which showed good response for MTX were treated with high-dose MTX, and the all cases have not recurred. Nineteen cases which lately showed resistance to MTX were initially treated with high-dose MTX but later with ESHAP, and among them 16 cases showed complete remission (CR) and progression free survival and the remaining cases showed partial remission. Three cases treated with ESHAP alone due to renal dysfunction showed CR, but later the all cases recurred. In conclusions, high-dose MTX therapy is very useful for MTX-responsive PCNSL, and ESHAP therapy is useful as a salvage therapy for the major of PCNSL that later show MTX-resistance. Combination with high-dose MTX and salvage ESHAP therapy would overcome the problems high-dose MTX therapy and possibly contribute to therapy for PCNSL.

P315

TREATMENT OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA WITH TEMOZOLOMIDE, VINCRISTINE, DOXORUBICIN, PREDNISONE AND RITUXIMAB

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Background: High-dose methotrexate is the mainstay of treatment for primary central nervous system lymphoma (PCNSL). However, it can cause major toxicities. **Materials and Methods:** Four patients with PCNSL were treated with systemic administration of temozolomide, vincristine, doxorubicin, prednisone and intravenous or intrathecal rituximab. **Results:** Complete responses were achieved in all the patients. The mean follow up date of the patients is 18 months till 10/15/2008. Grade I myelodepression was observed in all patients, but no other severe side effect manifested to date. The Karnofsky Performance Scores were 100 throughout the treatment, and the MMSE scales did not decrease in all the patients during all the cycles. **Conclusions:** The new combination has much less side effects compared to current standard therapy. Nearly equal effect can be achieved. Further efforts are warranted to evaluate this regimen.

P316

INTRAVENOUS RITUXIMAB COMBINED WITH TEMOZOLOMIDE AS A SECOND LINE THERAPY FOR CD20 POSITIVE PRIMARY CENTRAL NERVOUS SYSTEM MALIGNANT LYMPHOMA

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Recently, the combination of rituximab and temozolomide is reported to have synergistic effect in the treatment of primary central nervous system (CNS) lymphomas (PCNSL). We experienced, as a second line therapy, combination of rituximab and temozolomide in two patients with PCNSL. A 51-year-old man had a mass lesion in the left basal ganglia extending to the left temporal lobe. The tumor was partially removed, and was diagnosed as malignant lymphoma, diffuse large B cell type, CD20-immunopositive. High-dose methotrexate (MTX) chemotherapy followed by radiation therapy was carried out. After 2 years, the tumor recurred, and the patient was started on oral temozolomide, 300 mg for 5 days per 4 weeks, followed by intravenous administration of rituximab 540 mg. This combination of temozolomide and rituximab was repeated four times, and thereafter the patient was eight times on oral temozolomide, 300 mg for 5 days per 4 weeks. After this combination therapy, the tumor disappeared on MRI. A 65-year-old man had a mass lesion in the left basal ganglia extending to the left temporal lobe. The tumor was partially removed, and was diagnosed as malignant lymphoma, diffuse large B cell type, CD20-immunopositive. During high dose MTX chemotherapy, the tumor growth was progressive. After completion of radiotherapy, the patient was started on oral temozolomide, 300 mg for 5 days per 4 weeks, followed by intravenous administration of rituximab 540 mg. This combination of temozolomide and rituximab was repeated four times, and thereafter the patient was eight times on oral temozolomide, 300 mg for 5 days per 4 weeks. After this combination therapy, the tumor disappeared on MRI. The combination of temozolomide followed by rituximab demonstrated efficacy against recurrent PCNSL and PCNSL resistant to high-dose MTX-based chemotherapy. This immunochemotherapy combination can be a second line therapy for PCNSL.

P317

MAINTENANCE THERAPY USING RITUXIMAB FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Objective: The addition of high-dose methotrexate (MTX) to radiation has improved disease control and survival in patients with primary central nervous system lymphoma (PCNSL). However, most of patients eventually relapse, and uncontrolled PCNSL remains the primary cause of death. We designed a maintenance therapy using rituximab (anti-CD20 antibody) after induction of complete remission to suppress recurrence. **Methods:** Preradiation chemotherapy consisted of MTX 3.5 g/m2 for a total of three to five doses. Cyclophosphamide, doxorubicin, vincristine and predonisone were given concomitantly with each cycle of MTX. Chemotherapy was followed by 36-40 Gy of whole brain radiation. Older patients were given the option of deferring radiation. Within 3 months after induction of complete remission, patients received multiple courses of rituximab; each course consisted of rituximab 375mg/m2 given weekly for 4 consecutive weeks. Each course was repeated every 3 months. **Results:** Nine patients with a median age of 65 years were included in this study between 2004 and 2008. Six patients were older than 65 years-old. Median follow-up period was 21 months. All patients had histologic diagnosis of diffuse large B-cell lymphoma and positive immunohistochemical staining for anti-CD20 antibody. One patient died of recurrent disease, but 8 patients were alive (range, 18 to 38 months) and their Karnofsky performance status (KPS) were kept higher than 80. Eight patients experienced recurrence. The median time-to-recurrence was 15 months (range 6-34 months). No toxicity was seen in association with rituximab. **Conclusions:** In this study, maintenance therapy with rituximab did not suppress recurrence rate, however, it might prolong time-to-recurrence and suppress tumor growth rate, and eventually contribute to prolong overall survival. Complete remission was achieved in all patients who had recurrence. In older patients, deferring whole brain radiation by retreatment with high-dose MTX and maintenance with rituximab did not compromise overall survival but did reduce neurotoxicity.

P318

DIAGNOSIS AND MANAGEMENT OF SPINAL EXTRADURAL NON-HODGKIN LYMPHOMA:ANALYSIS OF THREE CASES AND REVIEW OF THE LITERATURE

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Non-Hodgkin lymphomas can involve central nervous system either primarily or secondarily. Spinal cord compression or neurolymphomatosis is reported in 0.1 to 10.2 % of patients, generally occurring late in the disease and often following an aggressive course. We reported three cases with histologically confirmed spinal non-Hodgkin lymphoma. The three are of female gender and median age at diagnosis is 54.3 years (range, 26-72 years); the time of symptom onset to the definite diagnosis averaged 6.5 weeks. Two of them were of B-cell origin and presented with thoracic cord compression and progressive paraplegia. The other sought treatment for axial neck pain and radiculopathy. Common neurological examinations demonstrated a discrete sensory level, hyperreflexia and present pathological reflex(Hoffman or babinski sign). Magnetic resonance imagings all revealed elongated intraspinal extradural tumors with good contrast enhancement but absent bony destructions. Differential diagnoses include meningioma, schwannoma or meningeal infiltration by metastatic tumors. All patients underwent a decompressive surgery(one corpectomy and two laminectomies) with instrumentation, subtotal tumor resection and systemic chemotherapy with adjuvant spinal irradiation. Two are alive and well. A rapid progressive spinal cord syndrome with consistent extradural compression and absent bony destruction on neuroimagings should alert the possibility of spinal lymphoma. Surgery for this diagnosis followed by chemotherapy and spinal irradiation could result in significant neurological improvement. The spinal non-Hodgkin lymphoma can be associated a favorable outcome if diagnosed and treated early.

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