also subjected to IgG4 immunostaining. IgG4 positivity (defined as >10 IgG4+ plasma cells/high power field) was correlated with clinical features (age, gender, presence of inflammatory bowel disease, PSC duration, PSC recurrence after transplant, and number of acute rejection episodes) and histologic findings in the liver explants (periductal fibrosis and degree of periductal lymphoplasmacytic inflammation).

Results: Twenty-three (23.2%) liver explants showed increased (>10/HPF) IgG4+ periductal plasma cell infiltration. Histologically, IgG4 positivity in the liver strongly correlated with moderate to marked periductal lymphoplasmacytic inflammation (p=0.007) but only loosely with the presence of increased IgG4+ plasma cells in the gallbladder (17.6% vs 5.3%, p=0.13). All cases showed dense periductal fibrosis; there was no storiform fibrosis (as often seen in IgG4-associated pancreatitis). Clinically, IgG4 positivity correlated with shorter PSC duration before transplant (5.3 ± 4.6 yrs vs 8.5 ± 6.2 yrs, p=0.03), but was not associated with age, gender, IBD, PSC recurrence, or rejection.

Conclusions: Nearly one quarter of explanted livers that carry a clinical diagnosis of PSC contain increased IgG4+ periductal plasma cells, a finding that correlates with more intense periductal inflammation. Whether these patients also have increased serum IgG4 levels and whether this is a distinct subtype of PSC or represents an early (more inflammatory phase) of "ordinary" PSC are questions that require further study. The shorter time to liver transplant in these patients could suggest either a more aggressive disease course of untreated IgG4+ sclerosing cholangitis, or simply an earlier phase of PSC.

1481 Frequent Aberrant Activation of the PI3K/Akt/mTOR Pathway in Pancreatic Endocrine Tumors

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Background: Mammalian target of rapamycin (mTOR), a serine-threonine kinase, functions in the regulation of apoptosis, proliferation, and cell growth. Signaling through the PI3K/Akt/mTOR pathway results in increased translation of key mRNAs governing cell cycle progression and metabolism. Aberrant activation of this pathway, either by signaling through growth factor receptors, activating mutations/amplification of kinases, or by loss of function of the tumor suppressor PTEN, has been described in a number of human cancers. Utilizing immunohistochemistry for PTEN, p-Akt, p-mTOR, and p-S6rp (a target of mTOR activation), we analyzed signaling through this cascade in a cohort of pancreatic endocrine tumors (PET).

Design: Tissue microarrays were constructed from formalin-fixed, paraffin-embedded blocks of 101 PET from our departmental archive and stained for PTEN, p-Akt, p-mTOR, and p-S6rp. Expression intensity was scored as 0 (absent), 1+ (modest), or 2+ (strong). Islets from 7 normal pancreata served as controls.

Results: The normal islets expressed PTEN (2+ in 4, 1+ in 3), with no demonstrable expression of p-Akt, p-mTOR, or p-S6rp. PTEN expression was detected in the majority of tumors (2+ in 77 and 1+ in 19). Twenty-three tumors showed modest (1+) p-Akt expression, which tended to inversely correlate with PTEN expression. Modest to high levels (1-2+) of p-mTOR expression were present in 79 tumors (2+ in 35, 1+ in 44), and modest to high levels of p-S6rp were detected in 37 (2+ in 8, 1+ in 29). Results are summarized in the table.

PI3K/Akt/mTOR Pathway Protein Expression

	Intensity	PTEN*	p-Akt*	p-mTOR*	p-S6rp*
PET	0	5	78	22	64
	1+	19	23	44	29
	2+	77	0	35	8
Islets	0	0	7	7	7
	1+	3	0	0	0
	2+	4	0	0	0

* data represents number of cases expressing the protein at a given intensity

Conclusions: Aberrant activation of the PI3K/Akt/mTOR pathway was detected in the majority of PET. In most of these cases this activation appears to be "downstream" (p-mTOR or p-S6rp immunoreactivity) and not attributable to PTEN loss/p-Akt activation. Given these findings, mTOR may represent a rational therapeutic target in PET.

Neuropathology

1482 Analysis of Natural Antisense Transcripts in Human beta-Site Amyloid Precursor Protein Cleaving Enzyme 1(*BACE1*)

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Background: Amyloid-beta (Abeta) is a pathologic hallmark of Alzheimer's disease (AD). BACE1 (beta-site APP cleaving enzyme1) is known as producing Abeta by processing the APP (amyloid precursor protein). BACE1 is a drug target for AD. Recently, natural antisense transcripts (NATs) are well known as functional RNA. If NATs possess overlap region in an opposite sense RNA, it is thought NATs have multiple functions, for example transcription or translation regulation, stabilization of sense-strand RNA, RNA editing, etc. But the functions of NATs have not been cleared in mammalian. Here, we identified some novel antisense-*BACE1* on the opposite strand of the *BACE1* loci, and investigated the interaction of the sense transcript and antisense transcripts of *BACE1*.

Design: We performed rapid amplification of cDNA end (RACE) and determined some novel natural antisense BACE1 in total RNA extracted from HEK293 cell line. Furthermore, to study the interaction of the sense and antisense RNA, HEK293 cells were transfected with siRNAs of *BACE1* and *AS-BACE1* including all splice variants to knockdown the expression of transcripts. The effect of the siRNAs on the expression of RNAs transcribed from the opposite strand was evaluated by the qRT-PCR.

Results: We identified 4 novel natural antisense transcripts. These all transcripts are spliced, and have poly (A) tail and conserved one exon against *BACE1* exon5. *AS-BACE1* has 135bp, 69bp and 36bp overlap regions against *BACE1* exon 5, 6 and 7, respectively. Interestingly, *AS4-BACE1* has long exon without splicing a part of intron. We suggested these transcripts have functions which regulate the *BACE1*. These novel transcripts were conserved one exon against *BACE1* exon5. Then, the expression of the *AS-BACE1* was suppressed by siRNA in order to analyze the role of AS in the regulation of *BACE1*. Though the knockdown of *BACE1* did not affect the expression of *AS-BACE1*, the expression of BACE1 was decreased by the knockdown of *AS-BACE1*. These results suggest that *AS-BACE1*is involved in the regulation of expression of the *BACE1*.

Conclusions: We identified 4 *AS-BACE1* which are spliced, have poly (A) tail, and conserved one exon against *BACE1* exon5. It is indicated that *AS-BACE1* equivalently expressed with *BACE1* and are concerned in the regulation of *BACE1*.

1483 Focal Myositis: A Clinicopathologic Study of 115 Cases

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Background: Focal myositis (FM) is an uncommon inflammatory pseudotumor of skeletal muscle that may be confused with a variety of neoplastic and inflammatory diseases.

Design: 206 cases coded as "focal myositis" were culled from our files. Only cases with adequate material, a solitary lesion, and correct diagnosis were included. IHC was performed.

Results: 115 FM cases were included, with 61 males, 52 females, and 2 of unknown sex. Ages ranged 7 -94 years (mean 41, median 36 years). All but 12 patients were otherwise healthy, and all but 10 lacked antecedent trauma. All patients presented with an intramusclular mass. Lesional sizes ranged from 1 .0 to 20.0 cm (median 3.0 cm, mean 3.9 cm). These were mainly located in specific muscles of the lower extremities [vastus lateralis /groin (n=39), gastrocnemius (n=22)], followed by the trunk (abdominal wall n=12), neck (submental n=8) and upper extremity. Histologically, these were composed of variable myopathic (93%) and neurogenic (89%) changes, fibrosis, and inflammation (97%) occasionally accompanied by prominent eosinophils (n=20). IHC: Most cases had CD163 positive macrophages that were negative for S100 protein and CD1a and CD3 positive lymphocytes, negative for CD20, EBER, ALK-1, TIA1 and granzyme. MHC-1 and weak IgG4 was focally positive in skeletal muscle. Cases with severe inflammation had CD20 positive/ CD123 positive cells. S100 is strongest in skeletal muscle fibers with vacuolar change. Initial pathologic diagnostic considerations ranged from malignant rhabdomyosarcoma, leiomyosarcoma, liposarcoma, lymphoma to benign rhabdomyoma, intramuscular lipoma, fibromatosis, myositis ossificans, proliferative myositis, polymyositis, and inflammatory myofibroblastic tumor. Follow-up to date reveals spontaneous regression.

Conclusions: FM occurs as a mass in specific muscle groups of young adults of both sexes without significant trauma. It is a largely unrecognized specific histologic entity that can be easily mistaken for an inflammatory myopathy or dystrophy, alternate reactive or even a neoplastic process. These appear to be macrophage and T-cell rich lesions that change to B-cell and dendritic plasmacytoid cells when markedly inflamed, but do not seem to have a known viral or molecular etiology. IgG4 presence may explain fibrosis in these lesions; IgG4 associated entities often have a autoimmune etiology. Careful attention to reproducible clinicopathologic features can aid diagnosis and spare patients from excessive surgery or adverse therapy.

1484 Pax-2(-)/Inhibin(+) Immunoprofile in Hemangioblastoma: A Helpful Combination in the Differential Diagnosis with Metastatic Clear Cell Renal Cell Carcinoma to CNS

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Background: Hemangioblstomas, which account for up to 2.5% of all intracranial tumors, may occur sporadically or as a part of the multi-system genetic syndrome von Hippel-Lindau disease (vHL). Approximately, 25% of hemangioblastomas occur as a part of vHL, which is caused by the inherited mutation of the vHL gene on chromosome 3p25-26. Patients with vHL are also at increased risk of developing clear cell renal cell carcinoma (cRCC). Distinguishing hemangioblastomas from metastatic cRCC to the central nervous system could be at times challenging on routine H&E sections. We propose a combination of Pax2 and Inhibin immunohistochemistry panel as a helpful approach to distinguishing the two lesions.

Design: Nine hemangioblastomas were retrieved from our surgical pathology archives (2005-2006). All H&E sections reviewed by two pathologists on the study. Clinical information was gathered from electronic medical records. Representative paraffin embedded section from each case was selected for immunohistochemical analysis. Eight metastatic cRCC to CNS represented on a Metastatic RCC Tissue Microarray were also used. IHC was performed using monoclonal antibodies for Pax2 (Zymed) and Inhibin (Serotec).

Results: Hemangioblastoma: Four lesions were located within the spinal cord and 5 in posterior fossa. No documented history of von Hippel-Lindau disease was seen in any of the patients. Pax2(-)/Inhibin (+) profile was exhibited by all 9/9 (100%) examined hemangioblastomas. Inhibin staining was cytoplasmic in nature. Nuclear Pax2 staining was not present in any of the 9 lesions. **Metastatic cRCC to CNS:** 5/8(63%) lesions demonstarted a Pax2(+)/Inhibin (-) immunoprofile while the remaining 3(37%) lesions were Pax2(-)/Inhibin (-).

Conclusions: We suggest a panel of Pax2 and Inhibin as a useful adjunct in the differential diagnosis of hemangioblastoma vs metastatic cRCC. In our pilot group of cases, the immuoprofile of Pax2(-)/Inhibin(+) supported the diagnosis of hemangioblastoma with a sensitivity and a specificity of 100% . On the other hand, a Pax2(+)/Inhibin(-)profile supported the diagnosis of metastatic cRCC with a sensitivity of 63% and a specificity of 100%. An expanded group of lesions is being evaluated.

1485 PAX8 as an Immunohistochemical Marker To Distinguish Metastatic Renal Cell Carcinoma from Hemangioblastoma

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Background: Renal cell carcinoma (RCC) has a predilection for distant metastasis. Metastatic RCC (mRCC) to the brain, especially clear cell type, can be a diagnostic challenge because of the overlapping histologic features with hemangioblastoma, and the coexistence of both entities in von Hippel-Lindau syndrome. Immunohistochemistry (IHC) is often needed to make the differentiation. However, most markers currently used have variable sensitivity and specificity. PAX8 is a transcription factor expressed in both fetal and adult renal epithelial cells and is required for the early development of renal lineage cells. PAX8 has been detected in renal epithelial tumors and is postulated to be a marker for tumors of renal origin. In this study, we investigate the diagnostic utility of IHC with PAX8 in the differential diagnosis of mRCC and hemangioblastoma in the brain.

Design: Twenty mRCCs and 18 hemangioblastomas were retrieved from archived material. IHC with PAX8 was performed on paraffin embedded tissue sections with the avidin-biotin peroxidase method after antigen retrieval. Distinct nuclear staining (intensity graded from 0 to 3) is considered specific. Cases with a score of 0 or 1 are considered negative and 2 or 3 positive.

Results: All mRCCs have a confirmed history of primary RCC. The mRCCs consist of 17 clear cell variants with Fuhrman nuclear grade 2 to 3, 2 with sarcomatoid features, and 1 with rhabdoid areas. Strong, diffuse PAX8 staining was detected in all 17 clear cell variants. Although no PAX8 was detected in the sarcomatoid or rhabdoid areas of the other three mRCCs, moderate to strong PAX8 staining was noted in the clear cell areas of these cases and they were considered to be positive. Of the 18 cases of hemangioblastoma, 17 were devoid of any PAX8 staining. In one case, a few scattered cells stained weakly for PAX8 in the tumor areas; the nature of these cells is under investigation. Purkinje cells and lymphocytes were noted to be positive for PAX8. Overall, PAX8 was found to have a sensitivity of 100% and a specificity of 94.4% in detection of mRCC when compared to hemangioblastoma.

Conclusions: We find that PAX8 is expressed in mRCCs in the brain and is a highly sensitive and specific marker to distinguish RCC from hemangioblastoma with similar clear cell morphology. We suggest that PAX8 should be included in the panel of differential markers for the diagnosis of mRCC in the brain.

1486 FISH-Based Molecular Assay for i17q REPA/REPB Rearrangement in Medulloblastoma; a Blinded Study

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Background: Medulloblastoma is the most common malignant brain tumor in children, accounting for 20 percent of all pediatric central nervous system tumors. Despite its relatively high incidence, the biology of this entity is poorly understood. Diagnosis is made based on morphology (histology), and prognosis is dependant on staging, tumor size, and age of onset. However, clinical factors do not accurately predict which standard-risk patients will have early relapse and die. Outcome is believed to be influenced by the presence of certain molecular markers, including N-MYC and C-MYC amplification, TrkC expression, and the presence of i17q chromosome. i17q has been shown to correlate with poor prognosis; however, data demonstrating that it is of prognostic value independently of anaplasia are lacking. Furthermore, i17q is typically identified by karotyping analysis, which necessitates the laborious process of culturing tumor cells and isolating mitotic figures. In a majority of cases, i17q is not a true isochromosome but an isodicentric chromosome (idic(17)(p11.2)), with rearrangement breakpoints within the REPA/REPB low-copy repeat element region in 17p11.2. Low-copy repeat elements are large DNA elements (larger than 1 kb) with greater than 90% homology that facilitate rearrangements by non-allelic homologous recombination.

Design: We have developed a FISH-based assay to identify rearrangements of the REPA/REPB region in formalin-fixed, paraffin-embedded tumor tissue specimens. This assay allows for the relatively rapid identification of i17q in archival specimens, as well as for the study of REPA/REPB rearrangements in medulloblastoma. We compared this novel FISH-based approach to standard karyotyping analysis in 17 archived medulloblastoma cases in a blinded fashion.

Results: Of 17 cases analyzed, the presence or absence of i17q was identified in 16 in agreement with the karyotype reports, with one false negative. Further investigation will determine the independent prognostic significance of REPA/REPB rearrangement in medulloblastoma.

Conclusions: This novel FISH-based assay provides a rapid and accurate method to detect i17q compared to karyotype analysis, and should facilitate further investigation of i17q in medulloblastoma.

1487 Evaluating Intraobserver Reliability in a Classification of Malformations of Cortical Development (MCD)

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Background: Malformations of cortical development (MCD) are a well-recognized cause of intractable epilepsy. A simplified histologic classification of MCD has been proposed (Palmini A et al. Terminology and classification of the cortical dysplasias. Neurology 2004(62)6;S3,S2-8). The current study examines intraobserver agreement using this classification system, and is a follow-up of a previous analysis of interobserver reliability.

Design: 26 epilepsy resections were selected representing the range of lesions in this classification system. 8 neuropathologists (NPs) classified each case as either: normal &/or gliosis, mild MCD (Types I & II), FCD Type IA, FCD Type IB, FCD Type IIA, or FCD Type IIB. After a minimum of 6 weeks (and after interobserver results had been returned), the material (differently coded) was resent for a second evaluation. Each NP's responses were compared to the original, and intraobserver rates of agreement using kappa analysis were determined.

Results: Intraobserver concordance among the eight NPs for the 26 cases ranged from moderate to very good (range k=0.4654-0.8504, mean k=0.6756). Of the 208 (8 NPs each classifying 26 cases) classifications originally made, 52 (25%) changed on a second viewing. Only 4.2% of the original FCD Type IIB diagnoses changed on second evaluation compared with other categories where 28.9-52.9% of diagnoses changed. Mild MCD (Types I & II) had the greatest incidence of intraobserver variability (52.9%).

Diagnosis variability in a classfication system for MCDs.

Category	# Original Classifications	# Changed on Reevaluation	% Change
Normal & / or gliosis	24	9	37.5
Mild MCD (Types I & II)	17	9	52.9
FCD Type IA	35	11	31.4
FCD Type IB	22	9	40.9
FCD Type IIA	38	11	28.9
FCD Type IIB	72	3	4.2
Total Classifications (8 NPs x 26 cases)	208	52	25

Conclusions: Intraobserver concordance using this classification system ranged from moderate to very good, underscoring limitations to this system. Intraobserver reliability was greatest for FCD Type IIB and least for mild MCD and FCD Type IB.

1488 1p/19q Deletion in Brain Tumors: An Innovative Approach for Automated FISH Analysis

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Background: The prognostic and predictive value of 1p/19q deletion in brain tumors has been addressed during the last few years. In particular, the 1p/19q combined loss has been described in more than 70% of oligodendrogliomas, and this genetic alteration is reportedly associated with a better chemo- and radiotherapy response and overall survival rates. Several technical approaches have been devised for the 1p/19q status assessment, including fluorescent in situ hybridization (FISH), comparative genomic hybridization**,** and loss of heterozygosity. Although FISH is most commonly used, the resulting analysis is labor intensive and time consuming. In addition, the cutoff deletion limits are often not standardized. Although an automated system for FISH analysis of hybridization patterns may represent a valid alternative to the shortcomings of visual counting techniques, published data comparing the systems are currently limited.

Design: FISH analysis for 1p/19q loss was performed on paraffin sections obtained from 83 primary brain tumors. Tumors included oligoastrocytomas (*n*=32, 39%), oligodendrogliomas (*n*=24, 29%), astrocytomas (*n*=15, 18%), high-grade gliomas (*n*=9, 11%), and other brain tumors (*n*=3, 3%). Commercial probes against 1p36/1q25 and 19q13/19p13 (Abbott) were used. An automatic system for FISH analysis (Metafer 4, Metasystems, Altlussheim, Germany) was applied for reevaluation of visually screened samples. We compared manual and automatic scoring systems in both pathologic and non-neoplastic perilesional tissues.

Results: The concordance rate of manual versus automatic score was near total (97%) for non-neoplastic areas and virtually total (>99%) for tumoral areas. In about 10% of scannings (17/166), the results were deemed not reliable because of microscopic features that were capable of preventing adequate signaling interpretation, such as tissue necrosis, suboptimal sample preparation, fluorochrome bleaching, and excessive tissue autofluorescence.

Conclusions: An automatic system for FISH analysis proved superior to visual counting because it allowed a faster and reliable evaluation of a greater number of tumor cell nuclei and prevented potential observer bias in the selection of both normal and tumor nuclei. Manual review of the image gallery after automatic analysis is effective in obtaining more reliable results. This system may improve the overall FISH testing efficiency, especially in high-volume laboratories.

1489 Neuronal Markers Expression and Outcome in Glioblastoma

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Background: Recent studies have shown that high grade gliomas, particularly glioblastomas (GBM) featuring giant cells, often demonstrate immunoreactivity for neuronal markers. Such tumors were said to be associated with better outcome.

Design: Expression for synaptophysin, neurofilament protein, Neu-N,chromogranin and GFAP was analyzed in 82 cases, including 11 fibrillary, 8 gemistocytic, 40 giant cell and 23 small cell GBM. Median time to recurrence and death (separately) was estimated using Kaplan-Meier methods. Time to recurrence (or death) was compared in tumors between presence (GBMpos) or absence (GBMneg) of markers via Cox Proportional Hazards regression. The outcome (recurrence or death) was also compared between GBM expressing only one versus two or more neuronal markers. Giant cell and small cell categories were also individually analyzed. P-values less than 0.05 were considered statistically significant. All analyses were performed using SAS v. 8 (Cary, NC).

Results: Patient population included 48 males and 34 females with mean age of 54 at the time of diagnosis. Fourty five of 82 cases (54.8%) of GBM (5 fibrillary, 5 gemistocytic, 30 giant cell and 5 small cell) expressed at least one neuronal marker, synaptophysin being the most common (95%). At least one neuronal marker was expressed in 75% of giant cell, 62.5 % of gemistocytic, 45% of fibrillary and 21% of small cell GBM. There was no statistical significant difference in recurrence and survival time intervals between overall GBMpos and GBMneg as well as between GBMpos and GBMneg within giant cell and small cell categories. However, among those with positive markers, there was

a significant difference in recurrence in the giant cell category between those with two or more markers versus a single marker (107 days vs. 265 days for two or more versus one marker, respectively, p = 0.02). Similarly, the risk of death was significantly higher for those with two or more markers as compared to those with a single marker (208 days vs. 258 days, p=0.03), and this difference looks to be driven by those within the giant cell category in particular (123 days vs. 295 days, p=0.026). This difference was not observed in small cell GBM category.
Conclusions: Neuronal markers expression is frequently present in various categories of GBM. Giant cell GBMs expressing 2 or more neuronal markers were associated with higher risk of recurrence and death as compared with giant cell GBM expressing a single neuronal marker.

1490 1p/19q Loss of Heterozygoty Patterns in Infiltrative Gliomas

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Background: Loss of heterozygoty (LOH) of 1p and 19q chromosomal arms has been mainly reported in oligodendrogliomas. 1p/19q codeletion is currently considered as a reliable predictive and/or prognostic marker. However, reported deletion patterns are variable: telomeric, interstitial, centromeric or involving whole arms. Consequently, their identification depends on the method used, particularly with LOH and FISH assays. In addition, prognosis could differ according to the 1p deletion pattern.
Design: We studied 1p and 19q deletion patterns in 42 gliomas. There were: 19 astrocytomas, 1 grade II (AII), 6 grade III (AIII) and 12 glioblastomas (GB); 14 oligodendrogliomas, 7 grade II (OII) and 7 grade III (OIII); 9 oligoastrocytomas, 4 grade II (OAII) and 5 grade III (OAIII). LOH was assessed by PCR and capillary electrophoresis on histologically selected areas from paraffin blocks vs. blood DNA, using 16 and 7 microsatellite markers spanning the entire arms (from centromere to telomere) of 1p and 19q chromosomes, respectively.
Results: We identified 5 molecular patterns: whole arm deletion (wLOH), telomeric deletion (tLOH), interstitial deletion, scattered deletion and no LOH. 1p wLOH was linked to the histological type (p=0.0006): presence in 64% of the oligodendrogliomas (9/14) without correlation with grade (p=0.35); presence in only one mixed OAIII; absence in all the AII, AIII and GB. 1p wLOH was strongly associated with 19q wLOH (p<0.0001) and this whole 1p/19q deletion was restricted to the 9 oligodendrogliomas. A 1p tLOH was found in 1 AIII, 1 OIII and 2 OAIII that was associated with a 19q tLOH in 1 OAIII. All but one of these tumors recurred within a year and had a worse prognosis than the other tumors of same histology. The other molecular patterns were not correlated with histological type and grade while 12 astrocytic tumors had no LOH.
Conclusions: Our study points out the usefulness of an entire 1p and 19q chromosome arms analysis. Our results confirm that whole 1p/19q codeletion is a marker of classical oligodendrogliomas although there is no strict concordance between histological and molecular data since this codeletion is absent in about one third of cases. The presence of a 1p/19q wLOH in OII as well as in OIII may indicate that this molecular alteration is an early event in oligodendroglioma carcinogenesis. Regarding prognosis, tLOH could be pejorative and we suggest that results of LOH and FISH assays targeted only on the telomeric regions should be carefully interpreted.

1491 Maternal Immune Activation Causes Overgrowth of the Fetal Cerebral Cortex

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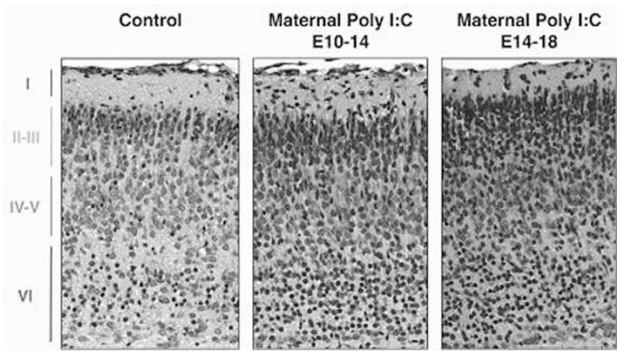
Background: Maternal infection during fetal brain development has been implicated as an important factor in the development of autism and schizophrenia, but a definitive link between the maternal immune response and altered fetal brain development has not been demonstrated histopathologically. At an early age, autistic children display a significant increase in the thickness of the cortical mantle. The cause of this increase remains unknown, but recent evidence indicates the presence of maternal-fetal antibodies in autism. Other cases of autism have been associated with perinatal viral infection. We investigated whether activation of the maternal immune system might be the common factor that is sufficient to promote a disturbance of fetal brain development using an agent that mimics a maternal viral infection, polyriboinosinic-polyribocytidilic acid (polyI:C).
Design: Daily intraperitoneal injections of polyI:C (5 mg/kg) were performed on E10-E14 or E14-E18. Mouse pups were sacrificed on the first day of life (P1), and the brains were processed for routine histological analysis. Photomicrographs of hematoxylin and eosin stained sections were analyzed using ImageJ (NIH) software to determine cell density in layers I, II-III, IV-V, and VI of the S1H1 area of the cerebral cortex.
Results: E10-E14 mice (n=16) displayed increased cell densities in all cortical layers when compared to controls (n=15): 49% in layer I, 23% in layers II-III, 15% in layers IV-V, and 25% in layer VI (p<0.01). E14-E18 mice (n=16) showed similar increases in layers I, IV-V, and VI: 50%, 17%, and 26%, respectively (p<0.0001). In layers II-III, however, there was a larger increase of 47% (p<0.0001).



Conclusions: The results support our hypothesis that maternal immune activation alone, potentially caused by a variety of insults (e.g. infection, autoimmunity), during pregnancy is sufficient to disturb fetal brain development. In addition, the data suggest the timing of immune activation has layer-specific effects, providing a potential explanation for different behavioral disorders (e.g. autism, schizophrenia). This cortical overgrowth may promote the development of autism and/or schizophrenia through disturbances of the canonical cortical neural circuit architecture and function.

1492 Expression of Annexin A1 and Constitutive Activation of the Akt/mTOR Pathway in High-Grade Diffuse Gliomas: A Potential Connection with S Phase Kinase-Associated Protein (Skp2) in Glioma Progression

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Background: Diffuse gliomas constitute the most common type of malignant primary brain tumor. Gliomas of astrocytic lineage have been shown to have a worse prognosis compared to those of oligodendrocytic lineage of similar grade. Annexin A1 (ANXA1) is a calcium binding protein implicated in the EGFR tyrosine kinase pathway. In glioblastomas, amplification of EGFR has been associated with Akt pathway activation. ANXA1 has been shown to be a binding partner of Akt by retrovirus-based protein complementation assay. Similarly, a downstream effector of the Akt/mTOR pathway, Skp2 is overexpressed in a number of malignancies and its downregulation is reported to induce apoptosis in T98G glioma cells. The objective of this study is to evaluate Akt and its downstream effectors, mammalian target of rapamycin (mTOR) and Skp2 in relation with ANXA1 in the different types and grades of diffuse gliomas.
Design: A tissue microarray was assembled from gliomas diagnosed according to the WHO 2007 classification which included: 24 oligodendrogliomas (OL)(Grade II), 10 mixed oligoastrocytomas (MOA)(Grade II), 22 anaplastic oligodendrogliomas (AO) (Grade III), 11 anaplastic mixed oligoastrocytomas (AMOA)(Grade III), 24 anaplastic astrocytomas (AA)(Grade III), 8 gliosarcomas (GS)(Grade IV) and 56 glioblastomas (GBM)(Grade IV). ANXA1, Akt, mTOR and Skp2 expression was determined by immunohistochemistry. Positive results were >30% stained with an intensity of 2 or higher.
Results: ANXA1 was expressed in 51% of GBM, 88% of GS and 27% of AA. In contrast, ANXA1 was expressed in only 9% of AO and none of the low-grade OL. Similarly, Skp2 was expressed in 54% of GBM, 29% of GS, 5% of AA, 6% of AO and none of the OL. ANXA1 and Skp2 expression showed a positive correlation and were present in higher grade gliomas (p<0.0002). Akt and mTOR were expressed in most of the tumor types evaluated and showed no significant correlation to tumor type or grade.
Conclusions: ANXA1 was coexpressed with Skp2 in tumors with an astrocytic component and in higher grade gliomas. Our results confirm the role of the Akt/mTOR pathway in gliomas and support an important role for ANXA1 and Skp2 in the progression to higher grade astrocytic gliomas.

1493 Comparision of EGFR Immunohistochemistry of wt-EGFR with EGFRvIII and Correlation with EGFR Gene Amplification in Glioblastoma Multiforme

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Background: Glioblastoma multiforme (GBM) is the most common and most malignant of glial tumors. Many studies have shown the presence of molecular genetic alterations in GBM, the most frequent of which is Epidermal growth factor receptor (EGFR) gene amplification. This leads to an overexpression of EGFR protein. Majority of GBMs with this amplification show a variety of qualitative EGFR alterations, resulting in different EGFR mutations. The most common EGFR mutation is EGFRvIII (deletion of exons 2-7) which presumably occurs through alternative splicing or gene rearrangements. Unlike wild type(wt) EGFR, EGFRvIII has a truncated extracellular ligand-binding domain which lends it a ligand-independent constituitive activity and enhanced tumorigenecity. EGFR amplification is a very strong indicator of poor survival prognosis. We have studied the expression of wt and mutated EGFRvIII and correlated the findings with gene amplification in a group of patients with GBM.
Design: Twenty three cases of Glioblastoma multiforme were selected, of which twelve were males and eleven were females, ages (20 to 84). We analysed the protein expression of wtEGFR, EGFR deletion mutant variant III (EGVRvIII) and performed gene amplification for EGFR. After histology review, 5 micron thin sections were cut from formalin fixed paraffin embedded tissue blocks and specific monoclonal antibodies for EGFR, EGFRvIII were used for immunohistochmical detection of the same. We then used fluorescent in situ hybridization (FISH) to assess the EGFR gene amplification in

these cases. The cases that were positive for EGFRvIII expression were selected and a dual probe FISH assay was performed on paraffin embedded sections with locus specific probes for EGFR and the centromere of chromosome 7 (CEP 7).
Results: Of the 23 patients of glioblastoma, eleven (47.3%) expressed EGFRvIII and nine (39.1%) expressed EGFR. When they were analysed for gene amplification by FISH, of the eleven patients that expressed EGFRvIII, six (54.5%) showed high amplification, three (27.2%) showed extra chromosomes and two (18.1%) were normal.
Conclusions: 1) EGFR over-expression is a common finding in Glioblastoma Multiforme. 2) Gene amplification is seen in about half of the cases with EGFRvIII expression. 3) High level amplification was seen in cases with positive wild type and mutant proteins. 4) Immunohistochemical analysis of wild type EGFR alone may not be sufficient to determine EGFR status in GBM.

1494 The Oncofetal Protein IMP3: A Novel Molecular Marker To Predict Aggressive Meningioma

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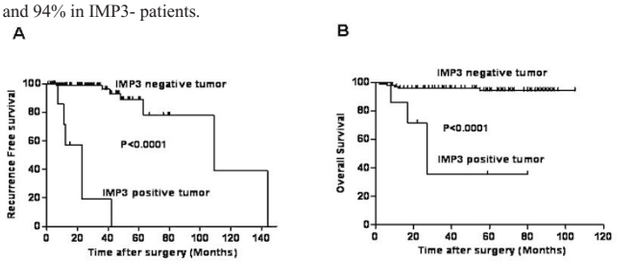
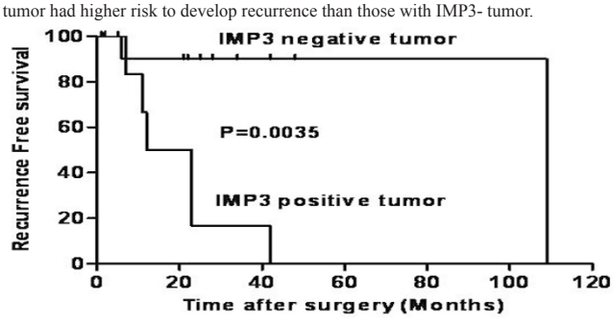
Background: One of major clinical challenges is to predict recurrence of meningioma. In this study, we investigated whether IMP3, an oncofetal RNA-binding protein, can be used as a new biomarker to predict the recurrence and overall survival of meningiomas.
Design: 107 patients with primary brain meningiomas were investigated the expression of IMP3 by immunohistochemistry, and were further evaluated for survival analysis.
Results: Tumor recurrence was found in 13 of 107 patients with primary meningioma. Seven (6.5%) of 107 patients' meningiomas expressed IMP3. Kaplan-Meier plots and log-rank tests showed that patients with IMP3+ tumors had higher recurrent rate (P=0.0035), poor overall survival (P<0.0001) than those with IMP3- tumors. The 5-year recurrence-free and overall survival rates were 0% and 36% in IMP3+ patients vs. 89% and 94% in IMP3- patients.



Multivariate analysis of IMP3 status in primary tumors showed hazard ratio of 21.68 for recurrence (P= 0.006) and 14.45 for overall survival (P=0.001) , which were much higher than hazard ratio associated with other risk factors. Analysis of recurrence-free survival in grade 2 and 3 tumor patients showed that grade 2 or 3 patients with IMP3+ tumor had higher risk to develop recurrence than those with IMP3- tumor.



The median recurrence-free survival was 17.5 months in patients with IMP3+ tumors vs 109 months in patients with IMP3- tumors.
Conclusions: IMP3 is an independent prognostic marker that can be used at initial diagnosis of meningioma to identify patients who have high potential to develop recurrence.

1495 Pathological-Clinical Evaluation of High-Grade Gliomas in Children with Neurofibromatosis Type I

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Background: Although Neurofibromatosis Type 1 (NF1) is one of the most frequent autosomal dominant disorders characterized by tumors of the central and peripheral nervous system, only very few pathologic studies analyze high grade gliomas in this group of patients. The gene product of the NF1 gene, neurofibromin, is an important regulator of cell proliferation and differentiation through negative interaction with the ras protein. Patients with NF1 are at increased risk of developing high grade malignant gliomas and glioblastomas, which are rare tumors in children and even less frequently observed in children with NF1. The objective of this study was to provide a clinicopathologic and molecular characterization of high grade gliomas in children with Neurofibromatosis Type I, and to determine whether specific pathologic or molecular features predict clinical behavior.
Design: We performed a retrospective review of patients with NF1 and high grade gliomas at the Childrens Hospital in Boston, which included the analysis of clinical records and pathological materials. Only patients who satisfied established current clinical criteria for NF1 and for whom pathologic material was available were included.
Results: A total of 6 patients were identified with NF1 and high grade gliomas. Clinical data demonstrate an average overall survival time of 5.2 years. The evaluation of H&E and immunohistochemical stains revealed highly pleomorphic GFAP-positive tumor cells with frequent mitoses in addition to necrosis and vascular proliferation. Molecular analyses displayed amplification of epidermal growth factor receptor copy numbers (EGFR-CISH), normal copy numbers for PTEN (PTEN-CISH), and hypomethylation of the MGMT locus.
Conclusions: This study provides preliminary evidence that children with NF1 develop high grade gliomas with unfavorable histological and molecular features, however, these patients have an overall increased survival time compared to children without NF1. These tumors will be studied with additional genomics/proteomics approaches to elicit molecular signatures unique for high grade gliomas in children with NF1.

1496 The Pathology of the Border of Glioblastoma Evaluated by 5 Aminolevulinic Fluorescence-Guided Resection

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Background: Gross total resection is increasingly recognized as an important first step and prognostic factor in the treatment of glioblastoma. The removal of the entire MRI enhancing lesion is accepted as the gold standard of gross total resection, although the frequency of verified total resection is suboptimal. Fluorescence-guided resection of glioblastoma using 5 aminolevulinic acid (5-ala) (Gliolan®), a fluorescent molecule which is incorporated in tumoral cells before the surgery, is a new surgical technique recently approved. Histopathological characterization of peripheral tumor cells (border zone), including the recently described CD133 positive cells (stem cells) were studied, and correlation with fluorescence was obtained.
Design: Eighteen consecutive glioblastomas, six were recurrent cases, of the same number of patients were resected by 5-ala guided technique. Volumetric MRI measurement was done. A total of 122 biopsies including 65 biopsies from the border of the lesion, as shown by the fluorescence, were studied. A conventional and immunohistochemical study including Ki-67 (Dako) and CD133 (Miltenyi Biotec) by amplification method (Novocastra) was applied. A semiquantitative evaluation of each case was obtained. Appropiate statistical tests were applied.
Results: In all cases, it was seen under the fluorescence three different zones: bright red, predominantly in the center of the tumor and a gradually less intense and non-fluorescent (blue areas) in the periphery. Interestingly, the pink areas always corresponded with atypical tumor cells, never with solid tumor, frequently less atypical than the tumoral cells in the center of the lesion. In general, blue areas corresponded with normal tissue or slight increased cellular density. In border areas, the presence of immunoreactive cells against Ki-67 was considered as tumoral ones. CD133 positive cells were non-observed in the border of tumor. The correlation with the histopathology was excellent: 100% specificity in non-recurrent cases and 94% sensitivity. A 100% resection was achieved as measured by MRI in 7 cases.
Conclusions: Border placed glioblastoma cells as it is identified by 5-ala guided resection are less atypical than the conventional ones, get a low proliferative index and are not immunoreactive against CD133 (stem cells). According to the pathological results, this surgical technique is a very efficient and safe method of achieving maximal safe resection of glioblastoma.

1497 The Spectrum of Pilomyxoid Astrocytomas (PMA): Transitional Pilomyxoid Tumors

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Background: The term PMA was first applied in 2004 to a generally pediatric brain tumor with small monomorphous bipolar cells, perivascular pseudorosettes, and abundant myxoid substance. The same tumor had been designated earlier as "infantile pilocytic astrocytoma". The 2007 World Health Organization considers the PMA a variant of pilocytic astrocyoma (PA), and grade II in light of the overall more aggressive nature. In spite of subsequent experience with the lesion, the pathological spectrum, degree of overlap, and transitions with PA are unclear.
Design: We evaluated 117 primary brain tumors that had either been given the diagnosis of classic PMA or had been specifically noted to have some features of PMA. We reviewed cases in a blinded fashion and classified the tumors as 'classic PMA' or 'similar to but different from the classic PMA' according to our interpretation of current diagnostic criteria. Classic PA and other WHO entities were excluded to leave 82 total cases. For each case we noted the presence of a number of histologic features, including: eosinophilic granular bodies (EGBs), Rosenthal fibers (RFs), microcysts, perivascular rosettes, mitoses, necrosis, vascular proliferation, oligodendroglial-like components, calcification, and myxoid substance.
Results: 38 of the cases were classic PMA. Some of the remaining 44 cases were more PMA-like in that they were predominantly monomorphous with myxoid substance and prominent perivascular-pseudorosettes. Some demonstrated prominent features more typically found in PA, such as areas of dense fibrillar structure, EGBs, RFs, and insipient biphasic architecture. Patients with transitional features were older at diagnosis than were patients with more classic PMA morphology (77 months vs. 40 months; p=0.01), and the presence of microcystic change was a feature that was more likely in tumors in older children within the group (56 months vs 33 months; p<0.05).
Conclusions: There was a wide spectrum of histologic features within this group of low grade astrocytic tumors with pilomyxoid features. Indeed a large number of the cases were histologically transitional—sharing features of both PMA and PA. The presence of features more often seen in PA may suggest a continuum of differentiation toward

pilocytic morphology. This observation supports the hypothesis that PMA and PA are variants of the same diagnostic entity.

1498 Induction of Ikaros Transcription Factors during Neuronal Hypoxia

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Background: During stroke, injury or global cerebral hypoperfusion, the amount of oxygen available to neurons of the brain is markedly reduced. Adaptation mechanisms to neuronal hypoxia may include the suppression of energy metabolism, global alterations in gene expression and ultimately the activation or repression of stress-responsive genes. Prolonged periods of hypoxia eventually lead to cell cycle arrest, apoptosis, and necrosis. Our preliminary observations have indicated that Ikaros (Ik), a family of zinc-finger transcription factors that is essential in the development and function of leukocytes and the pituitary, is expressed in neuronal cells under hypoxic-ischemic conditions.

Design: A neuronal cell culture model was established. P19 embryonal carcinoma cells were differentiated into mature neuronal cells by treatment with retinoic acid for 96h. Cells were then placed into a nearly anoxic environment of 0.2 % oxygen for 1, 2, 4, 6, 12 and 24h. They were assessed for Ik expression by immunohistochemistry, qRT-PCR and western blotting. For assessment in vivo, adult wild-type mice were subjected to low oxygen concentrations (5%) for up to 6 hours.

Results: Ik expression levels were markedly elevated in P19 cells after more than 12h in the ultra-low oxygen environment. Ik was also detected in the brains of mice that were subjected to low oxygen concentrations for over 4 hours. Cortical neurons, hippocampal neurons and cerebellar Purkinje cells all displayed strong nuclear and mild cytoplasmic Ik-immunoreactivity when compared to controls at normoxia. Highly similar observations were made in human brain tissue from neurosurgical procedures that showed morphologic evidence of hypoxia-ischemia.

Conclusions: Our finding that hypoxic states strongly induce Ik in several neuronal systems adds novelty to the still limited knowledge of the epigenetic events that occur in response to hypoxia. A better understanding of these chromatin changes could improve the treatment of stroke and CNS trauma. Future studies will clarify whether this response is limited to neuronal cells or whether it may represent a general cellular adaptation mechanism to a low oxygen environment.

1499 1p and 19q FISH Study of the Glioblastomas

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Background: The fluorescence in situ hybridization (FISH) of the 1p/19q used to be evaluated by the ratio of the numbers of red spots and the green spots(deletion: the ratio <0.8). Recently, however, Nagasaka et al. applied the evaluation guideline for neuroblastoma for investigating 1p/19q FISH status of the glioblastoma (GBM). By the guideline for neuroblastoma, the deletions by the ratio (<0.8) include definitive deletion, disproportional deletion and imbalance and the normal ratio of 1p/19q (ratio: 1) include true normal, monosomy or polysomy. The purpose of the present study is to verify the 1p and 19q status of 52 cases of GBMs. Serving as the control were 30 cases of anaplastic oligodendrogliomas.

Design: We performed a clinicopathologic review as well as immunohistochemical (GFAP, p53, EGFR, EGFRvIII, PTEN, MGMT, galectin-3 and MIB-1) and molecular studies (EGFR FISH, 1p19q deletion FISH, MGMT MSP) to characterize 1p and/or 19 abnormal GBMs.

Results: Using the simple ratio <0.8, 1p and19q deletions were 25% (13/52), and 19.2% (10/52) of GBMs, respectively, which included a 7.7% (4/52) of co-deletion. However, with the guideline for neuroblastoma, true 1p deletion was 15.3% (8/52), true 19q deletion was 13.4% (7/52) and 1p/19q co-deletion was 5.8% (3/52), which included definitive deletion and disproportional deletion. The remaining 5 cases (9.6%) of 1p deletion and 3 cases (5.8%) of 19q deletion by ratio were imbalanced. When they were evaluated by ratio, there were no statistically significant immunohistochemical and molecular markers of the above mentioned parameters to characterize 1p and/ or 19q detected GBMs except MGMT MSP and galectin 3; there was more MGMT MSP positivity (5/6, 83.3%) in 19q deletion and less galectin 3 protein expression in 1p and/or 19q deleted group ($p<0.05$). When they were evaluated by the guideline for neuroblastoma, 1p/19q deleted group showed male predominance and lower galectin-3 expression ($p<0.05$). In the Kaplan Meier survival analysis, there was no significant survival difference between 1p/19q deleted and non-deleted groups evaluated by any evaluation methods. However, anaplastic oligodendroglioma with 1p and/or19q deletion showed a significantly higher true deletion (78.6%) than glioblastoma (42.1%) ($p=0.011$).

Conclusions: From our study, we could not find any significant immunohistochemical or molecular markers to characterize 1p/19q deleted GBM except MGMT-MSP and galectin-3. In order to identify a more accurate status of 1p/19q, the guideline for neuroblastoma appears to be more appropriate than an evaluation just by ratio.

1500 Cerebral Amyloidoma: A Report of Three Cases

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Background: Amyloidomas, or tumor-like aggregates of amyloid, are very uncommon within the central nervous system, with approximately 30 cases reported to date. The most common clinical presentations of cerebral amyloidomas are seizures, hemiparesis, gait disturbance, visual abnormalities, and cognitive impairment.

Design: A review of the Emory University electronic medical record from 1988-2008 revealed three cases of primary cerebral amyloidoma. The slides, radiology and medical records were reviewed for each case.

Results:

Age	Sex	Symptoms	Location	Neuroimaging	Comorbidities	Treatment	Pathology
45	Female	Left-sided headache, sudden onset mental status changes, status epilepticus	Right posterior parietal	Contrast enhancing mass on MRI	Migraines	Biopsy only	Amyloid, Congo red +
46	Female	Nausea, slurred speech, "pressure" on right side of head, seizures with aura	Right temporal lobe extending into anterior parietal lobe	Contrast enhancing mass on MRI	Multiple sclerosis	Excision	Amyloid, Congo red +
72	Female	Ataxia, slurred speech	Left pons, right basal ganglia at Foramen of Monro	Contrast enhancing mass on MRI	Coronary artery disease, Hypertension, Hypothyroidism	Biopsy only	Amyloid, Congo red +

The mean age at presentation was 54.3 years. All cases presented as enhancing mass lesions confined to the brain. Prior to biopsy, patients 1 and 3 were felt clinically to have high-grade glioma and CNS lymphoma, respectively. Patient 2 was thought to have a demyelinating lesion. None of the patients had evidence of systemic amyloidosis. Serum protein electrophoresis performed on patient number 3 was within normal limits.

Conclusions: Cerebral amyloidomas are uncommon lesions that present with a variety of symptoms and may manifest clinically as malignant neoplasms. The second patient in our series is the third reported case of amyloidoma in association with multiple sclerosis. This may represent a rare, and as of yet unrecognized, sequela of multiple sclerosis.

1501 A Microregional Comparison of EGFR Amplification and MIB-1 Proliferative Index in High-Grade Astrocytomas

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Background: Amplification of the epidermal growth factor receptor (*EGFR*) gene is a genetic hallmark of glioblastoma (GBM), seen in approximately 40% of these biologically aggressive neoplasms. While it is widely assumed that *EGFR* amplification correlates with biologic activity, relationships with proliferation, invasiveness, and angiogenesis have not been clearly demonstrated in human tissue. In order to begin to address this issue, we examined the relationship between microregional *EGFR* amplification and MIB-1 proliferation indices in *EGFR*-amplified and non-amplified GBMs and non-amplified anaplastic astrocytomas (AA).

Design: Sections from 3 *EGFR*-amplified GBMs, 3 non-amplified GBMs, and 3 AAs were evaluated for regional variation in MIB-1 immunohistochemical staining indices. FISH was performed using dual color probes which hybridized to the *EGFR* gene and to the centromere of chromosome 7. Amplification was defined as a ratio of *EGFR*/ centromere signal ratio of ≥ 2. *EGFR*/centromere ratios were obtained from selected regions of each GBM and AA specimen (average 6 regions per section, 25 cells per region). *EGFR* amplification status and degree were correlated with MIB-1 proliferation in each tumor region.

Results: The degree of *EGFR* amplification varied considerably among the 18 regions of 3 *EGFR*-amplified GBMs, and did not show overall correlation with proliferative index ($r_s = 0.11$). Taken individually, one *EGFR*-amplified GBM showed a positive correlation between degree of amplification and MIB-1 index ($r_s = 0.61$) and two showed a negative correlation (r_s=-0.60 and -0.29). None of the correlations achieved statistical significance. The MIB-1 proliferation index was variable among the 18 selected regions in *EGFR*-amplified GBMs (mean +/- standard deviation = 24.9% +/- 17.6%, coefficient of variation = 73.1%), compared to non-amplified GBMs (14.4% +/- 9.4%, CV= 65.4%) and AAs (6.8% +/- 3.2%, CV=47.2%). The mean MIB-1 index and the CVs of *EGFR*-amplified GBMs were significantly higher than those of non-amplified GBMs ($p<0.05$).

Conclusions: From our limited data set, it appears that *EGFR* amplification in GBM is associated with an overall increase in MIB-1 proliferative index, but that the degree of amplification does not significantly correlate with the proliferative index within a given tumor microregion. High-grade astrocytomas show intratumoral heterogeneity of MIB-1 proliferation indices, and *EGFR* amplification appears to enhance this variability.

1502 Pituicytoma Histogenesis: TTF-1 Expression in Normal and Neoplastic Pituicytes

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Background: Pituicytomas are rare, generally low grade pituitary neoplasms of uncertain histogenesis. Their morphology and variable immunophenotype has led to speculation that they arise either from pituicytes of the neurohypophysis or from folliculostellate cells of the adenohypophysis. Given the role of TTF-1 in the developing rodent infundibulum and the expression of TTF-1 within the adult rat neurohypophysis, we speculated that TTF-1 expression may be a marker of human pituicytes and thereby help delineate the histogenesis of pituicytomas.

Design: Morphologic and immunohistochemical analysis was performed on normal and neoplastic pituitary tissue including pituitary glands obtained at autopsy (n=5), non-neoplastic neurohypophysis obtained incidentally at surgical biopsy (n=5), pituitary adenomas (n=21), pituicytomas (n=3) and atypical spindle cell tumors of the sellar region (n=2). Nuclear TTF-1 expression was assessed by immunohistochemistry using two monoclonal anti-TTF-1 antibodies (clones 8G7G3/1 and SPT24).

Results: TTF-1 is expressed in normal pituicytes of the human neurohypophysis, while no expression was seen in other cell types of the pituitary gland including folliculostellate cells. Furthermore, pituitary adenomas show essentially no TTF-1 expression with the exception of a single null-cell adenoma which exhibited rare individual cells expressing TTF-1. In contrast, all three pituictyomas available for examination showed robust TTF-1 expression. Two higher grade spindle cell tumors of the sellar region also demonstrated TTF-1 expression.

Conclusions: In addition to its use as a marker for lung adenocarcioma and thyroid carcinoma, TTF-1 immunohistochemistry can be useful in confirming the diagnosis of pituicytoma on histologic material. Furthermore, our findings indicate that pituictyomas are histogenetically related to the pituicyte lineage, and not folliculostellate cells of the

adenohypophysis. Finally, based on two index cases, we suggest that the category of pituictyomas should be expanded to include atypical spindle cell variants.

1503 Inflammatory Pseudotumors of the Central Nervous System

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Background: Inflammatory pseudotumor(IPT) is a disease with unsettled pathogenesis. The aim of this study is to investigate ALK-1 protein expression and IgG4-positive plasma cells(PC) in 3 intracranial IPTs.

Design: Three intracranial IPTs and the corresponding clinical information were retrieved from hospital archive.

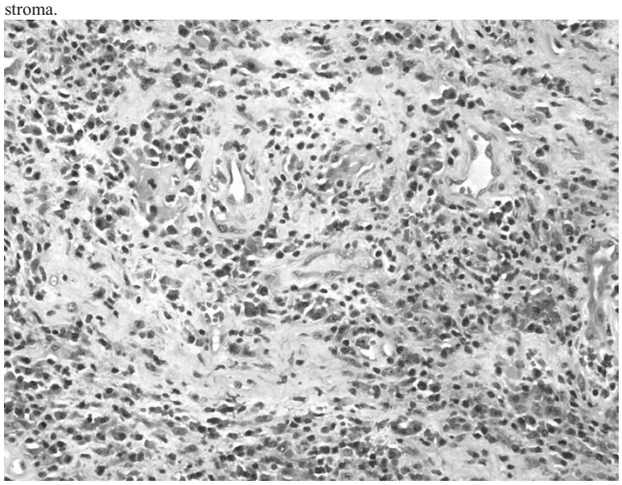
table 1

Location	Operation	No. of IgG4+ve PC/HPF(40x)
Cerebral falx & tentorium	Resection & radiotherapy	17
Right lateral ventricle	Resection	46
Right frontal region	Resection	41

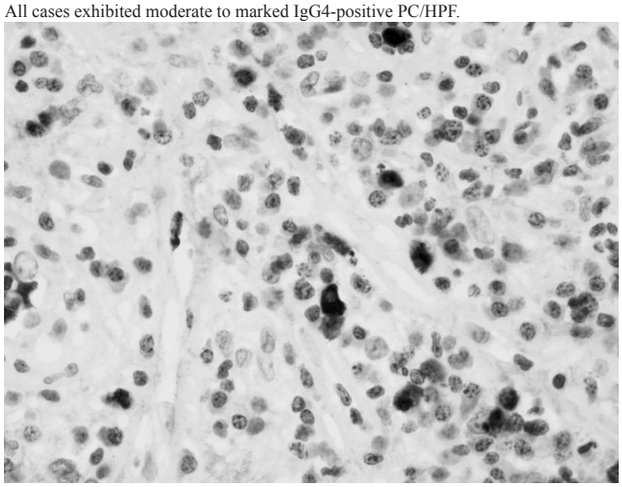
Clinical information and IgG4-positive plasma cell density in 3 patients.

Sections were examined under HE and immunohistochemical stainings including ALK-1 and IgG4.

Results: All cases displayed typical histologic features of IPT with dense lymphoplasmacytic infiltrate admixed with bland spindle cells in a collagenous stroma.



All cases exhibited moderate to marked IgG4-positive PC/HPF.



ALK-1 was negative.

Conclusions: ALK expression was absent in all cases and none of them recur. The detection of significant number of IgG4-positive PC in IPTs suggests that a considerable proportion of intracranial IPT may belong to the IgG4-related subgroup. Hence a trial of corticosteroid may be valid to avoid unnecessary risk taking neurosurgical procedures or in cases with incomplete tumor removal.

1504 Repeat Molecular Testing in Gliomas: A Retrospective Study of 53 Patients

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Background: Molecular testing for deletions on chromosomes 1p and 19q and for EGFR amplification has implications for the clinical management of certain glial tumors. The value of repeat testing in patients with multiple resections is unclear. The purpose of this study is to add to previously reported data assessing for evidence of molecular changes in gliomas which have undergone repeat testing.

Design: 53 patients (31 males; age mean 45.4 years) who had repeat molecular testing on specimens from two different resections for chromosome 1p deletion, chromosome 19q deletion and/or EGFR amplification by fluorescent in situ hybridization (FISH) were studied.

Results: Original diagnoses included 27 diffuse fibrillary astrocytomas (11 low grade, 3 anaplastic and 13 GBM), 16 oligodendrogliomas (11 low grade and 5 anaplastic), 6 mixed gliomas (4 low grade and 2 anaplastic) and 4 gliomas not otherwise specified. Nine tumors upgraded during the interval between the initial and the subsequent resection (4 astrocytomas, 2 oligodendrogliomas and 3 mixed gliomas).Paired results for 1p evaluation demonstrated a change in the profile from intact to loss in 1/50 patients (2%); the tumor upgraded from a low grade to anaplastic mixed glioma on the subsequent resection. Paired results for 19q evaluation demonstrated a change in profile in 4 of 41 patients (9.7%). Two of these tumors diagnosed as GBM on the initial and subsequent resections changed profile from 19q loss to intact. The remaining 2 tumors (1 astrocytoma and 1 mixed glioma) were initially 19q intact and changed to loss on the subsequent resection. The mixed glioma upgraded to anaplastic mixed glioma on the subsequent resection. There was no change in the EGFR expression in any of the patients tested (N= 34; 28 with no amplification, 6 with amplification). There was no change in the clinical management based on the repeated molecular tests in patients with discrepant repeat results.

Conclusions: There was only rare evidence of profile change in 1p and 19 q status (5/53 tumors) and no change in the EGFR amplification status with repeated testing. None of the tumors with change in molecular status were oligodendrogliomas. There appears to be no indication for repeat 1p/19q or EGFR FISH testing in gliomas at the time of repeat biopsy or resection.

1505 Isolation & Characterization of Brain Tumor Stem Cells (BTSC) in Human Glioblastomas (GBs)

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Background: Recent studies are suggesting that gliomas, develop from a subset of cells with self-renewal capacity stem-like cells. GB research, is showing evidences that brain tumor stem-cells (BTSC) drive tumorigenesis. BTSC isolated from GBs transplanted into immunodeficient animals generate GBs. However transplanted GBs cell lines devoid of BTSC do not generate tumors. BTSC are identified by their capacity to form neurospheres in culture, that express progenitor/ stem cell markers, like CD133, Nestin, Wnt, CXCR4, etc. Furthermore, BTSC expressing CD133 have been shown to be more resistant to radiation, being responsible of radiation treatment failure. Most studies were done in vitro or in animal models. We have addressed whether a population of BTSC exists in human GBs, that can be characterized phenotypically, to study patterns of expresssion & get insights into the biology of GBs.

Design: GBs disaggregated to single cells, were cultured with EGF & hFGF. Neuroespheres were harvested at 6 weeks. After centrifugation, the pellet was fixed, paraffin embedded and sectioned. Neuroespheres & original GB specimens from which they were generated, were immunostained with CD133, Nestin, Wnt1, CXCR4 & VEGFR3.

Results: CD133, Nestin, Wnt1, CXCR4 & VEGFR3 were expressed by neuroespheres with variable intensity, consistent with their heterogeneous nature. GBs displayed expression of these antigens by groups of neoplastic cells identified as BTSC with the following patters: -Frequent expression by perivascular cells, neoplastic vessels & perinecrotic palisades. -Increased expression by infiltrating marginal cells, versus the central tumoral areas. -High levels at the most anaplastic areas.

Conclusions: The patterns of expression of the BTSC subpopulation in GBs argues for their role as cancer driving cells. 1) The expression of stem/progenitor markers support their property as self-renewal neoplastic elements. 2) The perivascular and endothelial proliferating cells location of BTSC, argues for a role in neoplastic angiogenesis, a hallmark of glioma anaplasia. 3) The high levels of BTSC observed at infiltrating margins and perinecrotic palisades, are in keep with their suggested invasive function. This raises the importance of using BTSC as therapeutic targets to improve treatment success.

1506 Molecular Alterations of PDGFA and PDGFRA in Gliomas

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Background: Malignant gliomas are the most prevalent primary brain tumours, have an aggressive clinical course and lack effective treatment options. PDGF signalling is one of the key regulators of glioma development. The efficacy of anti-PDGFRA drugs for the management of patients with glioblastomas is currently being tested in clinical trials. The aims of this study were to determine the expression of PDGFRA and PDGFA and the underlying genetic mechanisms driving their expression in a large series of gliomas.

Design: We investigated the frequency of PDGFA and PDGFRA expression by immunohistochemistry in 169 gliomas and screened for *PDGFRA* gene mutations and gene amplification in 86 and 57 gliomas using a combination of direct sequencing, quantitative copy number PCR and microarray-based comparative genomic hybridisation.

Results: We found that PDGFA was largely expressed in different glioma histological types and its absence was associated with a poor prognosis. PDGFRA was significantly expressed at high levels in malignant astrocytic tumours. Moreover, we have observed the existence of putative PDGFA/PDGFRA autocrine/paracrine loops in glioblastomas. Finally, although *PDGFRA* gene activating mutations were not found, *PDGFRA* gene

amplification was observed in 21% of gliomas and was significantly associated with PDGFRA overexpression in diffuse astrocytomas.
Conclusions: The present study describes a comprehensive molecular analysis of PDGFA and PDGFRA in gliomas. Taken together, these results provide a molecular basis for anti-PDGFRA therapies in gliomas.

1507 Quantitation of Large Subsarcolemmal Mitochondrial Aggregates Improves Specificity for Diagnosis of Mitochondriopathy in Children

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Background: In the diagnosis of mitochondriopathy the presence of ragged red fibers and COX-negative fibers is helpful, but these features are uncommon in muscle biopsies from children. Until recently >2% of myofibers containing subsarcolemmal mitochondrial aggregates (SSMA) was proposed as a minor criterion for diagnosis of mitochondriopathy. The current authors suggested previously that only large SSMA (LSSMA), ≥4μm in thickness, are useful for the diagnosis of mitochondriopathy. The current study compares the sensitivity and specificity of % of myofibers containing LSSMA, SSMA, type 1 myofiber, and for each patient, the lowest individual electron transport chain (ETC) complex activity in the diagnosis of mitochondriopathy.
Design: Only patients with previously identified LSSMA and ETC testing in muscle were included in this study. The discriminative performances of LSSMA(%), SSMA(%), type I myofiber predominance(%), and lowest individual ETC complex activity (% of mean control) result were evaluated for the diagnosis of mitochondriopathy using receiver operating characteristic (ROC) analysis. Results are expressed as mean±SD.
Results: In 35 patients with LSSMA, 9 [age 7.3±7.8y] had mitochondriopathy (group 1) and 26 [age 4.3±2.9y] had no evidence of mitochondriopathy (group 2). Group 1 patients had increased LSSMA (p=0.007) compared to group 2, 4.7±3.4% vs. 1.7±1.0%, respectively. Type I myofiber (%) and SSMA (%) were similar between groups. ETC complex activities were similar except for decreased complex I+III activity in group 1 (p=0.008). ROC analysis results are summarized in the table. Logistic regression modeling indicated that the diagnostic performance was significantly improved (Area Under the ROC Curve=0.938) with the combined use of LSSMA and ETC testing

Comparison of ROC Results

	AURC	Cutoff	P-value	Sensitivity	Specificity
Lowest ETC activity (%)	0.80	≤40.0	0.0001	77.8	88.5
Large SSMA (%)	0.81	>3.3	0.0013	66.7	96.2
Type I Myofiber (%)	0.59	>60.0	0.44	55.6	68.0
SSMA (%)	0.54	>28.3	0.71	22.2	100.0

AURC; area under ROC curve **Conclusions:** Because of improved specificity, a LSSMA >3.3% should be considered as a potential major criterion for diagnosis of mitochondriopathy in children.

1508 Caveolin-1 Expression Predicts Outcome in Oligodendroglial Tumours Regardless of 1p/19q Status

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Background: Caveolin-1 (Cav-1) is the basic component of caveole, omega-shaped membrane microdomains involved in various cell functions. In tumours, Cav-1 can be either overexpressed, suggesting a pro-neoplastic role, or downregulated: therefore, its role in oncogenesis is still debated. Regarding brain tumours, our group demonstrated that Cav-1 is significantly more expressed in astrocytic-derived tumours than in oligodendrogliomas, suggesting how this marker could be used as a valuable tool in the differential diagnosis between these two categories. Moreover, in tumours of astrocytic origin, we reported that Cav-1 expression increases accordingly to tumour grade, thus suggesting a tumour-aggressiveness related phenotype and envisaging a possible role for Cav-1 in predicting patients' prognosis.
Design: We here studied Cav-1 expression in oligodendroglial tumours, such as oligodendrogliomas (OD), oligoastrocyromas (OA) and glioblastomas with oligodendroglial component (GBMO) to evaluate its potential role as a prognostic factor and to determine if its expression is related to 1p/19 deletion, to date the hallmark prognostic factor for these gliomas. Eighty-seven cases of ODs, OAs and GBMOs were collected, and studied for 1p/19q status and Cav-1 expression by FISH analysis and immunohistochemistry respectively.
Results: Cav-1 was expressed in a minority of cases (21.8%), mostly grade III OAs and GBMO; 1p/19q deletion was expressed in 45,97% cases, mostly grade II and III ODs. The correlation between 1p/19q deletion and loss of Cav-1 staining was proven to be statistically significative (p= 0.0002), as well as a single chromosome deletion (1p or 19q), defining a group of patients with better prognosis. Moreover, Cav-1 positivity independently recognized a subset of tumours with worse prognosis (Mantel-Cox=0.03), even if concurrently carrying 1p and/or 19q deletion.
Conclusions: We here provide the first evidence that Cav-1 is a new trustworthy, easy to manage, independent prognostic marker in oligodendroglial-derived tumours regardless of the 1p/19q status Since Cav-1 has been also associated with mechanisms underlying multi-drug resistance, we feel thus entitled to preliminarily suggest that the worse outcome in Caveolin-1 positive patients in our series could be at least partially related to an acquired chemotherapy resistance.

1509 Clinicopathological Study of Extraventricular Neurocytomas

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Background: From 1992, parenchymal counterpart of central neurocytoma (CN), extraventricular neurocytoma (EVN) started to be recognized, which share the histopathogical features of the CN but are known to show a wide morphological spectrum.
Design: Five recent cases of EVNs along with the clinicopathological and radiological findings are reviewed.
Results: The mean age of the patients was 36.2 years old (6 yrs~66 yrs) and a female predominance (M:F=1:4) was found. The most common symptom was seizure (n=4) and the tumors were located in the temporal lobe (n=3), frontal lobe (n=1), and hippocampus (n=1). MRI showed nonenhancing cystic lesion (n=2), infiltrating solid mass (n=2), and well circumscribed mass with focal high signal intensity lesion on T2 and FLARE (n=1). Near total resection of the tumors were performed in every case. The tumor cells in all cases, regardless of the tumor grade, were composed of small round cells with round nuclei and clear or eosinophilic cytoplasm. These cells were arranged in sheet, in association with broad zone of fine neuropils. They superficially mimicked oligodendroglioma, but fried egg appearing cells were only focally observed. The cellularity was variable area by area. Often smaller ganglioid cells with nuclei that are larger and paler than neurocytes were detected. Three high grade ones showed high mitotic activity (7 to 9/10 HPF) and high level of MIB-1 labeling Indices (6 ~29 %). Two of them had vascular endothelial hyperplasia and necrosis. Almost tumor cells were immuno-labelled for synaptophysin and NeuN, and, additionally, expressed the nestin. GFAP expressing cells were observed focally, but pseudopapillary configuration was not shown. Ultrastructurally, neural tubules and synapses with synaptic junctions and synaptic vesicles were well observed. 1p/19q FISH study perfomed in 2 cases revealed no deletions. Radiotheraphy was offered to three patients with high grade ones. There were no case of recurrence in the course of follow-up periods (3 ~26 months) even though the follow up duration was not sufficiently long enough to confirm the biologic behavior.
Conclusions: EVNs were occurred in the patients with broad age ranges and epilepsy was most common symptom. Without immunohistochemistry, EVNs had a diagnostic pitfall due to spectrum of tumor cell morphology and similarity to oligodendrogliomas. More reports about its clinicopathological, biological and genetic studies are needed to understand and to have confidence upon this tumor.

1510 Assessment of the 1p/19q Deletions at Different Areas in Biphasic Oligoastrocytomas by Using Chromogenic In Situ Hybridization

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Background: Oligoastrocytomas (OAs) are mixed gliomas composed of tumor cells morphologically resembling oligodendroglioma and diffuse astrocytoma. Albeit the status of 1p/19q deletions in oligodendrogliomas is well-known, isolated or combined losses of 1p/19q in OA remains to be elucidated. The goal of this study was to evaluate the 1p/19q deletions by using CISH at different tumor areas in a series of bona fide "biphasic" OAs of different grades of malignancy.
Design: CISH was performed on formalin-fixed paraffin-embedded cores in different tumor areas of 12 OA, intermingled ("diffuse") variant (4 grade II, and 8 grade III) by using a TMA block . The patient's mean age was 40 years (11-54 years) with 2:1 male/female ratio. The presence or absence of chromosome locus 1p36 and 19q13 was analized in different tumor areas [oligo *vs* astrocytic areas]. The cut-off values was established by counting 500 nuclei in normal brain tissue from patients with intractable epilepsy. It was analyzed a minimum of 200 tumor nuclei for each set of probes without the knowledge of the diagnosis.
Results: Were have found combined/isolated losses of 1p/19q in 50% of the tumor samples.

Table 1. Status of 1p/19q deletions in Oligoastrocytomas according different grades of malignancy.

Grade	1p/19q	1p	19q	No deletions	Total
II	1	1	—	2	4
III	1	2	1	4	8

The combined 1p/19q losses were detected in 2/12 cases and were present in both astrocytic and oligodendroglial components. The isolated loss of 1p was detected in 3/12 cases (25.0%). Interestingly, the 1p loss was present in both components in one grade III OA. The remainder exhibited isolated 1p loss in astrocytic but not in the oligodendroglial area (grade II) and vice-versa (grade III OA). The isolated loss of 19q was found in both components and it was present in one case.
Conclusions: 1p/19q deletions are not an uncommon event in OAs. Interestingly, in these cases, regardless if they have combined or isolated losses, the chromosomical deletions were found in both oligo and astrocytic components in 4/6 cases, which could reflect the clonal origin for both components. Furthermore, CISH is a low-cost technique, easy to perform and has a beneficial tool in the diagnosis assessment of 1p/19q status in gliomas.

1511 Adhesion Molecule Expression in Primary, Recurrent, and Metastatic Medulloblastomas

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Background: Medulloblastoma spreads by leptomeningeal dissemination rather than the infiltration that characterizes other CNS tumors. Adhesion of tumor cells to the meninges is necessary for this spread and may contribute to the survival and proliferation ofthe tumor implants. The mechanisms accounting for this are not known.
Design: This study represents an immunohistochemical study of molecules which may govern medulloblastoma adhesion to leptomeninges. Search of the UHN surgical archives uncovered 57 adult medulloblastomas from 42 patients. In addition to primary resections for all patients, there were 13 recurrences in the posterior fossa and 2 instances of leptomeningeal dissemination. Immunohistochemistry was performed with antibodies to transmembrane adhesion molecules: Beta1 Integrin, NCAM, L1CAM, NCadherin and extracellular matrix molecules: Collagens I and IV, Tenascin, Fibronectin, Vitronectin, Trombospondin and Laminin.
Results: Evaluation of the stained sections showed increased Beta1 Integrin reactivity in recurrent and leptomeningeal tumors compared to their matched primary resections.

There was little difference in the reactivity of NCAM, L1CAM and NCadherin between primary and recurrent tumors. Of the extracellular matrix molecules, the greatest difference was seen in Tenascin the staining of which was increased in recurrent tumors.

Conclusions: The current study supports previous *in vitro* studies from our group (Fiorrilli et al Laboratory Investigation 2008) demonstrating that Beta1 Integrin and Tenascin play critical roles in medulloblastoma adherence to the leptomeningeal extracellular matrix. Further studies in our laboratory will be directed toward elucidating the mechanism by which the expressions of Beta1 Integrin and Tenascin are increaed in these tumors and the importance of that expression to medulloblastoma metastasis.

1512 Do Microscopic Thrombi in Glioblastoma Multiforme (GBM) Predict the Development of Deep Venus Thrombosis (DVT)?

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Background: Patients with GBM are known to be at risk for hypercoagulable events. Tumoral intravascular thrombi likely contribute to the development of hypoxia and necrosis. The purpose of this study is to assess whether there is a relationship between the number of thrombi identified microscopically at the time of tumor resection and the subsequent development of extremity DVT.

Design: Retrospective review of 96 patients (pts) (53 males and 43 females; age range 21-92 years, age mean 60.2 years) with GBM (WHO grade IV). Thrombi were counted (number of thrombi/blood vessels evaluated/10 high power fields) in nonnecrotic areas of the resected tumor and correlated with a variety of clinical and pathologic parameters including the development of postoperative DVT, as detected by extremity ultrasound.

Results: Thrombi were identified in the resected GBM in 66 pts (69%). Of the tumors with thrombi, the percentage of blood vessels with thrombi ranged from 1.1-42.9% (mean 10.7%). DVT were discovered in 30 pts (31.3%). There was no correlation between the number of microscopic thrombi and the development of DVT. Eighty one pts died of tumor (1-66 months survival, mean 10.4 months), 12 pts were alive at last known follow-up, and 3 pts were lost to followup. Of pts with DVT, 26 pts died of tumor (survival 1-47 months, mean 11.0 months), 3 pts were alive and 1 pt was lost to follow-up. There was no correlation between the number of microscopic thrombi and the percent of resected tumor which was necrotic (range <5-90%), presence of palisaded necrosis (36% of tumors), presurgical (mean 78) or post surgical (mean 75). Karnofsky performance scores (KPS), or survival (mean 8.9 months in pts with no microscopic thrombi versus mean 11.5 months in pts with thrombi).

Conclusions: Microscopic thrombi were identified in about 2/3 (69%) of GBM and DVT developed in about 1/3 (31.3%) of pts with GBM. There was no correlation between the number of microscopic thrombi and the subsequent development of DVT in pts with GBM. Pts who developed DVT did not appear to have a worse survival.

1513 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Assay for Detecting Epidermal Growth Factor Receptor Variant-III (EGFRvIII) in Glioblastomas

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Background: The EGFRvIII is an oncogene generated by an in-frame genomic deletion that defines a prognostically distinct subgroup of glioblastomas associated with favorable response to tyrosine kinase inhibitor therapy. Detection of this mutation in clinical samples, therefore, is warranted. This is challenged by the unavailability of frozen tumor tissues, especially in a community hospital setting, and lack of a reliable EGFRvIII antibody for immunohistochemical detection. We developed a new, simple RT-PCR based assay for detection of EGFRvIII in formalin fixed, paraffin embedded (FFPE) tissues.

Design: From January 2007 to August 2008, 104 cases of newly diagnosed gliomas were retreived from the pathology information system database at our institution. Archival FFPE tissue blocks from 39 patients were selected based on tissue availability and adequate proportion of tumor for RNA extraction on histology review. RNA was exctrated using RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion, Austin, TX; Applied Biosystems, Foster City, CA). RNA quality and integrity was assesed by RT-PCR for B-actin transcripts. New primers were designed for RT-PCR using two-step RT-PCR method (Qiagen, Valencia, CA). We also used published primers for comparison. PCR products were run on 2.5% agarose gel for detection. All the positive samples were confirmed by bidirectional direct sequencing (BigDye terminator v3.1, Applied Biosystems, Foster City, CA).

Results: There were 19 glioblastomas, 3 anaplastic astrocytomas, 4 low-grade astrocytomas, 9 oligodendrogliomas, and 4 mixed oligoastrocytomas. Our RT-PCR assay detected the EGFRvIII mutation in 7 of 19 (37%) glioblastomas. In contrast, only 3 of these 7 were detected by using published primers. All the 7 positive cases were confirmed by direct sequencing. All the other cases (astrocytomas, oligoastrocytomas, oligodendrogliomas) were negative with either RT-PCR primers.

Conclusions: Our assay provides a simple, semi-quantitative, accurate detection of EGFRvIII mutation from clinical FFPE glioma samples. The increased sensitivity of our assay in the detection of this mutation may be explained by differences in the RNA extraction method and the PCR conditions. This assay may aid in stratifying patients with glioblastomas to predict response to EGFR kinase inhibitor therapy and further help in individualized targeted therapy strategies.

1514 *PTEN* (10q) Loss Occurs in a Subset of Pilocytic Astrocytomas with Anaplastic Transformation

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Background: Pilocytic astrocytoma (PA) is a low grade astrocytic tumor that occurs most commonly in children and young adults. Anaplastic (malignant) transformation of PA is an extremely rare event. No studies of the molecular characteristics of this phenomenon exist in the literature to our knowledge.

Design: We studied 12 PA obtained from 9 males and 3 females with a median age of 28.5 years at the time of malignant transformation (range 11-73). The tumors occurred at a variety of sites, including cerebellum (n=5), supratentorial brain (n=3), spinal cord (n=2), brainstem (n=1) and tectum (n=1). Relevant clinical features included NF1 (n=4) and prior irradiation (n=1). Histologic slides were evaluated by three (of 4) observers (EFR, CG, FJR, BWS). Anaplastic transformation was defined by the presence of brisk mitotic activity (≥5/10 HPF) with or without endothelial proliferation or necrosis. We performed dual color fluorescence in situ hybridization (FISH) studies, using LSI probes targeting *PTEN* (10q23)(n=12) and *EGFR* (7p12) (n=10) with respective CEP10 and CEP7 control probes. A total of100 cells/per case were counted by 2 independent observers. A third independent observer scored discordant cases. Immunohistochemical (IHC) studies for ki-67(MIB-1)(n=11) and P53 (n=10) were also performed.

Results: Clinical follow-up was available in 9 patients: 5 developed tumor recurrences and 4 expired 5 mos to 2 yrs after surgery. Histologic evaluation showed a median mitotic count/10 HPF of 8, necrosis (n=7) and endothelial proliferation (n=2). Results of FISH and IHC studies are summarized in table 1. There were no associations between molecular abnormalities and clinical features or survival. However, the only two PA arising in the spinal cord had *PTEN* (10q) deletion.

Conclusions: *PTEN* (10q) deletion is present in 25% of cases in our series of PA with anaplastic transformation. Although gains of chromosome 7 were frequent, *EGFR* amplification was not identified in any case. Further molecular studies using additional markers are necessary to better characterize the oncogenic mechanisms responsible for anaplastic transformation in PA.

FISH and IHC studies in the malignant component of pilocytic astrocytomas with anaplastic transformation

Marker	N (%)
PTEN (10q) heterozygous deletion	3/12 (25)
Chr 7 gain	8/10 (80)
EGFR amplification	0/10 (0)
P53 overexpression (3+)	3/10 (30)
MIB1 labeling index	Median 22% (range 4-63)

1515 Medulloblastoma Variants and Survival at a Children's Hospital

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Background: Medulloblastoma variants recognized by WHO- classic (NOS), desmoplastic (DES), large cell (LC), anaplastic (ANA), excessive nodularity (EN)- may have prognostic differences. ANA and LC may be poor, EN and DES may be more favorable.

Design: To assess the spectrum, frequency, and survival of variants, we reviewed our 25 year experience with medulloblastomas diagnosed, treated, and followed by Children's Hospital. Slides were reviewed and classified according to WHO . Follow-up was provided by the tumor registry. The Kaplan-Meier method was used for cumulative survival statistics.

Results: 35 children had slides for review, the variants included NOS 22 (63%), ANA 5(14%), DES 4(11%), EN 3(9%), and LC 1(3%). 34 had follow-up. Cumulative survival statistics were calculated.

Cumulative survival

Variant	N	1 yr survival	2 yr survival	5 yr survival	10 yr survival	15 yr survival
NOS	22		.7638	.6650	.6096	.6096
ANA	4	.7500	.5000			
DES	4	1.000	.1000	.5000	.2500	.0000
EN	3	1.000	1.000	1.000	1.000	1.000
LC	1	1.000	1.000	1.000	1.000	1.000
ALL	34	.9099	.8188	.6631	.5851	.5051
< 3 yrs	8	.875	.4379	.4379	.4379	.4379
3 yrs or older	26	.9200	.9200	.7375	.6392	.5114

8 children under 3 yrs at diagnosis included 4 NOS, 2 ANA, 2 EN. 4 died within 2 yrs. The 2 with follow-up more than 2 yrs have survived 17(NOS) and 19 (EN) yrs. In the 3yrs or older children, 2 deaths occurred between 5 and 10 yrs (NOS and DES, both at 8 yrs), and 1 recurred and died at 14 yrs (DES). All 4 with DES died, 2 with late recurrences (8 and 14 yrs). 2 EN have survived 19yrs (dx'd at 1 yr) and 17 yrs(dx'd at 4 yrs). 2/5 ANA were < 3yrs at dx. None of the 4 DES were < 3yrs at dx. The one LC is alive at 18 yrs. No second malignancies have occurred.

Conclusions: NOS variant was the most frequent. None of our DES survived, with 2/4 deaths occurring after 5 yrs. Our data supports that EN carries a good prognosis, with no deaths (follow-up of 1,17, and 19 yrs). Of the 4 ANAs, 1 died, but the others are recent without 5 yrs of follow-up. Those less than 3 yrs of age continue to do poorly, with deaths within 2 yrs, unless the variant is EN. Late deaths occur, and continued follow-up of these children is required.

1516 Chordoid Glioma of the Sacrum: A Case Report

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Background: Chordoid glioma is a rare glial neoplasm previously reported exclusively in the anterior third ventricle, with a strong female predilection.

Design: We present a case of a forty-one-year-old female who presented with a protuberant mass of the posterior sacrum. Magnetic resonance imaging showed a

heterogeneously enhancing, circumscribed, lobular mass protruding from the posterior sacrum and also extending internally into the lower abdomen. The external portion was excised for pathologic analysis.

Results: Examination of tissue from the mass showed a hypercellular lesion composed of clusters and cords of medium to large epithelioid cells in a background matrix of lightly basophilic, myxoid matrix, traversed by narrow fibrovascular bands with a mild lymphocytic infiltrate. The morphology was that of a chordoid neoplasm. No areas of papillary architecture, perivascular mucin, or collagen "balloons" were identified. Immunohistochemistry performed at two institutions demonstrated strong diffuse cytoplasmic positivity within tumor cells for glial fibrillary acidic protein (GFAP) and S100. Immunohistochemistry for synaptophysin, epithelial membrane antigen (EMA), CD99, desmin and pancytokeratin (AE1/3) was negative. Material suitable for ultrastructural examination was unavailable. Expression of GFAP and S100 could be consistent with a myxopapillary ependymoma. However, the morphology was not consistent with myxopapillary ependymomas, and these tumors are uniformally positive for CD99. Negative cytokeratin and EMA stains, as well as a positive GFAP, effectively rule out the possibility of a sacral chordoma.

Conclusions: Taken together, the morphologic and immunophenotypic evidence in this case are diagnostic for chordoid glioma, an entity not previously described outside of the third ventricle.

1517 Epstein-Barr Virus in Multiple Sclerosis

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Background: Multiple sclerosis (MS) is a chronic disease of the central nervous system thought to be autoimmune in nature, and characterized by inflammation and demyelination. Many viruses have been implicated in the pathogenesis of MS, including Human Herpes Viruses 6, 7, and 8, and Varicella Zoster Virus, but follow up studies have failed to confirm a role. Recent evidence has suggested Epstein-Barr Virus (EBV) as a candidate. MS patients have a higher incidence and higher titers of anti-EBV antibodies compared to controls. In addition, some T cells in MS patients can cross-react with both myelin basic protein and virus-derived peptides. EBV has the ability to latently infect B cells, which express specific EBV-associated proteins depending on the phase of infection, including latency membrane protein 1 (LMP1). In a recent study (Serafini et al., J Exp Med. 2007; 204: 2899), positive immunohistochemical (IHC) staining for LMP1 was found within demyelinated lesions in 18 of 20 postmortem specimens from MS patients. EBV-encoded RNA (EBER) was also detected by in-situ hybridization (ISH) in 19 of 20 of these specimens. The EBV immunostaining was mainly located in perivascular lymphocytic cuffs within white matter lesions. The goal of this study is to confirm these findings.

Design: We studied 17 formalin-fixed, paraffin-embedded MS lesions (16 surgical, 1 autopsy) for evidence of EBV infection. Control cases consisted of brain tissue from 15 inflammatory non-MS lesions (11 encephalitis, 4 progressive multifocal leukoencephalopathy (PML)) and from 12 autopsy cases with no histologic abnormalities. IHC was performed using monoclonal LMP1 antibody (Dako, Carpinteria, CA), and ISH was performed using an EBER probe (Ventana Medical Systems, Tucson, AZ). Positive controls included an RNA control on each case as well as Hodgkin's and non-Hodgkin's lymphomas. Negative controls were performed on all cases without antibody or probe.

Results: All MS and control cases were negative for EBV by both LMP1 IHC and EBER ISH. Positive controls showed strong diffuse positive staining by both IHC and ISH. Negative controls showed no staining with minimal background.

Tissue Type	LMP1	EBER
MS	0/17 (0%)	0/17 (0%)
Encephalitis	0/11 (0%)	0/11 (0%)
PML	0/4 (0%)	0/4 (0%)
Normal Control	0/12 (0%)	0/12 (0%)

Conclusions: The results of this experiment do not confirm previously reported evidence for a role of EBV infection in multiple sclerosis.

1518 CD68 vs. CD163, Which Is More Sensitive and Specific in Various Brain Lesions

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Background: CD68, also called KP1 is a 110-kD glycoprotein localizing to lysosomes and endosomes. It is highly expressed by monocytes and tissue macrophages. CD163 (scavenger receptor cysteine-rich type 1 protein M130 precursor) is a hemoglobin/haptoglobin scavenger receptor present on macrophages and monocytes. Two types of phagocytic cells are recognized within the central nervous system (CNS): innate microglial cells (microglia) and blood borne macrophages. Antibody to CD68 is used as a marker of both blood-borne as well as microglial cells. Less is known about the expression of CD163 in macrophages and microglia in various pathologic conditions in the CNS. The aim of this study is to compare and contrast immunoreactivity of CD68 and CD163 and to determine which antibody is more specific and sensitive in detecting macrophages and microglia in various CNS lesions.

Design: Immunostaining for CD68 and CD163 was performed on formalin-fixed paraffin embedded tissue. Forty-three cases were selected which included normal brain controls (autopsy cases without apparent CNS lesions, 10 cases), cerebral acute and subacute infarctions (10 cases), glioblastoma multiforme (10 cases), brain tissue adjacent to variety of tumors (9 cases), and demyelinating diseases (4 cases).

Results: In nearly all cases, CD163 showed a much stronger plasma membrane associated signal (3+) with a clean background, which allowed an easier visualization of reactive cells. In contrast, staining with the dilution of CD68 optimized for hematologic pathology diagnosis is consistently weaker than that of CD163 for both macrophages and microglia (1-2+). Further tittering of CD68 antibody with comparable sensitivity to CD163 (dilution from 1:20,000 to 1:2500) showed a higher background with nonspecific reactivity in endothelial cells, smooth muscle cells, and granular background staining.

Conclusions: This study shows that CD163 is superior in both sensitivity and specificity than CD68 in surgical neuropathology. CD163 consistently displayed strong membranous staining pattern outlining both macrophages and microglia with a clean background, which can be easily visualized. Although neither of the stains could be used to differentiate microglia from a blood borne macrophages (both stain macrophages and microglia), two populations can be readily separated based on their immunostaining patterns (round or ovoid plasma membrane pattern for macrophages vs. granular, stellate staining pattern for microglia. Therefore, we recommend CD163 should be the first choice for CNS lesions.

1519 Cancer-Associated Fibroblasts Colocalize with Microvascular Proliferation and Their Levels Inversely Correlate with Schwannian Stroma in Neuroblastoma

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Background: Cancer-Associated Fibroblasts (CAFs) promote tumor growth, angiogenesis and invasion in certain carcinomas. We have previously demonstrated inverse correlations between microvascular proliferation (MVP), a hallmark of angiogenesis in aggressive tumors, and Schwannian Stroma (SS) in neuroblastoma (NB). To investigate the relationship of CAFs to MVP and to SS in NB, we quantified CAF levels in 60 primary human NB tumors, and in NB xenografts with infiltrating murine Schwann cells and reduced vascular density.

Design: 46 SS-poor and 14 SS-rich/dominant human NB tumors were evaluated. Vascular morphology was examined for presence or absence of MVP. NB (SMS-KCNR) cells were inoculated either inside or outside the sciatic nerve of nude mice, with vascular density and Schwann cell infiltration levels previously reported. Quantitative (ACIS II) and semiquantitative scoring were utilized to assess CAF levels as reflected by percent of αSMA+ve tumor areas. Positive immunostaining for hMW-caldesmon distinguished pericytes from CAFs.

Results: In the human NB series, the level of CAFs reflected by percent αSMA+ve area were strongly associated with SS-poor histology ($p<0.001$) and colocalized with MVP ($p<0.001$).

Cancer-Associated Fibroblast Levels

TUMORS	1.1%αSMA	1.1-3.0%αSMA	3.0%αSMA	Av.αSMA+ve
5 NB Undifferentiated	1(20%)	2(40%)	2(40%)	3.40%±2.19
14 NB Poorly Differentiated	2(14%)	8(57%)	4(29%)	2.41%±1.64
23 NB Differentiating	6(26%)	9(39%)	8(35%)	2.39%±1.78
4 Ganglioneuroblastoma Nodular	0(0%)	4(100%)	0(0%)	2.48%±0.34
7 Ganglioneuroblastoma Intermixed	5(71%)	2(29%)	0(0%)	0.96%±0.31
7 Ganglioneuroma	7(100%)	0(0%)	0(0%)	0.79%±0.19
46 SS-Poor	9(20%)	23(50%)	14(30%)	2.48%±1.7
14 SS-Rich	12(86%)	2(14%)	0(0%)	0.88%±0.3
37 With MVP	6(16%)	18(49%)	13(35%)	2.7%±1.7
22 No MVP	15(68%)	7(32%)	0(0%)	1.13%±0.6

In the NB xenografts with infiltrating stromal Schwann cells (n=10), CAF accumulation was 7-fold less than in controls (n=9) with mean αSMA+ve cells/mm^2 of 51±30 vs. 368±105, respectively; $p<0.001$.

Conclusions: Our findings suggest that CAFs promote angiogenesis in NB and that Schwann cells may prevent CAF infiltration. Novel anti-CAF therapeutic strategies may benefit children with aggressive NB.

1520 Increased Inhibitor of Differentiation 4 (Id4) Expression in Glioblastoma: A Tissue Microarray Study

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Background: Inhibitor of differentiation protein family (Id1-4) is involved in negative regulation of helix-loop-helix transcription factors as well as cell cycle control, tumor genesis and angiogenesis. Among these proteins, Id4 is known to have an important role in governing neural stem cell differentiation and increased Id4 mRNA level has been detected in glioblastoma multiforme (GBM) samples and glioma cell lines. Here we report the differential expression of Id4 in astrocytomas of various grades including GBM using tissue microarrays (TMA) and immunohistochemistry (IHC).

Design: The GBM TMA was constructed from 53 cases of archival GBM (grade IV) specimens at Georgetown University Hospital. The normal brain and grade II, III astrocytoma TMA was obtained from Cybrdi (Rockville, MD). TMA sections were stained with Id4 antibody (Chemicon International, Temecula, CA) using a DAKO autoimmunostainer and Envision Flex detection system (DAKO, Carpinteria, CA). The slides were scored according to percentage of the staining nuclei (<9% -, 10-50% +, >51% ++). Fisher Exact test was used to test for statistical significance.

Results: Nuclear staining for Id4 was seen in astrocytoma tumor cells. There was positive staining in 39 of 53 (73.58%) GBMs, 2 of 8 (25%) grade III and 1 of 8 (12.5%) grade II astrocytomas. None of the normal brain tissue (0/16) shows nuclear staining. There was a statistically significant difference between GBM and normal brain tissue, GBM and grade II, III astrocytoma ($p<0.01$). No statistically significance was detected between normal brain tissue, grade II and grade III astrocytoma ($p>0.05$).

Conclusions: Our study demonstrates frequent upregulation of Id4 in GBM. The tendency was noted that the higher the grade the more frequent Id4 expression in astrocytomas and none of the normal brain tissue showed positive Id4. This suggests that Id4 overexpression plays an important role in tumorigenesis of astocytomas, possibly in transformation of low to high-grade (i.e. GBM). Further studies of Id4 are warranted to determine its precise role in brain tumorigenesis and its possible use as a target for directed therapy in GBM.